International Journal of Microbiology and Biotechnology
2021; 6(4): 104-118
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20210604.12
ISSN: 2578-9678 (Print); ISSN: 2578-9686 (Online)

Antimicrobial Susceptibility and Minimum Inhibitory Concentration of *Salmonella enterica* Isolates from Chickens in Yobe State

Ahmed Idriss Jajere[1, *], Ahmed Mahmud Jajere[2], Mustapha Bala Abubakar[3], Mailafia Samuel[4], Ahmed Ibrahim Jajere[5], Isa Mohammed Bammami[6]

[1]Department of Biological Sciences, Federal University Gashua, Gashua, Nigeria
[2]Department of Chemical Engineering, University of Maiduguri, Maiduguri, Nigeria
[3]Department of Veterinary Microbiology, University of Maiduguri, Maiduguri, Nigeria
[4]Department of Veterinary Microbiology, University of Abuja, Abuja, Nigeria
[5]Department of Geography, Federal University Gashua, Gashua, Nigeria
[6]Nigeria Field Epidemiology and Laboratory Training Programme, Damaturu, Nigeria

Email address:
Idrissjajere@gmail.com (A. I. Jajere)
[*]Corresponding author

To cite this article:
Ahmed Idriss Jajere, Ahmed Mahmud Jajere, Mustapha Bala Abubakar, Mailafia Samuel, Ahmed Ibrahim Jajere, Isa Mohammed Bammami. Antimicrobial Susceptibility and Minimum Inhibitory Concentration of *Salmonella enterica* Isolates from Chickens in Yobe State. *International Journal of Microbiology and Biotechnology*. Vol. 6, No. 4, 2021, pp. 104-118. doi: 10.11648/j.ijmb.20210604.12

Received: August 18, 2021; **Accepted**: September 1, 2021; **Published**: October 12, 2021

Abstract: *Salmonella* of poultry are zoonotic microorganisms transmitted to humans and other animals via contact with infected poultry feces, meat, eggs and formites. This study was conducted to phenotypically characterize *Salmonella enterica* from samples collected from chickens presented for slaughter in some selected Local Government Areas of Yobe State, Nigeria, as well as carry out antimicrobial susceptibility and minimum inhibition concentration on the isolates. A cloacal swab and blood samples were collected and transported on ice pack to Veterinary Microbiology Laboratory, University of Maiduguri and analyzed for the presence of *Salmonella enterica*. Samples were then inoculated onto Xylose Lysine Deoxycholate agar for morphological identification of *Salmonella* blackish colonies. A total of 600 (300 cloacal swab and 300 blood), consisting (202 males, 98 female chickens, 150 local and exotic each) were randomly sampled in 16 weeks for the isolation of *Salmonella enterica*. The presumptive *Salmonella* isolates were further characterized using the Microbact^{TM} GNB 24E System kit, with 40 randomly selected presumptive isolates (8 from blood and 32 from cloacal swab) tested using Microbact 24E GNB Computerize system, with 10 samples found to be positive for *Salmonella* organisms out of which 9 (22.5%) were from cloacal swab and 1 (2.5%) from blood. All the blood samples were tested for haemagglutination using slide method, 255 were found to be positive, where agglutination was observed. Where as only 8 (2.7%) were positives after blood culture 8 (2.67%). Exotic chickens showed the highest resistance level of (35%) to commonly used antibiotics (Amoxixillin and Ampicillin). The isolates from exotic chickens are susceptible to Ciprofloxacin 11 (68.8%), Ofloxacin 10 (62.5%), Gentamicin 2 (12.5%), Levofloxacin and Erythromycin 6 (37.5%), while intermediate to Norfloxacin 5 (31.3%) and Amoxicillin 7 (43.8%) but were resistant to Ampicillin 6 (37.5%%), Cefuroxime 10 (62.5%) and Amoxicillin 4 (25.0%). The MIC was carried out on all the 10 *Salmonella* isolated that showed positive on microbact 24E computerized system. All the 10 isolates from microbact 24E computerized system showed susceptibility to amoxicillin, ofloxacin, and ciprofloxacin on MIC. The MIC of ofloxacin and ciprofloxacin was distributed within 0.00175–2 µg/ml each, and for amoxilin, the MIC ranged between 0.00175-3.00 µg/ml. It is therefore, concluded that *Salmonella* organisms phenotypically characterized in the study area had antimicrobial susceptibility to routinely used antimicrobial drugs. As a result, it is suggested that the medications with high susceptibility be used to treat poultry salmonellosis in the study area.

Keywords: Antimicrobial, *Salmonella enterica*, Minumum Inhibitory Concentration, Chickens

1. Introduction

The genus *Salmonella* is rod shaped, Gram-negative, flagellated, facultative anaerobes, bacteria of family Enterobacteriaceae [39]. The genus consists of two separate species; *Salmonella bongori* and *Salmonella enterica* and encompasses over 2500 known serotypes, all of which are considered potential human pathogen [39, 9]. *Salmonella* species that cause human disease are traditionally divided into a small number of human restricted invasive typhoidal serotypes and thousands of non typhoidal *Salmonella* serotypes, which typically have a broad vertebrate host range and cause various presentations that usually include diarrhoeal disease [18].

Salmonella enterica infection (Salmonellosis) infects about 1.4 million people annually in United State of America (USA), with an estimation of 30% of all food borne diseases, costing about 500 lives and has an estimated cost of $2.4 billion dollars annually [23]. Typically, people with *Salmonella* infection show no symptoms because *Salmonella* infections usually clear up without medical treatment. Others develop diarrhea, fever and abdominal cramps within eight to 72 hours, other additional symptoms includes; bloody diarrhoea, vomiting, headache and body ache. Most healthy people recover within a few days without specific treatment [23].

Food-borne diseases caused by non-typhoidal *Salmonella enterica* serovars represent an important public health problem and an economic burden in many parts of the world [37]. *Salmonella* is an important cause of foodborne infections with a broad host spectrum [23]. It is frequently isolated from environmental sources that serve as relay for the bacteria and play a major role in its spread between different hosts [23]. *S. enterica* remains a formidable public health challenge [23] and with a reported increase in its incidence. Salmonellosis can result in a number of diseases, with the following symptoms; gastroenteritis, bactaeremia, typhoid fever and focal infections such as tonsillitis, upper respiratory tract infections, and sinusitis [23]. Certain cases of Salmonellosis are severe and often require antimicrobial therapy for treatment, thus, resistance to antimicrobial drugs is a great concern [31].

The main sources are foods of animal origin, such as eggs, milk, poultry, beef, and pork meat. In addition, fruits and vegetables have been accused as vehicles in *Salmonella* transmission by ingestion. *Salmonella* is considered as a global problem ranking first among food borne diseases others are *Clostridium perfringes, Staphylococcus aureus* and *Campylobacter jejuni*. All motile *Salmonella* of poultry are zoonotic, as they can be transmitted to humans via contact with infected poultry feces, meat, eggs and handling of young chickens particularly by children, or anything in the area where they live [37].

Hospital acquired infection also known as nosocomial infection have reported to be associated with the outbreaks of *Salmonella* diseases. The disease has been reported in many parts of the world hospitals, particularly African countries including Nigeria [23]. It occurs among patients who are admitted with different cases [23]. Outbreaks of hospital-acquired *Salmonella* can be particularly severe on young children in developing countries, where children may be malnourished and have other host risk factors [28]. In African hospitals, including Nigeria, Kenya, and Egypt, it has been tradition for food to be provided by a patient's relatives. Although few studies have examined risk factors for infection in hospital outbreaks, contaminated food and person-to-person transmission have been considered as source of *Salmonella* transmison [23]. High death rates are frequently observed, especially when outbreaks are caused by strains of *Salmonella* that are resistant to the local medical treatment based on experience [23].

