Phenotypic characterizationof virulence factors in Multidrug-Resistant*Salmonellatyphi* serovars isolated from clinical specimens in Bauchi Metropolis

ABSTRACT

Background: Typhoid fever is one of the most common infectious diseases in developing countries including Nigeria.Globally, typhoid fever is an important cause of morbidity and mortality in many regions of the world.Peoplemostatriskfor seriouscomplicationsdue to*Salmonella*infectionsinclude older adults,pregnantwomen, infants,children,andpeople who have compromisedimmune systems.

Design and Duration: Thestudy involvesthecollectionofbloodandstoolspecimensacrossall agesandgenderbetweenages0-70yearswhopresentwithfeveranddiarrhoeaamong other symptomsoftyphoidinselected hospitals within Bauchi from January 2019 to August 2020.

Aim: The aim of this research is to phenotypically characterize virulence factors in Multidrug-Resistant*Salmonellatyphi* serovars isolated from clinical specimens in selected hospitals within Bauchi.

Materials and Methods: A total of 518 blood and stool specimens were collected from selected health facilities within Bauchi metropolisto determine the presence of *Salmonellae* pathogens. Phenotypic identification of*Salmonellatyphi*was performed using standardmicrobiologicalprocedures, virulence factorswere investigated and the Kirby Bauer Disk Diffusion method was used for the determination of the antimicrobialsusceptibility and MultidrugResistant patternoftheisolates.

Results and Discussion: Highest number of specimens collected was among patients with fever. Age groups 31-40 and 0-10 had the highest frequencies of occurrence respectively while age group 61-70 had the least. There was no significant difference between the age group and the number of isolates as p>0. 05. Highest frequency of *S. typhi* was found within the Female gender while Males recorded the lowest. Stool had the highest number of positive samples 31(21.6%) and blood had the least 17(4. 5%). Flagella was present in 18(37.5%) of 48 virulence isolates and was the most prevalent. Haemolysin was the least prevalent 4(8.3%) in all the isolates in our study. In the present study, 37(77.0%) of *S. typhi* isolates were resistant to 2 or more antimicrobial agents (Multidrug resistance). Highest resistance was observed in Oxacillin 46(95.8%). The isolates were sensitive to Ciprofloxacin 31(64.5%), Colistin Sulphate 29(60.4%), and Ceftriaxone 28(58.3%). All isolates 48(100%) were Multidrug-resistant and sensitive to Ciprofloxacin, Colistin Sulphate, Ceftriaxone, and Amikacin.

Conclusion: This study established that *Salmonella typhi* was more prevalent in the middle age group, female out-patient with mostcases of fever, diarrhoea, and sometimes both. Most of the *Salmonellatyphi* recovered from this study were more from stool than blood. Of all of the isolates in this study 48(9.2%) produced two or more virulent factors, with flagella as the commonest across all the MDR isolates; which is indicative of a significant relationship between virulence factors and multidrug resistance. The emergence of multidrug-resistant strains of *Salmonella* has added to the urgent need for the development of more effective control measures.

Keywords: *Salmonella typhi* Serovars, Phenotypic,flagella, virulent factors, Multi-Drug Resistance.

