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

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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 blackshick colonies. A total of 600 (300 cloacal swab and 300 blood), consisting (202 males, 98 female chickens, 350 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 Microbact12® GNB 24E System kit, with 40 randomly selected presumptive isolates (8 from blood and 32 from cloacal swab) tested using Microbact 24E GNB Computerized 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 (Amoxicillin 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 amoxicillin, 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 • Minimum inhibitory Concentration • Chickens

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 enteric 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 diarrheal disease [18].

Salmonella enteric 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 enteric 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. enteric 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, bacteraemia, 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 infections have been associated with the outbreaks of *Salmonella* diseases. The disease has been reported in many parts of the world, 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* transmission [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].

*Salmonella* 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 [16]. In the last two decades, multidrug-resistant *Salmonella enterica* isolates have increasingly become a major health hazard [16]. 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 transposons [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 States 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 states between 2009 and 2012 includes Aminoglycosides, Lincosamides, Cephalosporins, Penecillins, Sulfonamides, Crystal Macrolides antibiotics, Tetracyclines and Ionosopires [47].

Fowl typhoid and pullorum disease, caused by *Salmonella enterica* subspecies *enteric* serovars *gallinarum* and *Salmonella 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 enteritis* by gene deletion events [44]. The terms serovar *gallinarum* or *pullorum* will be used, as this more usefully distinguishes the two biotypes 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 medicaiton. 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 hosts 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 feacces easily carried by healthy birds and may be passed to raw animal origin food as a result of contamination during slaughtering and processing [15]. Generally, *Salmonellae* 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 *pullorum* 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 Pullorumisolation and identification [43].

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 poult [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.

**Materials and Methods**

**Study Area**

Yobe is a state located in Northeast Nigeria Coordinates: 12°00’N 11°30’E
Therefore, six hundred samples from 300 chickens were collected from approximately 400.

Thus, \( N = \frac{1000}{1 + 1000 	imes 0.0025} = 399.99 \)

E = 0.05 at confidence level of 95%

\[
Z = \frac{N}{1 + Ne^2}
\]

Where,

N = population size = unknown

\[
E = 0.05 \text{ at confidence level of 95%}
\]

Thus, \( N = \frac{1000}{1 + 1000 	imes 0.0025} = 399.99 \)

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.

**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 A, Potiskum as Area B, Damaturu as Area D, and Bade as Area B.

**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.

**Collection of Cloacal Swabs**

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 five times against the cloacal mucosal surface with gentle pressure to take test fluid sample. Then immediately transfer into 10ml 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].

**Collection of blood samples**

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 5 minutes to separate the serum from blood cells. The sera were collected in the plain vacutainer tube after centrifugation using Pasteur pipette.

**Laboratory culture and identification**

The laboratory identification in this study involves; enrichment, selective plating, preliminary identification and complete biochemical identification with some modification (Mailafia et al., 2017). The samples were analyzed by using semisolid modified Rappaport Vassiliadis medium as the selective enrichment medium, showing turbidity 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 (Duerrden et al., 1998).

**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 (Duerrden et al., 1998).

**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].

**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 biblious paper. Finally the smeared slides were viewed using a microscope under oil immersion at 100× Magnification [16].

Salmonella organisms are gram negative. Thus, they appeared pinkish red [23].

**Catalase Production Test**

The enzyme catalase breakdowns 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 platinum 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.

**Oxidase Test**

This test depends on the presence, in bacteria, of certain oxidases.
that catalyze the oxidation of reduced tetramethyl-p-phenylenediaminedihydrochloride (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.

### 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.

#### 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 indication of urease negative result [29].

#### 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].

#### Tripplr Sugar Iron (TSI) Test

(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 [28].

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].

#### 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].

#### Indole Production Test

Certain bacteria which possess enzyme tryptophanase, 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].

#### Voges-proskauer (VP) test for acetoil 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 c-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 ofo-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.

### Microbact™ 24E GNB Computer Identification System

The Microbact 24E system (Oxoid™Microbact™GBN 24E System kit, manufactured by Thermo-Fisher Scientific, Waltham, Massachusetts, USA) is a miniaturized identification system for the identification of microorganisms. The Microbact™ is a commercially used Microsysytem 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.

### Preparation of Innoculums and Inoculations

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 bacterial suspension was added. Using a dropper bottle, the substrates underlined on the holding tray were overlayed 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.

### Antimicrobial Resistance Profile

Antimicrobial resistance profile of *Salmonella* isolates were determined by the disc diffusion method of Kirby Bauer, [28] 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 transferred 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, norfloxacain, ofoxacin, Chloramphenicol, erythromycin, ciprofloxacin, levofloxacin, nitrofuranton and cefuroxime.

**Determination of Minimum Inhibitory Concentration (MIC)**

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 (oxefoxacin, ciprofloxacin and amoxicilin) 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].

**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.

**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.

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

Table 1.1 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 3 (in Damaturu) where 35.71% (15) of the samples examined found to be positive, followed by week 4 (in Potiskum) with 42 (17.5%).