Salmonellosis is a bacterial disease affecting both humans and animals worldwide and Nigeria is not an exception. Although most of the infections in humans cause mild gastroenteritis, and or life-threatening systemic infections are common especially among high risk categories [15]. In the last two decades, multidrug-resistant *Salmonella enterica* isolates have increasingly became a major health hazard [15]. This resistance can be acquired by mutations in the chromosomal DNA or by the acquisition of extra-chromosomal genomic material by means of plasmids and transpoosons [46]. The growing resistance of pathogenic bacteria to antimicrobial agents has raised the concern that the widespread use of antimicrobial agents in animal's production allowed in some countries of European Union, England, France, Wales, and United State of America (USA), and Brazil may promote the development of resistance bacteria or resistance genes that can be transferred to bacteria that cause disease in humans [47]. The antimicrobial drugs approved for use in food-producing animals actively in the United state between 2009 and 2012 includes Aminoglycosides, Lincosamides, Cephalosporins, Penecillins, Sulfonamides, crystal Macrolides antibiotics, Tetracyclines and Ionospores [47].

Fowl typhoid and pullorum disease, caused by *Salmonella enterica* subspecies *enterica* serovars *gallinarum* biovars *gallinarum* and *Salmonella enterica* subspecies *enterica* serovar *pullorum,* are widely distributed throughout the world, especially in developing countries including Egypt, South Africa, Indonesia and India [10] where increasing antimicrobial resistance in these strains has also become a problem [37]. They have been extirpated from commercial poultry in many developed countries of Western Europe. The United States of America serovar is referred to as *pullorum* [20], even though the strains are now considered to be the same serovar that is derived from *Salmonella enteritidis* by gene deletion events [44]. The terms serovar *gallinarum* or *pullorum* will be used, as this more usefully distinguishes the two biovars that cause clearly distinct clinical syndromes and are therefore, epidemiologically different. *Salmonella gallinarum* recurred in some European countries in the first decade of the 21st century [21]. *Salmonella pullorum*

remains as a constant reservoir in wild and game birds.

In food-producing animals and particularly poultry, Salmonellosis is among the leading infection, and has a direct effect on the world marketing of the specific food-producing animals and animal-derived food products [15]. Poultry salmonellosis related to host adapted serovars stand still as a main constraint on poultry production in whole parts of Nigeria [15]. Famers still experience major losses (due to mortality, morbidity, and fall in egg production) caused by host adapted *Salmonella* serovars despite huge amounts of money spent on vaccination and medication. In early life, *Salmonella pullorum* causes very high mortality rates of both broilers and commercial laying chickens. Older birds also give up heavily to other serovars of *Salmonella* and it is believed that *Salmonella* infections of this category of birds are mainly as a result of *Salmonella gallinarum* [15]. In addition, to these host adapted *Salmonella* serovars resulting systemic disease, poultry can also harbor the organism in their gastrointestinal tracts as commensal. Hence, these *Salmonella* serovars can be present in feaces excreted by healthy animals and may be passed to raw animal origin food as a result of contamination during slaughtering and processing [15]. Generally, *Salmonella* in food producing animals, including poultry, displays as long as period of latent carriage with occasional faecal shedding, which is the leading source of contamination of feed, water and environment [15].

In chickens, turkeys, and several other avian species, *Salmonella gallinarum* and *pulorum* cause fowl typhoid and pullorum disease, respectively [42]. In North America, Western Europe, and other developed countries like Australia and Japan, these biovars have been eradicated from commercial poultry. However, they continue to play a significant economic role in the poultry business in many African, Asian, Central, and South American countries [25]. A confirmed case of chicken typhoid and Pullorum disease necessitates biovar Gallinarum or Pullorum isolation and identification [43, 17, 22].

The pullorum disease normally reaches its peak between 2-3 weeks old birds with high death rate and minor condition in matured birds. Susceptibility is high in breeding and laying birds [47]. There is low egg production and hatchability of birds infected with *Salmonella pullorum*. One of the major routes for *S. pullorum* and *S. gallinarum* to be transferred into eggs is trans-ovarian infection ensuing infection of the egg and hatched chicks or poults [35].

Salmonellosis in poultry is endemic globally, causing morbidity, mortality and economic losses [1, 4, 7]. The disease is very significant by virtue of the fact that *Salmonella* can be transmitted vertically from parent to offspring [4]. The control of salmonellosis in the poultry industry is complicated because, in addition to vertical transmission from parent stock to offspring, horizontal transmission on farms is also common; this makes its control a challenge [4, 11, 19]. Poultry can become infected by the horizontal route via infected litter, faeces, feed, water, dust, fluff insects, equipment, fomites, diseased chicks and rodents, contaminated with *Salmonella* [4]. They can also be transmitted by other animals, wild birds and personnel [4]. *Salmonella* may infect young chicks directly via ovarian transmission or invade the eggshell after the egg has been laid [32]. Poultry farms and poultry products are the main sources for *Salmonella* contamination [4]. Reports on various poultry diseases occurring in most parts of Nigeria showed that salmonellosis is the major menace facing poultry production in Nigeria [30], and poultry droppings have been shown to be a potential reservoir for many enteric species [4]. Hence, consumers of poultry and poultry products are at risk of contracting *Salmonella* infections via consumption of contaminated products.

Although vaccination to prevent salmonellosis has been practised successfully on layer poultry farms in many countries [11], vaccines produced from local isolates are still not readily available in the market, especially in developing countries. Hence, the control of salmonellosis is predominantly dependent on good sanitary practices and the use of antimicrobial drugs for prevention and treatment of the disease [1, 7]. This subsequently leads to abuse of antimicrobial drugs in poultry settings, culminating in the development of antimicrobial resistance and the eventual limitation of the therapeutic outcome in the treatment of the diseases [4].

It is usually difficult to report the occurrence of salmonellosis and antimicrobial resistance in developing countries like Nigeria because of a lack of coordinated surveillance systems. Studies so far in Nigeria have only included a limited number of samples or isolates from a single or a few reservoirs and limited geographical coverage [6].

Salmonella is considered as a global problem ranking first among food borne illness that are commonly found in the gastrointestinal tract of humans and animals. It can also be found in raw meats and poultry eggs. *Salmonella enterica* infections result in many cases of abdominal complications like gastroenteritis. The bacteria can bind to the cells lining the intestines where they produce toxins and attack the intestinal cells. It is regarded as one of the most serious infectious disease menace to public health on a global scale and Nigeria is not an exception.

2. Materials and Methods

2.1. Study Area

Yobe is a state located in Northeast Nigeria Coordinates: 12°00′N 11°30′E / 12.000°N 11.500°E. It was carved out of Borno State on August 27, 1991. The capital of Yobe state is Damaturu. The state shares borders with the Nigerian states of Bauchi, Borno, Gombe, and Jigawa. It borders the Diffa Region and the Zinder Region to the north in the Republic of Niger. Because the state lies mainly in the dry savanna belt, weather is hot and dry for most of the year, with exception of the southern part of the state which has a pleasant climate.

Yobe state is mainly an agrarian state where most of the

population depends largely on agricultural production for their livelihood. It also has rich fishing grounds and mineral deposits of gypsum, kaolin, and quartz in Fune Local Goverment Area. The state's agricultural products are; gum Arabic, millet, maize rice, groundnuts, beans, and cotton. The state have one of the largest cattle markets in West Africa, located in Potiskum Local Government Area. Yobe's ground consists of plains that are drained by the seasonal Komadugu Yobe River and its tributaries in the north and by the Gongola River in the south. The state's vegetation is primarily of the Sudan savanna type, with scattered shrubs (acacia). There is also an area of Sahel savanna, comprising of sandy soils and thorn scrub, which is located in the northern part of the state. The Kanuri are the primary and major ethnic group in the state. Sorghum, millet, peanuts (groundnuts), cowpeas, corn (maize), sesame, and cotton are the primary crops. Cattle herding and farming are the major occupations. Damaturu is the state capital, and Nguru, Potiskum, Geidam and Gashua are sizable market towns. The state is served by trunk roads joining Potiskum, Damaturu, and Maiduguri (Borno state). (Pop, "2006" PHC, 2006). Yobe State comprises 17 local government areas (or LGAs). They are: Bade, Bursari, Damaturu, Geidam, Gujba, Gulani, Fika, Fune, Jakusko, Karasuwa, Machina, Nangere, Nguru, Potiskum, Tarmuwa, Yunusari and Yusufari [49].