1. INTRODUCTION

Typhoid fever,alsoknown asenteric fever, isanacute systemic infectioncausedby *Salmonellatyphi*,a Gram-negativebacterium[1].Itislargely adiseaseof developingnationsduetotheirpoorstandardofhygieneandunavailability ofcleanwater which contributes greatly to its spread.The disease is transmittedfaeco-orally throughcontaminatedfoodandwater. Theinappropriateandover-useofantibioticsand otherantimicrobialstotreatinfectionsandconsequentantibioticselection pressurearethoughtto bethemajorcausativefactorscontributingtotheappearanceofstrainswithreducedsusceptibility toantibiotics*Salmonella* species possess a number of specific structural and physiological virulence factors enabling them to cause acute chronic diseases in humans. Such factors that improve their pathogenicity/antigenicity includes, polymorphic surfaces carbohydrates, multiple fimbria adhesion, phase variable flagella, well-structured mechanisms for invasion and survival in host macrophages and resistance genes profiles [2]. *Salmonella*,aprimaryinhabitant ofthegastrointestinaltract,isrecognizedasoneofthemostcommonbacterial causesofdiarrheainfections worldwide,resulting inmillionsofinfectionsandsignificanthuman mortality [3].Symptomsamong others areheadache,nausea,vomiting,fatigue,gastroenteritis, abdominalcramps,andbloody diarrheawithmucusandsometimesreactivearthritis [4].
The diseaseissystemic,withouttherapy,theillnessmaylastforthree tofourweeks. Althoughtheglobalburdenoftyphoid feverhasreduced,emergenceofmultidrug-resistant*Salmonellatyphi*(MDRST)isstillathreatto public health. *Salmonellatyphi*infectionhasbeenrecognizedasamajorpublichealthproblem[5].Generally,feveri sthemostcommoncauseofconsultationsinthetropicsandsub-Saharan Africa where mostfeverareof infectiousoriginofwhichtyphoidaccountsfor a majority.Typhoidfever is amongthemostendemicdiseasesinthetropics,itisassociatedwithpoverty andunder developmentwithsignificantmorbidity andmortality [6].
Theemergenceofmultidrugresistancetothecommonlyused antibioticshasfurther complicated the treatmentandmanagementof enteric fever andthisis recognizedasoneofthegreatestchallengesinthemanagementofthisdiseaseThe disease is anindicationofneglectofcontroloftheenvironment,whileitis goingextinctinthewiderworld, thecaseinAfricaremainsanalarming oneasitisbeingrecordedtoconstituteamajorcauseof hospital admissionsinAfrica[7].Thegeneticcharacterizationofantimicrobialresistancegenesaswell astheirlocationanddiversity isimportantinidentifyingfactorsinvolvedinresistance, understandingthediversity ofMDRstrains,identifyinggeneticlinkagesamong markers, understanding potentialtransfermechanisms,anddeveloping efficientdetectionmethods. Investigatingtheantibioticsusceptibilitypatternsofpathogensisimportant towardtailoring treatmenttotheever-changingresistancepatternsanddistributionofpathogenicbacteria*Salmonella typhi* possesses many kinds of virulence factors that play an important role in the process of pathogenesis [8]. Ability of *Salmonella typhi* toproduce multiple virulence factors contribute to its pathogenicity and also enables it to elicit an infection by overcoming the host defence mechanisms this is further escalating with the combination of resistance genes [9]. Most of the clinical species of *Salmonellatyphi* produced double or multiple virulence factors which increases their chances of overcoming host defence system. of MDR *Salmonellatyphi*strains produced. Virulence factors play a key role in conferring resistance to antibiotics. A robust and wide array of virulence factor repertoire may be essential for a pathogen to overcome intact host defences, whereas it may be unnecessary in a compromised host.

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2. MATERIALS AND METHODS

2.1 Study area

The study was carried out in selected hospitals across Bauchi metropolis. They include Abubakar Tafawa Balewa University Teaching Hospital, State Specialist Hospital, Bauchi,

Infectious Disease Hospital Bayara and Comprehensive Health Centre, Tashan Babiye. The study was conducted on both Febrile and Diarrhoeic patients of all ages and sex attending these hospitals. The specimens were collected from patients diagnosed by clinicians with either fever, gastroenteritis or both. Demographic information and important Bio data was also recorded.

2.2 Specimen collection

A total of 518 blood and stool samples were collected from both inpatients and outpatients in the selected hospitals of the study area to determine the presence of *Salmonellae* pathogens. Diarrhoea is defined as the presence of at least three loose stools or one watery stool per day. Fever is defined as a rise in temperature higher than 37°C [10]. Fluid Tetrathionate [8ml] broth in McCartney bottles was used to collect 2ml of venous blood. [11].