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

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

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

<table>
<thead>
<tr>
<th>Salmonella species isolated</th>
<th>No. (%) +ve isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhi</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>S. paratyphiA</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>S. gallinarum</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>S. pullorum</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Salmonelllasub. 3B*</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (20.0)</td>
</tr>
</tbody>
</table>

**Table 2.2 shows Salmonella species, isolated from chickens using Microbact 24E GNB Computer Identification system.**

<table>
<thead>
<tr>
<th>Species isolated</th>
<th>No. (%) +ve isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella typhi</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>Salmonella paratyphiA</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>Salmonella gallinarum</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>Salmonella pullorum</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (100.0)</td>
</tr>
</tbody>
</table>

**Antimicrobial Susceptibility Studies of Salmonella enteric isolates from Local and Exotic Chickens to CommonUsed Antimicrobial Agents**

Table 3(a) 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 (60) 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 Nitrofuranton, 4 (5.0%) to Cefuroxime & Tetracycline. While 54 (67.5%) were intermediate to Gentamicin & Ampcillin, 46 (57.5%) to Nitrofuranton, 40 (50.0%) 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 Nitrofuranton, 28 (32.5%) to Ampcillin 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.

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

Table 3(b) shows Antimicrobial Susceptibility Studies of S. Enteric 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 Ampcillin, Norfloxacin 26 (40.6%), and Cefuroxime 20 (31.3%). However, 34 (53.1%) were resistance Chloramphenicol and 32 (50.0%) to Tetracycline.
Table 3(a). Antimicrobial Susceptibility Studies of *Salmonella enterica* isolates from local and exotic chickens to commonly used antimicrobial agents.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Antimicrobials</th>
<th>Drug Conc.(ug/mL)</th>
<th>No. (%) Susceptible</th>
<th>No. (%) Intermediate</th>
<th>No. (%) Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
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</tr>
<tr>
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<td>40 (50.0)</td>
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<td>30 (37.5)</td>
</tr>
<tr>
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<td>CRP</td>
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<td>22 (27.5)</td>
<td>8 (10.0)</td>
</tr>
<tr>
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<td>36 (45.0)</td>
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<tr>
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<td>TE</td>
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<td>4 (5.00)</td>
<td>32 (40.0)</td>
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N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicillin (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 3(b). Antimicrobial Susceptibility Studies of *S. enterica* isolates from Local Chickens to commonly used antimicrobial agents.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Antimicrobials</th>
<th>Drug Conc.(ug/mL)</th>
<th>No. (%) Susceptible</th>
<th>No. (%) Intermediate</th>
<th>No. (%) Resistance</th>
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<tr>
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</tr>
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N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicillin (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 3(c). Antimicrobial Susceptibility Studies of *S. enterica* isolates from Exotic Chickens to commonly used antimicrobial agents.

<table>
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<tr>
<th>S/N</th>
<th>Antimicrobials</th>
<th>Drug Conc. (ug/mL)</th>
<th>No. (%) Susceptible</th>
<th>No. (%) Intermediate</th>
<th>No. (%) Resistance</th>
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<td>10 (62.5)</td>
</tr>
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<td>GEN</td>
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</tr>
<tr>
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<td>12 (75.0)</td>
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</table>

N- Norfloxacin (10), CRX- Cefuroxime (30), GEN- Gentamicin (10), AM- Ampicillin (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).

Antimicrobial Susceptibility Studies of *Salmonella enterica* Isolates from Exotic Chickens to Commonly Used Antimicrobial Agents

Table 3(c) shows Antimicrobial Susceptibility Studies of *S. enterica* isolates from exotic chickens to commonly used antimicrobial agents. Even though, 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 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.
Minimum Inhibitory Concentration Distribution of Salmonella Isolates

The MIC of Salmonella positive samples are presented in Table 4. 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 0.007 µg/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 MIC of amoxicillin ranges between 0.00175 and 3.00 µg/ml where 2 Salmonella Typhi showed MIC of 0.00175 µg/ml and 1 with MIC 0.0035 µg/ml. The MIC of amoxicillin with MIC 0.00175 µg/ml and the other 3 Salmonella gallinarum isolates the MIC of 0.0035 µg/ml, 0.007 µg/ml and 0.015 µg/ml respectively. Again of the 2 Salmonella pullorum isolates 2 were resistant to amoxicillin with MIC 0.00175 µg/ml, another 2 of the Salmonella 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 amoxicillin with MIC of 0.00175 µg/ml, MIC 0.0035 µg/ml and MIC 0.007 µg/ml.

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 an isolation rate of 7% [38], and Ibadan, southwestern Nigeria, with an isolation rate of 10% [18]. 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 metropolitan 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 enteric 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 enteric isolates from chickens presented for slaughter in Yobe state using conventional method in this research show that 80 presumptive positive isolates are positive for Catalase, Citrate, H2S, Mortility test, Methyl red and Tripple sugar ion tests. While negative for Gram stain, Oxidase, VP, Indole and Ureaese 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 fecally contaminated waters [14].

Several studies have shown that Salmonella exhibit multidrug resistant patterns [2]. Multiple drug resistance was observed in all isolates of

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

**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 is 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%. Whereas, 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 highest 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.

References