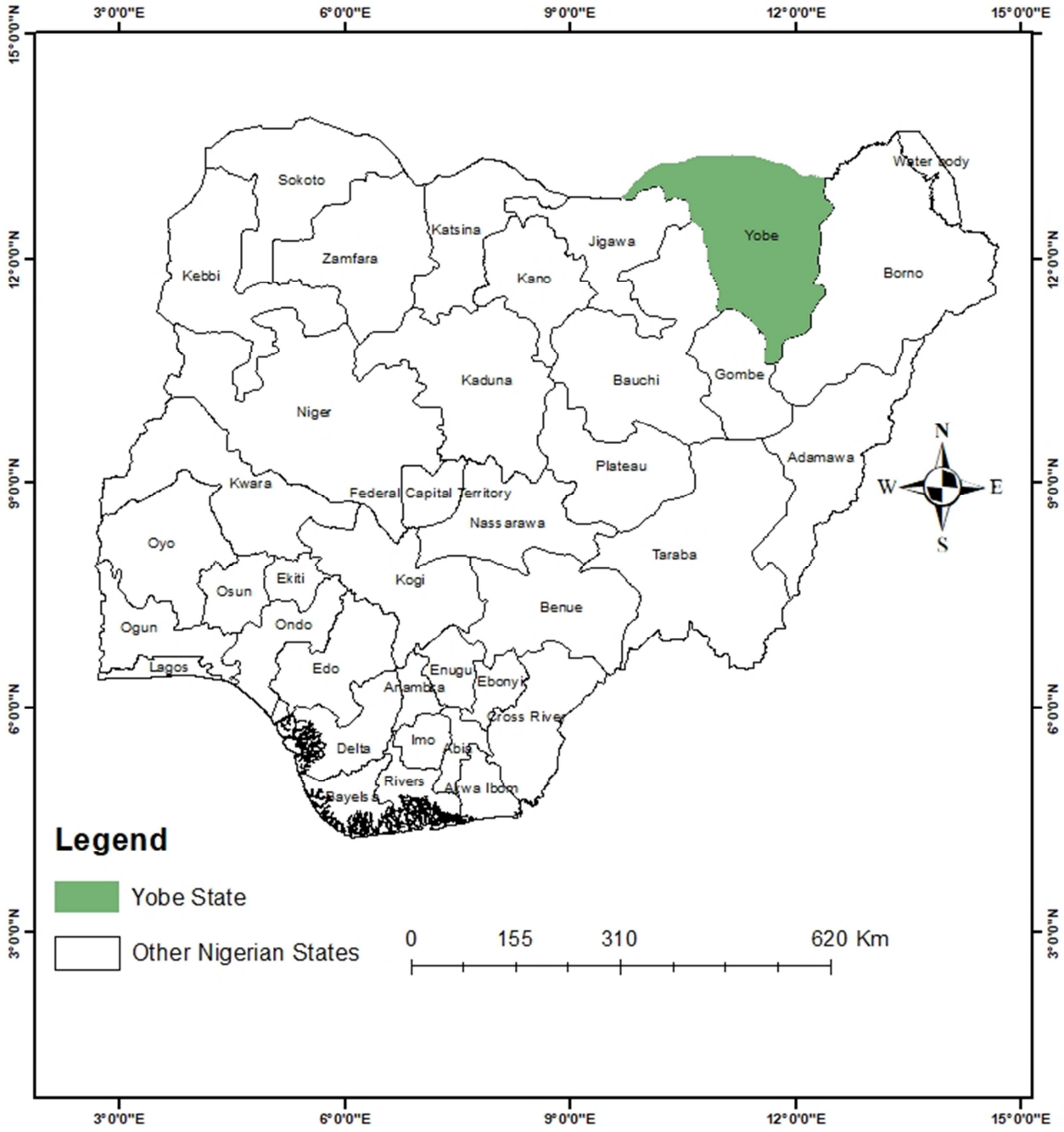

Figure 1. *Map of Nigeria showing Yobe State Cartography Laboratory, Yobe state University Damaturu (2021).*

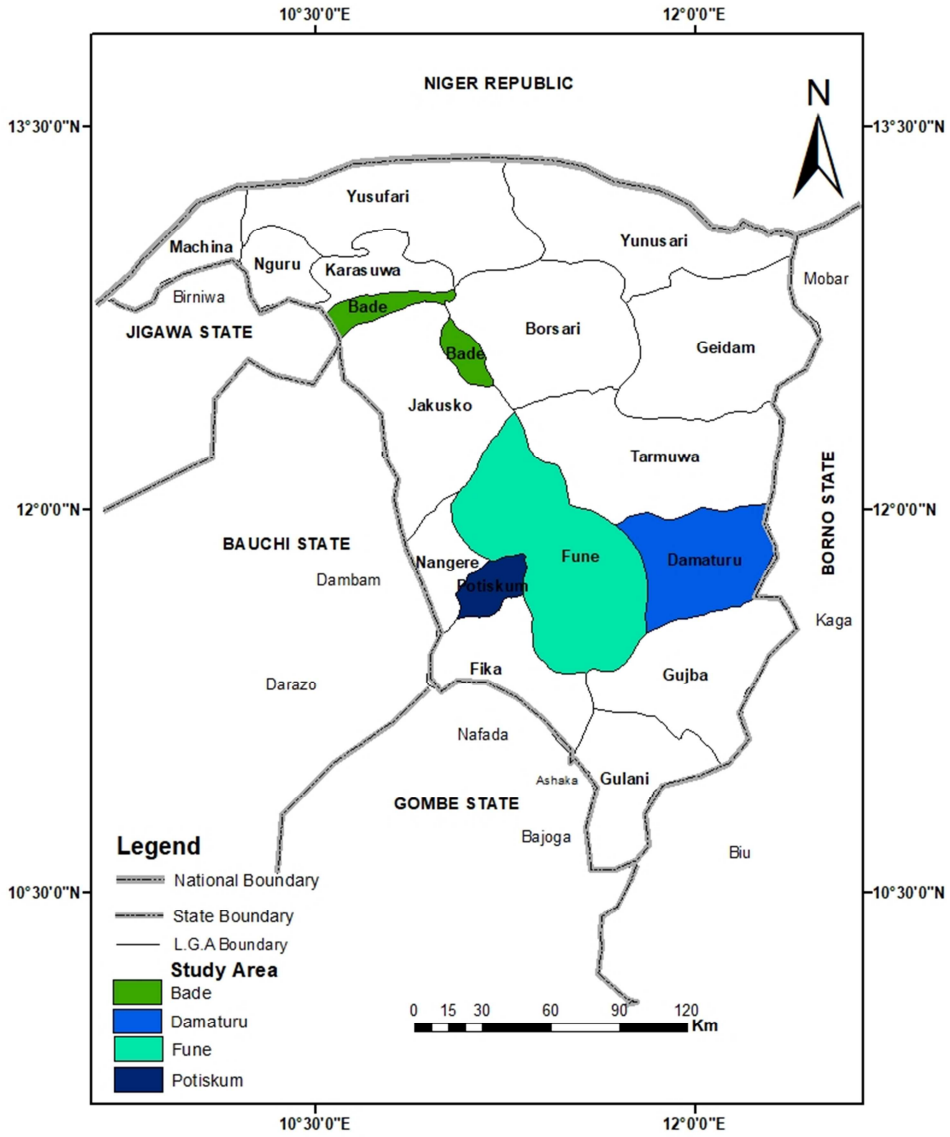

Figure 2. *Map of Yobe showing study areas of sample collection. Cartography Laboratory, Yobe state University Damaturu (2021).*

2.2. Sample Size Determination

The sample size was determined by using Thrush field sample size calculation formulae

$$Z=N/(1+Ne^2)$$

Where,
N=population size=unknown
e=0.05 at confidence level of 95%
Thus, N=1000/(1+1000×0.0025)=399.99
Approximately=400 [45].

Therefore, six hundred samples from 300 Chickens were collected from apparently healthy chickens in Yobe state to increase precision of the study.