2.3 Specimen processing

2.3.1 Isolation of *Salmonellatyphi* from stool specimen

Exactly 1gram of faecal specimen was dispensed in nine [9ml] of Selenite F Broth [PART I and PART II], [Oxoid Limited, Hampshire, England] and used for pre-enrichment to allow for the multiplication of bacteria and incubated for 24hrs at 37°C. Turbidity was observed for bothmedia, thensub cultured onto Salmonella-Shigella Agar, MacConkey agar and Brilliant Green agar and incubated for 24hrs at 37°C. The representative *Salmonella* colonies were characterized morphologically using Gram staining method according to standard protocols [12].

2.3.2 Isolation of *Salmonellatyphi* from blood specimen

Exactly 2ml of venous blood was drawn aseptically from each patient using a needle and syringe by cleaning the skin using a swab and placed into 8ml of tetrathionate broth (Oxoid Limited, Hampshire, England) [13]. Blood culture broths were incubated at 37°c and checked for signs of bacterial growth daily for up to ten days. Bottles which showed signs of growth were sub cultured onto Salmonella-Shigella Agar, Brilliant Green agar and MacConkey agar.

2.4 Biochemical screening

2.5 Sugar fermentation tests

Nutrient broth cultures were prepared and used in Bijou bottles containing the basal medium. Carbohydrate [mannitol, maltose, dulcitol, sucrose and glucose] was inoculated with drop of the nutrient broth suspension of the test isolate and loosely capped and incubated at 37ºC overnight. They were observed for change in colour from amber to red and for gas production (in the medium filled inverted Durham tube) [14].

2.6 Urease test

The test organisms were inoculated heavily on the entire slope surface of the urea agar slants prepared in caped tubes. The tubes were placed in racks and incubated at 37ºC for up to 48 hours. Tubes were thereafter examined for change of colour from plain to pink [15].

2.7 Serotyping of identified *Salmonella* species

Colonies considered to be of *Salmonella* Spp. Were further tested for Somatic [O] and Flagella [H] antigens with polyvalent antisera (Oxoid,UK), [16]. Colonies of *Salmonella typhi* pure culture were picked using a sterile wire loop and placed on polyvalent antisera on a tile. This was then agitated for about 3minutes until agglutination reaction is seen. The agglutination antibody levels against O and H antigens of the *S. typhi*were then noted.

2.8 Phenotypic detection of *Salmonellatyphi* virulence factors

All the *Salmonellatyphi* isolates were screened for flagella, fimbriae, hydrogen sulphide gas production, haemolysin and biofilm formation.

2.8.1 Motility test [flagella test]

Motility agar was prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility was examined after 24 hours for 37°C.

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Motility was indicated by the presence of diffuse growth [appearing as colouring of the medium] away from the line of inoculation [17].

2.8.2 Detection of fimbriae

This was done using Mannose Sensitive Agglutination (MSA) of Bakers' yeast (*Saccharomycescerevisiae*) as described bySchembri *et al.*[18]. The *S. typhi* cells were mixed with a drop of 5% yeast cells suspension on glass slide and observed for agglutination.

2.8.3 Haemolysin production test

A plate haemolysis test was performed for the detection of haemolysin produced by *S. typhi* as described bySu and Liu[19]. The isolates were inoculated onto 5% sheep blood agar and incubated overnight at 37^{o}C. haemolysin production was detected by the presence of a zone of complete lysis of the erythrocytes around the colony and clearing of the medium.

2.8.4 Hydrogen sulphide production

Test organisms were inoculated into the Triple Sugar Iron agar slants contained in test tubes. These were incubated at 35° – 37°C for up to 48 hours. After incubation the TSI agar media was checked for blackening and change in colour from amber to red at the bottom (butt) of the tube [20].

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2.8.5 Biofilm production

Bacterial biofilm production was tested by a qualitative tube method as described by [21]. A loopful of test organism was inoculated in 10ml of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 35° – 37°C for up to 24 hours. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.

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2.9 Antibiotics susceptibility testing and Multidrug Resistant screening

Antibiotic susceptibility testing was carried out by disc diffusion method using Mueller-Hinton agar against the following antibiotics (Oxoid Ltd, UK), Ampicillin (AMP, 10mg), Amoxicillin (AMC, 30mg), Augmentin (AUG, 30mg), Amikacin (AMK, 10mg), Ceftriaxone (CFX, 30mg), Cefotaxime (CTX, 30mg), Ciprofloxacin (CIP, 10mg), Neomycin (NEO, 30mg), Erythromycin (ERY, 25mg), Chloramphenicol (CHL, 5mg), Nalidixic acid (NAL, 5mg), Cefuroxime (CXM, 30mg), Cephalothin (CEF, 5mg), Cotrimoxazole (COT, 25mg), Fusidic acid (FA, 5mg), Colistin sulphate (CST, 25mg), Gentamycin (GEN, 10mg), Novobiocin (NOV, 5mg), Oxacillin (OXA, 5mg) Methicillin (MET, 5mg) , Tetracycline (TET, 30mg) and Imipenem (IPM, 10mg) using. Prepared broth of colony suspension matching turbidity standard (0.5 McFarland) of the isolate was evenly streaked onto freshly prepared Muller-Hinton agar. Inoculated plates allowed to stay for 5mins, and subsequently incubated at 37°C for 18 hours. Determinative antibiotics susceptibility chart was used to interpret the zone sizes of inhibition. *Salmonella typhi* that are resistant to up to three (3) recommended antibiotics of choice were regarded as Multi-Drug Resistant (MDR) isolates.

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3. RESULTS AND DISCUSSION

Salmonellatyphi is more prevalent in developing countries than in developed regions. [22] reported a decrease in cases of typhoid fever in developed countries (due largely to adequate sanitary measures as compared to developing countries). The differences in the pattern of typhoid fever in developed and developing countries is multifactorial.In recent years, emergence of ever-increasing number of antibiotic resistant microbial strains has become a severe health threat to human-kind and one of the biggest challenges to global drug discovery programs [23]. Multidrug Resistant (MDR) strains of *Salmonella* aided by the presence of multiple virulence factors are now encountered frequently worldwide and the incidence of Multi-Drug Resistance have increased considerably in recent years [24].

Studies on the distribution of *S. typhi* among patients in selected Hospitals from the study area according to clinical diagnosis (Table 1), indicated a total of 518 specimens were collected with 48 of the specimen positive for *S. typhi*. Specimens collected from patients who presented with Fever was 308 out of which 18(5.8%) were positive for *S. typhi*. Patients with diarrhoea were 189 with 26(13.7%) positive. Patients with symptoms of Fever and diarrhoea were 21 with 4 (19.0%) of positive specimens. Patients with typhoid fever typically present with fever characterized by high temperature rise, diarrhoea and sometimes both.

Comment [KP11]: specimens

Table 1:Distribution of *Salmonella typhi*among patients in the study area according to clinical diagnosis

Cases	No.(%) of Specimens Collected	No. (%) of Isolates Positive	No. (%) of Isolates Negative
Fever	308	18 (5.8)	290 (94.1)
Diarrhoea	189	26 (13.7)	163 (86.2)
Fever and Diarrhoea	21	04 (19.0)	17 (80.9)
Total	518	48 (9.27)	470 (90.7)

The present study observed the frequency distribution of *S. typhi* isolates and MDR according to patient's demographic information and background (Table 2), where a total of 48 positive isolates with 37 MDR were recovered from 518 specimens collected from different age group (0 to above 70years), Age group 31-40 and 0-10 had the highest frequencies of occurrence (17) and (11) while age group 61-70 had the least (1). There was no significant difference between the age group and the number of isolates as $p>0.05$. [25] showed that prevalence of typhoid fever in endemic areas is considered high in young adults and school aged children. Older adults are presumably relatively resistant due to frequent boosting of immunity.

The study according to gender showed that 317 specimens were collected for males and 201 for females, highest frequency of *S. typhi* was found within the Female gender [26] while Males recorded the lowest (22). Females are more vulnerable to such diseases due to the physiological status, hormonal imbalance and changes as well as environmental factors associated to these women. Both Out-patients (31) and In-patients (17) were recorded in the present study. It also indicated that outpatients had the most percentage of *Salmonellatyphi* isolated than the in patients. The high rates among the out-patient was attributed to self-medication and indiscriminate use of antibiotics before coming to the hospital thereby engaging in irrational treatment that may not be effective. The report agrees with previous reports of [27] who reported sampling of typhoid fever from out patients as 63.2 % and 36.8% inpatients.