2.3. Experimental Design

A total of 600 samples (300 Cloacal swab and 300 Blood samples) were obtained from chickens from four different local government areas of Yobe state. The samples were collected for period of 16 weeks from July to October, 2019. The locations for the samples collection sites are designated as follows: Fune local government designated as Area F, Potiskum as Area P, Damaturu as Area D, and Bade as Area B.

2.4. Sampling

Convenient sampling was conducted based on availability and willingness of the butchers, where, cloacal swab and blood sample were collected from 75 chickens in four batches from four different areas. Before taking the samples each butcher, was interviewed orally to obtain information on sex of each chicken, and was briefed on purpose of sampling.

2.4.1. Collection of Cloacal Sample

The swabs samples were carefully obtained, to avoid contamination from the outside environmental contamination after removal from the cloaca. A sterile swab was inserted into the cloaca of the chicken and then rotated two to fivetimes against the cloacal mucosal surface with gentle

pressure to take test fluid sample. Then immediately transfer intoa10ml sterile transport/pre-enrichment medium contained in a tube until delivery to the laboratory for further processing. The swab samples were kept at refrigerating temperature by keeping it on Ice Park in a cooler and transported to the laboratory [23].

2.4.2. Collection of Blood Sample

Three milliliters of the blood were collected from each chicken into plain vacutainer tube and EDTA contained vacutainer each, during slaughtering. Those in plain vacutainer tubes were centrifuged at high speed (2500rpm) for 5minutes to separate the serum from blood cells. The sera were collected in the plain vacutainer tube after centrifugation using Pasteur pipette.

2.5. Laboratory Culture and Identification

The laboratory identification in this study involves; enrichement, selective plating, preliminary identification and complete biochemical identification with some modifacation (Mailafia *et al.,* 2017). The samples were analyzed by using semisolid modified Rappaport Vassiliadis medium as the selective enrichment medium, showing tubdity and color changes in the medium. The sample from enrichment medium were streaked into xylose lysine desoxycholate agar medium (selective solid medium) and incubated at 37°C for 24h. The *Salmonella* Isolates colonies, appear red with black centers on xylose lysine desoxycholate medium (Duerden *et al.,* 1998).

2.5.1. Enrichment Medium

Cloacal swabs and blood samples (from EDTA containers) were analyzed by using semisolid modified Rappaport Vassiliadis medium as the selective enrichment medium, where the presumptive *Salmonella* isolates from pre-enrichment (buffered peptone water) transport medium were inoculated unto test tubes containing prepared Rappaport vassiliadis medium (Duerden *et al.,* 1998).

2.5.2. Isolation of Salmonella enterica (Selective Plating)

The sample from enrichment medium were streaked into xylose lysine desoxycholate agar medium (selective solid medium) and incubated at 37°C for 24h. The *Salmonella* colonies presumed to appear red with black centers on xylose lysine desoxycholate medium according to [16].

2.5.3. Gram Staining

Gram staining method is most frequently used in Diagnostic Bacteriology of bacteria. Clean slides with heat fixed smears were placed on a staining tray. The smears were flooded with crystal violet gently and let stand for 1 minute, before they were tilted slightly and gently rinsed with tap water or distilled water using a wash bottle. The smears then were flooded with lugals/Gram's iodine and let stand for 1 minute, and thereafter gently rinsed with tap water or distilled water using a wash bottle. The smears appeared as purple circle on the slide, and were decolorized using 95% ethyl alcohol. Drop by drop of alcohol was applied to the slides for 5 to 10 seconds until the alcohol runs almost clear. The slides were rinsed immediately so as not to over-decolorize and flooded gently with safranin to counterstain and let stand for 45 seconds. They were then tilted slightly and gently rinsed with tap water using a wash bottle and blot dry slide bibulous paper. Finally the smeared slides were viewed using a microscope under oil immersion at 100x Magnification [16]. *Salmonella* organisms are gram negative. Thus, they appeared pinkish/ red [23].

2.5.4. Catalase Production Test

The enzyme catalase breakdown of hydrogen peroxide into oxygen and water. This principle is used for detection of catalase enzyme in a bacterial isolate. A loopful of 10% hydrogen peroxide was putted on colonies of the test organism on nutrient agar. Alternatively, a few colonies of the organism were picked up with platuim wire loop from nutrient agar plate and dipped in a drop of 10% hydrogen peroxide on a clean slide. The production gas bubbles from the culture, indicates a positive reaction. A false positive result may be obtained if the growth is picked up from the medium containing catalase e.g blood agar or if an iron wire loop is used (Duerden *et al.,* 1998).

2.5.5. Oxidase Test

This test depends on the presence, in bacteria, of certain oxidases that catalyze the oxidation of reduced tetramethyl-*p*-phenylene-diamine dihydrochloride (oxidase reagent) by molecular oxygen. A drop of freshly prepared 1% solution of oxidase reagent was put on a piece of filter paper. Then a few colonies of the test organism were rubbed on it. Oxidase positive isolates produced a deep purple colour within 10 seconds. Alternatively, oxidase reagent will be poured over the colonies of the test organism on culture plate. The colonies of oxidase positive rapidly develop a deep purple colour (Duerden *et al.,* 1998).

2.6. Complete Biochemical Characterization

The biochemical identification tests used for this study for pathogenic identification and confirmation of *Salmonella enterica* isolates includes: lysine iron agar test, urease test, citrate test, TSI test, Sulfur Indole Motility test, Methyl red test, and Voges-Proskauer test.

2.6.1. Urease Test

Urease test is performed to check the capability of microbes to produce urease. During the test, the straight wire containing pure culture was streaked over the surface of urea agar slant. The tubes were further kept in the incubator overnight at 37°C, maintaining the yellow coloration of the media is an indicaton of urease negative result [29].

2.6.2. Citrate Test

Citrate test is carried out in labs in order to check the ability of microbes to utilize citrate as a sole source of carbon and energy. Citrate agar medium contains a pH indicator called bromothymol blue, which is green at normal pH, yellow at acidic pH and blue at basic pH. If citrate is utilized

by the microbes, alkaline by-products will be formed which changes the medium colour from green to blue. Pure culture was taken using sterile straight wire and streaked over the surface of citrate agar slant. The tubes were further kept in the incubator overnight at 37°C [29]. The media that turn to royal blue are indicative of positive results [23].

2.6.3. Triplate Sugar Iron (TSI)

(TSI) agar is used to test the ability of microbes in sugar fermentation and hydrogen sulfide production. TSI agar consists of glucose, sucrose, lactose, pH indicator phenol red and ferrous sulfate [29].

TSI agar was kept in the butt and the slant form in a test tube. The bacterial cultures from the colonies formed in agar medium were taken using a sterile straight wire. Then the needle containing cultures were stabbed into the butt of the TSI agar tube and streaked the needle back and forth along the surface of the slant. The tubes were further kept in the incubator overnight at 37°C, the hydrogen sulfide is produced, as it react with the iron in the agar to form ferrous sulfide, and were observed as a black precipitate in the butt [29].

2.6.4. Methyl Red (MR) Test

This test detects the production of sufficient acid by fermentation of glucose so that the pH of the medium falls and it's maintained below 4.5. The isolates were inoculated in glucose phosphate broth and incubated at 37°C for 2-5 days. Then five drops of 0.004% solution of methyl red were added, mixed well and the result was read immediately. Positive tests are bright red (indicating low pH) and the negative are yellow [12].

2.6.5. Indole Production

Certain bacteria which possess enzyme tryptopanase, degrade amino acid tryptophan to indole, pyruvic acid and ammonia. Indole production was detected by inoculating the isolates into peptone water and incubating it at 37°C for 48-96 hours. Then 0.5 ml of Kovac's reagent was added gently. A red colour in the alcohol layer indicates a positive reaction [12].

2.6.6. Voges-Proskauer (VP) Test for Acetoin Production

Many bacteria ferment carbohydrates with the production of acetyl methyl carbinol (acetoin). In the presence of potassium hydroxide and atmospheric oxygen, acetoin is converted to diacetyl, and α-naphthol serves as a catalyst to form a pink complex. *Salmonella* isolates were inoculated in glucose phosphate broth and incubate at 37°C for 48 hours. Then 1ml of potassium hydroxide and solution ofα-naphthol was added in absolute alcohol. A positive reaction is indicated by the development of pink colour in 2-5 minutes and crimson in 30 minutes (Duerden *et al.,* 1998).