Table 2*:* Distribution of *Salmonellatyphi* isolates and MDR according to patients' demographic information

Patients' information	No. of Samples collected (n = 518)	No. (%) of *S. typhi* isolates (n = 48)	No. (%) of MDR *S. typhi* isolates (n =37)
Age (years)			
0-10	152	11(22.9)	09(24.3)
11-20	73	05(10.4)	03(8.1)
21-30	78	09(18.8)	06(16.2)
31-40	89	17(35.4)	14(37.8)
41-50	63	03(6.2)	03(8.1)
51-60	38	01(2.0)	01(2.7)
>70	25	02(4.1)	01(2.7)
Gender			
Female	317	26(54.1)	21(56.8)
Male	201	22(45.9)	16(43.2)
Group			
Out-patient	396	31(64.6)	24(64.9)
In-patient	122	17(35.4)	13(35.1)

The percentage of *Salmonellatyphi* isolated from blood and stool shows that there was no significant difference ($p>0.05$) between blood and stool samples (3). Stool had the highest number of positive samples 31(21.6%) and blood had the least 17(4.5%).Stool and blood can be collected from acute patients and they are especially useful for the diagnosis of typhoid carriers. The isolation of *S. typhi* from stools and bloods is suggestive of typhoid fever. However, the clinical condition of the patient should be considered. A failure to isolate the organism from blood may be caused by several factors such as inadequate laboratory media, prior use of antibiotics, inadequate volume of blood, the time of blood collection and incubation conditions. Widal test which is a serological test is the most widely requested by clinicians.The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture-proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibody response. On the other hand, *S. typhi* shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriaceae, and this can lead to false-positive results. Such results may also occur in other clinical conditions, such as malaria, typhus, bacteraemia caused by other organisms, and cirrhosis. In areas of endemicity, there is often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas. Hence, the gold standard for *Salmonellatyphi* identification is in blood and stool cultures.The health facilities where the samples were collected does not determine the number of positive isolates. Health facilities have differences in the availability of standard laboratories and test facilities which also play a major role in diagnosis and control of diseases.

Table 3:Distribution of specimens according to type and frequency of *Salmonella typhi* isolated from different health centres in the study area

Health facility	No. /type of specimen		No. (%) of *S. typhi* isolated	
	Blood	Stool	Blood	Stool
ATBUTH	58	31	04(6.9)	06(19.3)
IDHB	82	37	02(2.4)	05(13.5)
SHB	103	23	05(4.8)	09(39.1)
CHCTB	132	52	06(4.5)	11(21.1)
Total	375	143	17(4.5)	31(21.6)

Key

ATBUTH – Abubakar Tafawa Balewa University Teaching Hospital
IDHB – Infectious Disease Hospital Bayara
SHB – Specialist Hospital Bauchi
CHCTB – Comprehensive Health Centre Tashan Babiye

Distribution of *S typhi* isolates based on virulence phenotype in this study (Table 4) shows that all 48 isolates had one or more virulence factors. Flagella was present in 18(37.5%) of 48 virulence isolates and was the most prevalent. This is followed by Hydrogen Sulphide (H_2S) gas production 16(33.3%) of 48 virulence isolates. Biofilm production and Fimbriae both showed virulence factors in 5(10.4%) each of the isolates. Haemolysin was the least prevalent 4(8.3%) in all the isolates in our study. The difference in virulence factors production among MDR isolates from the entire sample source is not statistically significant ($p > 0.05$). Although haemolysin is not too essential for the establishment of acute pyelonephritis, it may contribute to tissue injuries, to the survival of the microorganism and entry into the blood stream including sepsis. Haemolysin is known to confer selective advantage to the pathogen by releasing iron from lysed erythrocytes and enhances pathogenicity by destroying phagocytic cells and epithelial cells. Haemolysin production has also been shown previously to influence pathogenicity.

Most of the isolates in our study produced double or multiple virulence factors. These results are consistent with other findings [28], where majority (82.5%) of MDR *Salmonellatyphi* strains produced. this supports the hypothesis that virulence factors play a key role in conferring resistance to antibiotics. A robust and wide array of virulence factor repertoire may be essential for a pathogen to overcome intact host defences, whereas it may be unnecessary in a compromised host.

Biofilms are a group of microbes which are encased in an exopolysaccharide matrix on both biotic and abiotic surfaces. This causes a number of persistent infections which respond poorly to conventional antibiotics therapy. Biofilm production was high in this study. The ability to form biofilms has been related to persistence of bacteria in the blood and high rate of fever associated with typhoid. In our study Hydrogen Sulphide (H_2S) gas production was present in 16(33.3%) of 48 virulence isolates. *Salmonella* strains are high Hydrogen Sulphide gas producers, this enhances pathogenicity, antibiotics resistance and host defence tolerance. A few species have been reported to show low Hydrogen Sulphide (H_2S) gas production.

Fimbriae and Flagella are virulence factors that aid the organism in the process of pathogenesis and binding to specific receptor structures thereby able to colonise specific surfaces. They promote adhesion to surfaces in the host cell. Flagella is essential for chemotaxis and motility in microorganisms and also enhances movements to preferred sites of infection.

Table 4: Virulence phenotypes of *S. typhi* isolate according to MDR pattern

Virulence factor	No. (%) of virulent isolates (n = 48)	No. (%) of MDR isolates (n = 37)
Biofilm formation	05 (10.4)	5(13.5)
H_2S gas production	16 (33.3)	12 (32.4)
Haemolysin	04 (8.3)	02 (5.4)
Fimbriae	05 (10.4)	04 (10.8)
Flagella	18 (37.5)	14 (37.8)

MDR – Multi-Drug Resistant

Enteropathogens are among the leading causes of diarrhoea and fever which is still the most common illness among children and young adults causing the highest number of morbidity and mortality and hospitalization in developing countries including Bauchi, Nigeria.

Comment [KP12]: Bauchi and Nigeria.

Table 5:Frequency of occurrence of Enterobacteriaceae isolates from the specimens collected from patients in the study area

Isolates	Number of occurrences (n = 518)	Percentage (%)
Escherichia coli	138	26.6
Klebsiella sp.	61	11.7
Shigella sp.	42	8.1
Salmonella typhi.	48	9.2
Pseudomonas sp.	77	14.8
Proteus sp.	83	16.0
Enterobacter sp.	69	13.3

Comment [KP13]: *typhi*

Diarrhoea and fever is more prevalent in developing world due in part, to lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status [29]. This study revealed that other organisms isolated from the specimens collected are *Escherichiacoli* with the highest prevalence rate of 26.6%, *Klebsiella* species 14.1%, *Shigella*

species 8.1%, *Salmonella* species 9.2%, *Pseudomonas* species 14.8%, *and Proteus* species 16.0%. *Enterobacter* species had the least percentage of 13.3%.

Multi-Drug Resistant (MDR) strains of *Salmonella* are now encountered frequently worldwide and the rates of Multi-Drug Resistance have increased considerably in recent years. Drug resistance is highly linked to virulence of microorganisms. Some variants of *Salmonella* have developed Multi-Drug Resistance and virulence genes as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant and virulent genes even when antimicrobial drugs are no longer used [30].