2.6.7. MicrobactTM 24E GNB Identification System

The Microbact 24E system (Oxiod^{TM} Microbact^{TM} GNB 24E System kit, manufactured by Thermo-Fisher Scientific, Walham, Massachusetts, USA) is a miniaturized identification system for the identification of microorganisms. The Microbact^{TM} is a commercially used Microsystem for identification of common clinical isolates of Enterobacteriaceae and non-fermenting Gram-negative bacilli and consists of dehydrated substrates distributed in wells of microtitre trays. This system assists in final identification of fresh isolates from cloacal swabs; the system is easy to use and comes with complete computerized profile registers to assist in identification of the isolates. This system proves to be accurate and convenient in the identification of microorganisms.

(1) Interpretation of Microbact 24E GNB Identification System

An octal coding system has been adopted for Microbact™ 1. Each group of three reactions produces a single digit of the code. Using the results obtained, the indices of the positive reactions are circled. The sum of these indices in each group of three reactions forms the code number. This code is entered into the computer package.

(2) Microbact™ Computer Aided Identification Package

The Microbact™ Computer Aided Identification Package was consulted for the identification choices. The percentage figure shown against the organism name is the percentage share of the probability for that organism as a part of the total probabilities for all choices.

(3) Preparation of Inoculums and Inoculation

Isolated colony from XLD culture was picked and emulsify in 5ml of sterile saline solution (0.85%). Then mixed thoroughly to prepare a homogeneous suspension.

The wells containing individual substrate sets were exposed by cutting the end tag of the sealing strip and slowly peeling it back.

Each plate was placed in the holding tray and using a sterile Pasteur pipette 100 µl of the bacterial suspension was added. Using a dropper bottle, the substrates underlined on the holding tray were overlayyed with sterile mineral oil, that is, wells 1, 2 and 3. Whereas, kovac's reagent to well 8, VP1 and VP2 to well 10 and TDA to well 12. The inoculated rows were resealed with the adhesive seal and incubated at 35°±2°C for 18-24 hours. The 12A (12E) strips were read at 18-24 hours. The 12B/24E strips were read at 24 hours to identify *Salmonella* specie.

2.7. Antimicrobial Resistance Profile

Antimicrobial resistance profile of *Salmonella* isolates were determined by the disc diffusion method of Kirby Bauer, [26] and zones of inhibition interpretation was carried out as described by the Clinical Laboratory Standard Institute. The antibiotic disks used are manufactured by Oxoids. Each *Salmonella* isolate was transfered into Muller Hinton broth and incubated at 37°C for 24 hours. The turbidity of the suspension was adjusted asceptically with sterile saline to obtain turbidity of 0.5 McFarland standards. Then, pour on the Muller Hinton agar plate to cover the entire surface and then drained the excess media. The antibiotic disks were placed on the surface of agar at equal distance, sufficient to separate them from each other to avoid overlapping of the inhibition zones. Each plate carries a

maximum of six discs and each test was performed in duplicate. After 30 seconds of pre-diffusion, the plates were incubated at 37°C for 24 hours followed by the diameter of inhibition zones measurement and then adjusted to the nearest rounded number [38]. A total of 12 antimicrobial agents were used in this study namely: Amoxillin/clavulanic acid, Ampicillin, tetracycline, gentamicine, norfloxacin, ofloxacin, Chloramphenicol, erythromycin, ciprofloxacin, levofloxacin, nitrofurantion and cefuroxime.

2.8. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the least amount of antimicrobial agent that inhibits visible growth of an organism after overnight incubation. Three antimicrobial agents (ofloxacin, ciprofloxacin and amoxixillin) were incorporated into the culture medium in the concentration of 0.0017, 0.0035, 0.007, 0.015, 0.03, 0.06, 0.125, 0.5, and 2 µg/ml by serial dilutions (1/10) of the antibiotics (representing different concentrations of the antibiotics) and are added to growth medium in separate test tubes. These tubes are then inoculated with the bacterial isolates and allowed to incubate overnight at 37°C. The broth tubes that appear turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. The inoculums prepared as in case of disc diffusion method by comparing with 0.5 McFarland opacity standards. 1-2 µl of the inoculums were applied on the Muller-Hinton agar surface. Incubated at 37°C for 18hr and the results were read [8].

2.9. Statistical Findings

The data obtained in this research were analysed using descriptive statistics such as plates, figures, percentages and tables using Microsoft word and excel 2013, and the locations were compared by using Chi-square at level of significance $p<0.05$ using SPSS.

3. Result

The isolation of *Salmonella* organism obtained from six hundred (600) blood and cloacal swabs from poultry presented for slaughter in some selected local governments of Yobe state is presented in tables below.

3.1. Prevalence of Salmonella Isolated on XLD Agar from Chickens in Four Local Government Areas of Yobe State

Table 1 shows the number of samples collected and prevalence of *Salmonella enterica* from poultry in different Locations of Yobe state. The highest isolation rate was observed in Week 1 (in Damaturu) where 35.71% (15) of the samples examined found to be positive, followed by week4 (in Damaturu) with 22.22% (8), then week 2 (in Damaturu) with 19.44% (7), week 14 (in Fune) with 16.67% (6), week 10 with 13.89% (5), week 3, 5, 8, 9, 12, 15 and 16 (in Damuturu, Potiskum, Potiskum, Bade, Bade, Fune and Fune respectively) both with 11.11% (4) each, while week 6, 11 and 13 (in Potiskum, Bade and Fune respectively) with 8.33% (3) each. The lowest isolation rate in the weeks was observed in week 7 (in Potiskum) with only 5.56% (2) out of the samples examined. The isolates per locations rate were compared using Chi-square test on SPSS at P value <0.05 and level of significance found to be 0.001, showing highly significant at $P<0.05$

3.2. Salmonella Species Isolated from Chicken's Faeces and Blood Using Microbact 24E GNB Computer Identification System

Table 2 shows *Salmonella* species, isolated from chickens using Microbact 24E GNB Computer Identification system. *Salmonella typhi* had high rate of isolation with 3 (30.00%) of the 10 (100%) *Salmonella* species, isolated, followed *Salmonella paratyphi* 2 (20%), *Salmonella gallinarum* and *Salmonella pullorum* with 2 (20.00%) each. The lowest isolated rate was observed in *Salmonella* subs. 3B with only 1 (10.00%).

3.3. Antimicrobial Susceptibility Studies of Salmonella enterica Isolates from Local and Exotic Chickens to Common Used Antimicrobial Agents

Table 3 shows Antimicrobial Susceptibility Studies of *Salmonella enterica* isolates from local and exotic chickens to commonly used antimicrobial agents. Among all the samples from chickens that were positive (80) for *S. enterica,* 50 (62.5%) of the positive isolates are susceptible to Ciprofloxacin, 50 (62.5%) to Ofloxacin, 30 (37.5%) to levofloxacin, 28 (35.5%) to Amoxicillin, 28 (35.0%) to Erythromycin, 24 (30.0%) to Norfloxacin, 23 (28.8%) to Gentamicin 4 (5.0%) to Nitrofurantion, 4 (5.0%) to Cefuroxime & Tetracycline. While 54 (67.5%) were intermediate to Gentamicin & Ampicillin, 46 (57.5%) to Nitrofurantion, 40 (55.0%) to Amoxicillin, 30 (37.5%) to Ofloxacin, 38 (47.5%) to Erythromycin, 36 (45.5%) to levofloxacin, 34 (42.4%) to Chloramphenicol, 32 (40.0%) to Norfloxacin and Tetracycline, 26 (32.5%) to Cefuroxime, and 22 (27.5%) to Ciprofloxacin. Whereas, 50 (62.5%) were resistance to Cefuroxime, 46 (57.5%) to Chloramphenicol 44 (55.0%) to Tetracycline, 30 (37.5%) to Nitrofurantion, 26 (32.5%) to Ampicillin 24 (30.0%) to Norfloxacin, 14 (17.5%) to levofloxacin and Erythromycin, 12 (15.0%) to Amoxicillin, 8 (10.0%) to Ciprofloxacin, 3 (3.75%) to Gentamicin, and 0 (0.00%) to Ofloxacin.