Table 6: Distribution of isolates according to antimicrobial susceptibility pattern

Antimicrobial Agent (µg)	**Number (%) of isolates (n = 48) and susceptibility pattern (%)**	
	Sensitive	**Resistant**
Ampicillin (10)	09 (18.7)	39 (81.2)
Augmentin (30)	18 (37.5)	30 (62.5)
Amikacin (30)	10 (20.8)	28 (79.1)
Ceftriaxone (30)	28 (58.3)	20 (41.6)
Cefuroxime (30)	15 (31.2)	33 (68.7)
Cefotaxime (30)	11 (22.9)	37 (77.0)
Cephalothin (5)	18 (37.5)	30 (62.5)
Chloramphenicol (5)	23 (47.9)	25 (52.1)
Ciprofloxacin (5)	31 (64.5)	17 (35.4)
Cotrimoxazole (25)	22 (45.8)	26 (54.1)
Colistin Sulphate (25)	29 (60.4)	19 (39.5)
Erythromycin (25)	08 (16.6)	40 (83.3)
Fusidic acid (5)	08 (16.6)	40 (83.3)
Gentamycin (10)	13 (27.0)	35 (72.9)
Imipenem (10)	04 (8.3)	44 (91.6)
Novobiocin (5)	07 (14.5)	41 (85.4)
Oxacillin (5)	02 (4.1)	46 (95.8)
Methicillin (5)	18 (37.5)	30 (62.5)
Tetracycline (30)	11 (22.9)	37 (77.0)

Resistance to ampicillin, chloramphenicol, cotrimoxazole, and ciprofloxacin was observed in this study, the resistance could be as a result of indiscriminate use of antibiotics. The inclusion of preventive doses of antimicrobial agents in poultry feed as growth promoters is often associated with the development of resistance in enteric bacteria. These resistant

bacteria contribute to the reservoir of resistant bacteria found in the human intestinal tract including resistant *Salmonellatyphi* [31].

In the present study, 37(77.0%) of *S. typhi* isolates were resistant to 2 or more antimicrobial agents (Multidrug resistance). Highest resistance was observed in Oxacillin 46(95.8%), Imipenem 44(91.6%), Novobiocin 41(85.4%), Erythromycin 40(83.3%), and Ampicillin 39(81.2%). The isolates were sensitive to Ciprofloxacin 31(64.5%), Colistin Sulphate 29(60.4%), and Ceftriaxone 28(58.3%). The use of antimicrobials for growth-promotion, prophylaxis and treatment of animal of animal's food increases the prevalence of resistance in human pathogens. High susceptibility of *Salmonellatyphi* was observed against Fluoroquinolones (Ciprofloxacin). This finding is similar to a report by… [32] in Lagos, Nigeria, reported 18% reduced susceptibility of *Salmonella*spp to Ofloxacin and Ciprofloxacin. The high susceptibility of enteric bacteria to the Fluoroquilones recorded in this study may be connected to relatively high cost of Ciprofloxacin. Therefore, fluoroquinolones are not used indiscriminately because not many could afford them. The selection for resistance almost certainly comes from availability of cheaper generic drugs for the treatment of this invasive bacterial infection. The relatively low cost of generic medicine is partly the major reason for antibiotics abuse [33].

With the emergence of resistance towards traditional antibiotics, fluoroquinolones and extended-spectrum cephalosporins have been introduced as the antimicrobial agents of choice in treating MDR *S.* In countries with a higher incidence of MDR isolates, *S. paratyphi* displays a higher level of resistance towards fluoroquinolone compared to *S. typhi* [34]. Nalidixic acid resistance, which is used as an indicator of reduced susceptibility of ciprofloxacin and other fluoroquinolones is displayed by isolates from Pakistan, India and Vietnam, with high incidence rate of 59%,57% and 44%, respectively [35].

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4. CONCLUSION

This study revealed a high occurrence of multidrug resistant and virulent *Salmonellatyphi* isolated from blood and stool specimensof both active adults and young ages and sex, which reaffirm the importance of proper diagnosis and drugs administration in the treatment of typhoid fever.

Consent and Ethical Approval

Ethical approval was obtained from the Government of Bauchi State, Ministry of Health research and ethics committee, with written informed consent also sought from all patients prior to specimen and data collection.

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Comment [KP15]: 1(1): 153-157.

Comment [KP16]: Umar AF, Sahal MR, Inusa T, Agbo EB. Detection of Resistance genes in Multidrug Resistance *Salmonellatyphi* and its overall clinical implications; a case study.Adv. Appl. Microb. Biol.2019; 1(1): 153-157.

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