3.4. Antimicrobial Susceptibility Studies of Salmonella Isolates from Local Chickens to Commonly Used Antimicrobial Agents

Table 4 shows Antimicrobial Susceptibility Studies of *S. enterica* isolates from local chickens to commonly used antimicrobial agents. Of the isolates from local chickens 40 (62.5%) isolates were susceptible to Ofloxacin, Ciprofloxacin 39 (60.9%), Levofloxacin 24 (37.5%), Gentamicin 21 (32.8%). While, 44 (68.7%) were intermediate to Ampicillin, Norfloxacin 26 (40.6%), and Cefuroxime 20 (31.3%).

However, 34 (53.1%) were resistance Chloramphenicol and 32 (50.0%) to Tetracycline.

3.5. Antimicrobial Susceptibility Studies of Salmonella Isolates from Exotic Chickens to Commonly Used Antimicrobial Agents

Table 5 shows Antimicrobial Susceptibility Studies of *S. enterica* isolates from exotic chickens to commonly used antimicrobial agents. Eventhough, exotic chickens showed the highest resistance level of (35%) to commonly used antibiotics, the isolates from exotic chickens were susceptible to Ciprofloxacin 11 (68.8%), Ofloxacin 10 (62.5%), Levofloxacin & Erythromycin 6 (37.5%), and Gentamicin 2 (12.5%). While, 7 (43.8%) were intermediate to Amoxil and 5 (31.3%) to Norfloxacin. Where as, 10 (62.5%) were resistant to Cefuroxime, 6 (37.5%) to Ampicillin and 4 (25.0%) to Amoxicillin.

3.6. Minimum Inhibitory Concentration Distribution of Salmonella Isolates

The MIC of *Salmonella* positive samples are presented in Table 6 Among 3 *Salmonella Typhi* were resistant to ciprofloxacin with MIC 0.00175 µg/ml, 2 with MIC 0.0035 µg/ml and 1 with MIC 0.007µg/ml. The MIC of ofloxacin was distributed within 0.00175–2 µg/ml. 3 of the *Salmonella Typhi* were resistant to ofloxacin with MIC 0.00175 µg/ml and 1 with MIC 0.0035 µg/ml. Similarly, the MICof amoxilin ranges between 0.00175 and 3.00 µg/ml where 2 *Salmonella Typhi* showed MIC of 0.00175 µg/ml and 2 of the *S. Typhi* displayed MIC 0.0035. Where as, 2 of the *S. paratyphi* were resistant to amoxicilin with MIC 0.00175 µg/ml, 2 showed MIC 0.0035 µg/ml and 1 with MIC 0.007 µg/ml, among the 2 *S. gallinarum* isolated 2 were resistant to amoxacilin with MIC 0.00175 µg/ml and the other 3 *S. gallinarum* solates the MIC of 0.0035 µg/ml, 0.007µg/ml and 0.015 µg/ml respectively. Again of the 2 *S. pullorum* isolates 2 were resistant to amoxacilin with MIC 0.00175 µg/ml, another 2 of the *S. pullorum* showed MIC of 0.0035 µg/ml, and 1 of the isolates revealed MIC of 0.007 µg/ml. Further more 1 isolate *Salmonella* subs. 3B showed resistance to amoxacilin with MIC of 0.00175 µg/ml, MIC 0.0035 µg/ml and MIC 0.007 µg/ml.

Table 1. *Weekly Isolation of Samples Collected and prevalence of Salmonella on XLD agar from Poultry from Sampling areas.*

Location (s)	Week (s)	No. Sampled	No. (%) Positive
Damaturu	1	42	15 (35.7)
	2	36	7 (19.4)
	3	36	4 (11.1)
	4	36	8 (22.2)
Fune	1	42	4 (9.5)
	2	36	3 (8.3)
	3	36	2 (5.6)
	4	36	4 (11.1)
Bade	1	42	4 (9.5)
	2	36	5 (13.9)
	3	36	3 (8.3)
	4	36	4 (11.1)
Potiskum	1	42	3 (7.1)
	2	36	6 (16.7)
	3	36	4 (11.1)
	4	36	4 (11.1)
Total		600	80 (13.3)

Significant P<0.05

Table 2. *Salmonella species isolated from chicken's faeces and blood Using Microbact 24E GNB Computer Identification system.*

***Salmonella* species isolated**	**No. (%) +ve isolates**
S. typhi	3 (7.5)
S. paratyphi A	2 (5.0)
S. gallinarum	2 (5.0)
S. pullorum	2 (5.0)
Salmonel̈9la subs. 3B*	1 (2.5)
Total	10 (25.0)

*Blood

Table 3. *Antimicrobial Susceptibility Studies of Salmonella enterica Isolates from local and exotic chickens to commonly used antimicrobial agents.*

S/N Antimicrobials	Drug Conc.(ug/mL)	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistance
1 N	10	24 (30.0)	32 (40.0)	24 (30.0)
2 CRX	30	4 (5.00))	26 (32.5)	50 (62.5)
3 GEN	10	23 (28.8	54 (67.5)	3 (3.75)
4 AM	25	0 (0.0)	54 (67.5)	26 (32.5)
5 OFL	5	50 (62.5)	30 (37.5)	0 (0.0)
6 AX	30	28 (35.5)	40 (50.0)	12 (15.0)
7 NIT	300	4 (5.00)	46 (57.5)	30 (37.5)
8 CRP	5	50 (62.5)	22 (27.5)	8 (10.0)
9 LEV	20	30 (37.5)	36 (45.0)	14 (17.5)
10 E	30	28 (35.0)	38 (47.5)	14 (17.5)
11 CH	10	0 (0.00)	34 (42.5)	46 (57.5)
12 TE	50	4 (5.00)	32 (40.0)	44 (55.0)

N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicilin (25), OFL-Ofloxacin (5), AX Amoxicilin (30), NIT- Nitrofurantion (300), CPR- Ciprofloxacin (5), LEV Levofloxacin (20), E- Erythromycin (30), CH- Chloramphenicol (10), and TE- Tetracycline (50).

Table 4. *Antimicrobial Susceptibility Studies of S. enterica Isolates from Local Chickens to commonly used antimicrobial agents.*

S/N Antimicrobials	Drug Conc. (ug/mL)	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistance
1 N	10	19 (29.7)	26 (40.6)	19 (29.7)
2 CRX	30	4 (6.25)	20 (31.3)	40 (62.5)
3 GEN	10	21 (32.8)	43 (67.2)	0 (0.00)
4 AM	25	0 (0.0)	44 (68.7)	20 (31.3)
5 OFL	5	40 (62.5)	24 (37.5)	0 (0.0)
6 AX	30	23 (35.9)	33 (51.6)	4 (6.25)
7 NIT	300	4 (6.25)	36 (56.3)	24 (37.5)
8 CRP	5	39 (60.9)	20 (40.6)	5 (7.81)
9 LEV	20	24 (37.5)	30 (46.9)	10 (15.6)
10 E	30	22 (34.4)	30 (46.9)	12 (18.8)
11 CH	10	0 (0.00)	30 (46.9)	34 (53.1)
12 TE	50	4 (6.25)	28 (43.8)	32 (50.0)

N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicilin (25), OFL-Ofloxacin (5), AX Amoxicilin (30), NIT- Nitrofurantion (300), CPR- Ciprofloxacin (5), LEV Levofloxacin (20), E- Erythromycin (30), CH- Chloramphenicol (10), and TE- Tetracycline (50).

Table 5. *Antimicrobial Susceptibility Studies of S. enterica Isolates from Exotic Chickens to commonly used antimicrobial agents.*

S/N Antimicrobials	Drug Conc. (ug/mL)	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistance
1 N	10	5 (31.3)	6 (37.5)	5 (31.3)
2 CRX	30	0 (0.00)	6 (37.5)	10 (62.5)
3 GEN	10	2 (12.5)	11 (68.6)	3 (18.8)
4 AM	25	0 (0.00)	10 (62.5)	6 (37.5)
5 OFL	5	10 (62.5)	6 (37.5)	0 (0.00)
6 AX	30	5 (31.3)	7 (43.8)	4 (25.0)
7 NIT	300	0 (0.00)	10 (62.5)	6 (37.5)
8 CRP	5	11 (68.8)	2 (12.5)	3 (18.8)
9 LEV	20	6 (37.5)	6 (37.5)	4 (25.0)
10 E	30	6 (37.5)	8 (50.0)	2 (12.5)
11 CH	10	0 (0.00)	4 (25.0)	12 (75.0)
12 TE	50	0 (0.00)	4 (25.0)	12 (75.0)

N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicilin (25), OFL-Ofloxacin (5), AX Amoxicilin (30), NIT- Nitrofurantion (300), CPR- Ciprofloxacin (5), LEV Levofloxacin (20), E- Erythromycin (30), CH- Chloramphenicol (10), and TE- Tetracycline (50).

Table 6. *Minimum Inhibitory Concentration distribution of Salmonella isolates.*

Antibiotic Breakpoint	Bacteria isolates	MIC (µg/mL) 0.00175	0.0035	0.007	0.015	0.03	0.06	0.125	0.5	1	2
AX (≤3 µg/mL)	*S. Typhi*	2	2	0	0	0	0	0	0	0	0
	S. paratyphi A	2	2	1	0	0	0	0	0	0	0
	S. gallinarum	2	1	1	1	0	0	0	0	0	0
	S. pullorum	2	2	1	0	0	0	0	0	0	0
	S. subs. 3B	1	1	1	0	0	0	0	0	0	0
OFL (≤0.5 µg/mL)	*S. Typhi*	3	1	0	0	0	0	0	0	0	0
	S. paratyphi A	2	0	0	0	0	0	0	0	0	0
	S. gallinarum	2	1	0	0	0	0	0	0	0	0
	S. pullorum	2	1	1	0	0	0	0	0	0	0
	S. subs. 3B	1	1	0	0	0	0	0	0	0	0
CIP (≤0.5 µg/mL)	*S. Typhi*	3	2	0	0	0	0	0	0	0	0
	S. paratyphi A	2	1	0	0	0	0	0	0	0	0
	S. gallinarum	2	2	0	0	0	0	0	0	0	0
	S. pullorum	2	1	1	0	0	0	0	0	0	0
	S. subs. 3B	1	1	0	0	0	0	0	0	0	0

S.: *Salmonella*, AX: Amoxicillin, OFL: Ofloxacin, CIP: ciprofloxacin, and MIC: Minimum Inhibitory Concentration

4. Discussion

Salmonella is an enteric pathogen that is shed primarily in faeces resulting in faecal contamination of food and water. *Salmonella* infection is a major public health interest and proceeds to have a serious economic importance in the poultry industry around the world with the great expansion of the poultry industry, the wide spread occurrence of the poultry salmonellosis has positioned it as one of the most important egg-borne bacterial diseases of poultry [33]. The present study was conducted to determine phenotypic characters, antimicrobial susceptibility patterns and Minimum inhibitory Concentration of *Salmonella* isolates from chickens presented for slaughter in four selected local government areas of Yobe state. The *Salmonella* serovars isolates were prevalent from cloacal swab and blood in the study area. It was understood that the organism is an

important zoonotic pathogen and its occurrence in animals posses a continuous menace to man [33]. The isolation rate of *Salmonella* from this study collaborated a common study from Maiduguri, northeastern Nigeria, with a isolation rate of 7% [38], and Ibadan, southwestern Nigeria, with an isolation rate of 10% [16]. A higher isolation rate of (37%) of *Salmonella* in broiler farms had been reported from Algeria [13] therefore, suggesting chickens and poultry habitats as important reservoirs of *Salmonella* in Nigeria. This is the first comprehensive study on the isolation, biochemical characterization, Antimicrobial Susceptibility and Minimum inhibitory concentration of *Salmonella* in commercial chicken presented for slaughter from all the four regions (Sample sites) of Yobe state.

This study revealed the presence of *Salmonella* in chickens from blood and cloacal swab samples analyzed with an overall isolation rate of 13.33%. This finding, in itself, is not surprising since *Salmonella* is reported to be an environmentally persistent pathogen capable of living and replicating in diverse environments [48]. The 13.33% prevalence of *Salmonella* obtained in this study is however lower than the 40% isolation rate by [34] in Dakar, Senegal and higher than the 5% isolation rate by [24] in Maiduguri metropolis Borno State, Northeastern Nigeria. The prevalence reported in this study is higher than those documented for chickens in EU countries, with overall prevalence of zoonotic *Salmonella* serovars of 2.5%. The high prevalence observed in this study may be attributed to lack of implementation of control programmes on poultry farms and differences in terms of *Salmonella* status among countries but could be influenced by housing system, local environmental conditions, sample types, collection seasons, isolation methodologies and culture media.

The prevalence of *Salmonella enterica* isolated from chickens presented for slaughter show that 42.66% isolated from local chickens is higher than 10.66% isolated from exotic chickens. This is because local chickens in the study locations depend largely on contaminated wastewater sources and underground feeds and vegetable as previous studies report that *Salmonella* can persist in the farm environment for extended periods of time due to movement within the farm from animals, human and livestock excrement [27]. The *Salmonella* isolates obtained from local (42.66%) chicken were higher than the 10.66% from exotic isolates obtained in this work and this could be attributed to management and husbandry practices as well as the climatic conditions of the study areas. It has been reported by [38], reported that the emergence or resurgence of numerous infectious diseases is strongly influenced by environmental factors such as climate, weather, topology and hydrology. The existence of the diseases especially in these local breeds is of great concern as the diseases have the potential for horizontal and vertical transmission. The prevalence might have gone even higher if the sample size was increased and samples were taken from dead chickens.

The Biochemical reactions for pathogenic identification and confirmation of presumptive *Salmonella enterica* isolates from chickens presented for slaughter in Yobe state using conventional method in this research show that All 80 presumptive positive isolates are positive for Catalase, Citrate, H_2S, Mortility test, Methyl red and Tripple sugar ion tests. While negative for Gram stain, Oxidase, VP, Indole and Urease tests. This result show the efficiency and specificity of Microbact 24E GNB Computer Identification System as against the conventional biochemical test.

The distribution of serotypes of *Salmonella* in the study using Microcat 24E GNB Computerize system, comprised *S. typhi* 4.0%, *S. paratyphi* A 2.66%, *S. gallinarum* 2.7%, *S. pullorum* 2.7% and *Salmonella subs. 3AB* 4.1%. *S. typhi* had the highest prevalence rate of 4.0%. This result is quite worrisome as *S. typhi* is strictly a human pathogen that causes invasive fever (typhoid fever), whereas most other *Salmonella* serotypes cause mainly gastrointestinal symptoms without systemic invasion [8]. Its high prevalence could be attributable to poultry feed as *S. typhi* has been reported to be frequently isolated from sewers and feacally contaminated waters [14].

Several studies have shown that *Salmonella* exhibit multidrug resistant patterns [2]. Multiple drug resistance was observed in all isolates of *Salmonella* tested in this study. The emergence of *Salmonella* isolates with high multiple antibiotic resistance indicates that these isolates must have originated from environments where antibiotics are abused and often used as therapeutic measures in humans and growth promoters in livestock [41]. The detection of *S. typhi* is an indication of contamination of human origin, which was mostly detected in the study was also observed to be most resistant and hence implies human use/misuse of antibiotics. Although, it is possible that isolates may have acquired the genes for resistance to multiple antibiotics from other enteric bacteria. Some isolates from exotic chickens were particularly observed to be resistant to eight of twelve antibiotics tested. This represents a great public health issue as certain cases of poultry salmonellosis are severe and often require antimicrobial therapy for treatment [31]. Hence, these multidrug resistant *Salmonella* strains obtained from exotic chickens commonly eaten is a major concern for food safety. The detection of these resistant *Salmonella* strains in this study calls for attention. These findings indicate that these isolates have the capability to develop resistance for routinely prescribed antimicrobial drugs and pose considerable health hazards to consumers, hence the need for institution of sensible control measures. It has been reported that *Salmonella* strains contain both antimicrobial resistance and virulence genes as factors such as colonization and survival in the host may select for resistance [31].

The worldwide spread of multidrug-resistance plasmids has been lifted by selective pressure from usage of antimicrobial in human and veterinary medicine (Schultsz and Geerlings, 2012). Plasmid mediated resistance is a transfer of antibiotic resistance genes which are carried on plamids. The plasmids can be transferred between bacteria within the same species or between different species by conjugation. Plasmids often carry multiple antibiotic

resistance genes, contributing to the spread of multidrug-resistance (MDR). Antibiotic resistance mediated by MDR plasmids severely restricts the treatment options for the infections results by *Salmonella* and other members of *enterobacteriaceae* [40]. The high resistance level to most of the antimicrobials tested in this study, especially nalidixic acid and ciprofloxacin, is worrisome because fluoroquinolones are used strategically in the treatment of salmonellosis in the study area. This resistance may be because of indiscriminate use of antimicrobials at recommended doses or at subtherapeutic doses in feed as growth promoters, and as chemotherapeutic agents to control epizootics on the farms; however, it is important to inquire the types of antimicrobials the farmers administer to their poultry either as prophylaxis or therapeutics before studying the antimicrobials resistance in future studies. The lack of policy to prevent the use of antimicrobials, especially fluoroquinolones, including ciprofloxacin, enrofloxacin and ofloxacin in poultry in the study area, may have contributed to the past spread of resistance in the poultry industries [36]. These findings agreed with the report of [16] which equally reported a high level of resistance to nalidixic acid and reduced susceptibility to ciprofloxacin. The resistance to cephalosporins (ceftazidime and cefotaxime) is in agreement with [46; 3; 5]. This is worrisome, in view of the high level of resistance observed for all of the *Salmonella* serovars isolated in this study. Cephalosporins are major antimicrobials used to treat chronic *Salmonella* infections in humans. However, their effectiveness is being compromised by the emergence of extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated cephalosporinases [46]. The low level of resistance by most of the isolates to neomycin might be because of the fact that the farmers in the study area have neglected this drug and opted for some alternate effective antimicrobials like ciprofloxacin. *SalmonellaTyphi*, which is of zoonotic significance, was one of the most prevalent serovars in this study and showed a high level of resistance to most of the commonly used antimicrobials. These observations call for regulation of antibiotic usage in poultry in the study area to improve spread of resistance to antimicrobials.

The minimum inhibory concentration (MIC) is the least amount of antimicrobial agent that inhibits visible growth of an organism after overnight incubation. The MIC of ofloxacin was distributed within 0.00175–2µ g/ml 3 *Salmonella Typhi* were ofloxacin resistant with MIC 0.00175 µg/ml and 1 with MIC 0.0035 µg/ml. Similarly for amoxilin, MIC ranged between 0.00175 and 3.00 µg/ml were 2 *Salmonella Typhi* were amoxicilin resistant with MIC 0.00175 µg/ml and 2 with MIC 0.0035, 2 *Salmonella paratyphi* were amoxicilin resistant with MIC 0.00175 µg/ml, 2 with MIC 0.0035 µg/ml and 1 with MIC 0.007µg/ml, 2 *Salmonella gallinarum* were amoxacilin resistant with MIC 0.00175 µg/ml, 1 with MIC 0.0035 µg/ml, 1 with MIC 0.007 µg/ml and 1 with 0.015 µg/ml, 2 *Salmonella pullorum* were amoxacilin resistant with MIC 0.00175 µg/ml, 2 with MIC 0.0035 µg/ml, and 1 with MIC 0.007 µg/ml, and 1 *Salmonella* subs. 3B were amoxacilin resistant with MIC 0.00175 µg/ml, 1 with MIC 0.0035 µg/ml, 1 with MIC 0.007 µg/ml. The Minimum Inhibitory concentration determination in this study revealed the least amount of susceptible antimicrobial agent that inhibits visible growth of *Salmonella* organism isolated. Hence its paramount important as it could helps in known drug dosage for the treatment of poultry Salmonellosis in the study area.

The continuous contact with infected poultry feces, meat, eggs and handling of young chickens particularly by children, or anything in the area where they live is therefore, calls for concern, as *Salmonella* is one of the major causes of intestinal diseases globally as well as the etiologic agent of more severe systemic diseases such as typhoid and paratyphoid fever [38]. Due to the lack of facilities to offer crucial tests for the detection of Salmonella infections, it is difficult to get a good image of the true condition of poultry salmonellosis in Nigeria, as well as the rest of Africa. However, there has been a limited amount of research on non-typhoidal Salmonella serovars that cause human infections in Africa, with S. Enteritidis and S. Typhimurium and *S. Typhimurium* as the most prevalent serovars. Moreover, a recent study monitoring *Salmonella* from diverse sources, including humans, in the north-eastern regions of Nigeria reported *S. Eko*, *S. Enteritidis* and *S. Hadar* as the most common serovars that infects humans, whereas these serovars did not enter among the most common serovars found in chicken in Nigeria based on the data collected.

Grandparent stocks are frequently imported from Europe, according to an FAO study, however the lack of regulations and stringent implementation of laws against the importation of uncertified poultry and poultry products may be a concern in Nigeria. It's unclear how much of the elevated Salmonella prevalence seen in this study was caused by the entrance of diseased birds from other countries or due to the infection of the animals once they were farmed in Nigeria. The high prevalence and presence of multiple *Salmonella* serovars throughout the country may be due to poor sanitary conditions of poultry farms, frequent movement of people and lack of enforcement of monitoring programmes particularly for imported animals as well as the poorly managed borders with neighboring countries. Improving these conditions together with improved cleaning and disinfection could have a significant impact on reducing *Salmonella* infections level on farms in Nigeria. Although vaccination is still regarded as an important part of the overall peventive strategy for *Salmonella*, it is, however, advocated that routine vaccination for *Salmonella* control should not stop at fowl typhoid control alone, but rather, should also include other serotypes which could be easily transmitted in eggs and poultry meat meant for human consumption. The circulation of zoonotic *Salmonella* in Nigeria, as in other developing countries, may have a global impact in terms of public health because of movements beyond the area of origin, thanks to trade and travel. Knowledge about the extent of the phenomenon is important

in order to find possible control measures at global level. Moreover, comparison of livestock and human isolates could discern the feasible contribution of diverse sources to the burden of human salmonellosis.

The findings of this study suggest that free-range village poultry production (local hens) and intensive poultry production may suffer fowl typhoid and/or Pullorum disease in the future in the study area unless adequate attention is paid to disease prevention and control. As a result, systematic national regulatory survey programs for both free-ranging and captive animals should be established. Farmers should be instructed and trained on the use of Salmonella-free parents in both free-range village hens and intensively produced chickens to prevent losses and control infections.

5. Conclusion

In the present study, the phenotypic characterization of *Salmonella* organism in blood and cloacal samples from chickens in some selected areas of Yobe State, were observed and reported for the first time using Microbact 24E GNB computer identification system. The results showed that *Salmonella* organism are prevalent in all the four (4) selected Local Government areas of Yobe State. Five (5) *Salmonella* species were isolated and these are *S. typhi, S. paratyphi, S. gallinarum, S. pullorum* and *S. subs. 3B*. The overall percentage of isolates was 13.3%. Where as, percentage of isolates from local and exotic chickens are 42.7% and 10.7% respectively, while 32.7% from males and 24.5% from female chickens, significance was observed within the different locations with Damaturu having the higest percentage (22.7%) and Fune having the lowest (8.7%). *Salmonella* isolates were found to be susceptible to ofloxacin, amoxicillin, ciprofloxacin and gentamicin amongst the commonly used antibiotics in the study area. On Minimum Inhibitory concentration determination *Salmonella* isolates showed susceptibility towards ofloxacin with MIC of 2-0.007, amoxicillin with MIC of 2-0.015 and ciprofloxacin with MIC of 2-0.007.

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