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Phenotypic Characterization of *Salmonella enterica* from Chickens in Some Selected Local Governments of Yobe State

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ABSTRACT

Salmonella of poultry are zoonotic, microorganisms 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. This study was conducted to determine phenotypic characters of *Salmonella enterica* from chickens presented for slaughter in some selected LGA of Yobe State, Nigeria. 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 plate on Xylose Lysine Deoxycholate agar showing blackish colonies. A total of 600 (300cloacal 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 result show that72 (24.00%) *S. enterica* were isolated from cloacal swab where 8 (2.66%) from blood samples while, 24 (24.48%) from female chickens, 66 (32.67%) from male chickens and 64 (42.66%) from local chickens, Similarly 16 (10.66%) from exotic chickens and. The overall isolation rate was 80 (13.33%).All the 80 presumptive positive isolates showed 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. The presumptive *Salmonella* isolates were subjected to Microbact^{TM} GNB 24E System kit, where 40 selected presumptive isolates (36 from blood and 4 from cloacal swab) tested using Microbact 24E GNB Computerize system 10 (9 (45%) from cloacal swab and 1 (5%) from blood) samples were found to be positive for *Salmonella* organism. All the blood samples were tested for agglutination using slide method, 255 were found to be positive, showing a wide range with positives after blood culture 8. It is therefore, concluded that *Salmonella* organisms are prevalent in some selected local governments of Yobe State. It is therefore, recommended that more research should be conducted on other poultry such as ducks and goose which could reservoir of *salmonella* organism in the study area.

Keywords: *Salmonella*, Phenotypic, Characterization, Chickens.

INTRODUCTION

The genus *Salmonella* consists of rod shaped, Gram-negative, flagellated, facultative anaerobes, and belongs to the family Enterobacteriaceae (Salehi *et al.*, 2005). 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 (Baggesen *et al.*, 2000). *Salmonellae* causing 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 (Gordon *et al.,* 2012).

Salmonellosis, caused by *Salmonella enterica*, occurs in about 1.4 million people per year in United State of America alone, an estimated 30% of all food borne illness, causing about 500 deaths and has an estimated cost of $2.4 billion dollars per year (Callaway *et al*., 2008). Typically, people with *Salmonella* infection have 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 are; bloody diarrhea, vomiting, headache and body ache. Most healthy people recover within a few days without specific treatment (Callaway *et al*., 2008).

Food-borne diseases caused by nontyphoidal *Salmonella enterica* serovars represent an important public health problem and an economic burden in many parts of the world (Parvej 2016). *Salmonella* is an important cause of foodborne infections with a broad host spectrum (Wei et *al.,* 2011). 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 (Liljebjelke *et al.,* 2005). *S. enterica* remains a formidable public health challenge (Cummings *et al.,* 2012) and with a reported increase in its incidence (CDC, 2011). Salmonellosis can result in a number of disease symptoms including gastroenteritis, bacteremia, typhoid fever and fecal infections (Darwin and Miller, 1999). Certain cases of Salmonellosis are severe and often require antimicrobial therapy for treatment, thus, resistance to antimicrobial drugs is a great concern (Marrero-Ortiz *et al.,* 2012).

The main sources are foods of animal origin, such as eggs, milk, poultry, beef, and pork meat. In addition, fruits and vegetables have been incriminated 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 (Parvej 2016).

Hospital acquired infection occurs known as nosocomial infection have been associated with the outbreaks of *Salmonella* diseases. The disease, have been reported in hospitals in many parts of the world, particularly African countries including Nigeria (Lepage *et al.,* 1990). It occurs among patients who are admitted with a different diagnosis. Outbreaks of hospital-acquired *Salmonella* can be particularly severe on pediatric wards in developing countries, where children may be malnourished and have other host risk factors (Lepage *et al.,* 1990). In African hospitals, including Nigeria, Kenya, and Egypt food is often provided by a patient's family. Although few studies have examined risk factors for infection in hospital outbreaks, contaminated food and person-to-person transmission have been considered. High death rates are frequently observed, especially when outbreaks are caused by strains of *Salmonella* are resistant to the local empirical therapy (Lepage *et al.,* 1990). Out of 360 adult and pediatric patients with hospital-acquired diarrhea in a Kenyan hospital in 1988, 10% of cases were due to *Salmonella* species and 2.5% to *Shigella* species. Among children aged 6 months to 6 years, recent antimicrobial use and crowded living conditions at home were associated with hospital-acquired diarrhea due to *Salmonella* and *Shigella* species. Among adults, sharing a hospital room with somebody who has diarrhea and a history of previous hospitalization were associated with hospital-acquired *Salmonella* or *Shigella* diarrhea (Paton *et al.,* 1991). Prevention strategies could include patient and visitor education regarding personal

hygiene and food preparation and storage, provision of safe drinking water, hand washing before and after patient contact by health care workers, thorough cleaning of the hospital environment, reduction in crowding, avoidance of sharing beds, increasing the number of health care workers, adequate disinfection of reusable equipment, surveillance of *Salmonella* infection, and isolation of identified cases (Lepage *et al.,* 1990, Paton *et al.,* 1991).

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, life-threatening systemic infections are common especially among high risk categories (Fagbamila *et al.,* 2017). In the last two decades, multidrug-resistant *Salmonella enterica* isolates have been increasingly became a major health hazard (Fagbamila *et al.,* 2017). This resistance can be acquired by mutations in chromosomal DNA or by the acquisition of extra-chromosomal genomic material by means of plasmids and transposons (Vazquez *et al.,* 2005). The growing resistance of pathogenic bacteria to antimicrobials has raised the concern that the widespread use of antimicrobials in animal's production may promote the development of resistance bacteria or resistance genes that can be transferred to bacteria that cause disease in humans (Wegener *et al.,* 1997). The use of antimicrobial agents in food producing animals for different purposes, including infection treatment, disease prevention, and also growth promotion (allowed in some countries of European Union, England, France and Wales and United States of America (USA), Brazil, has been a major factor in widespread dissemination of antimicrobial-resistant bacteria that could be transferred to humans through the food chain. The antimicrobial drugs approved for use in food-producing animals actively in the United states between 2009 and 2012 includes Aminoglycosides, Lincosamides, Cephalosporins, Penicillins, Sulfonamides, crystal Macrolides antibiotics, Tetracyclines and Ionospores (Wegener *et al.,* 1997).

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 (Barbour *et al.,* 2015) where increasing antimicrobial resistance in these strains has also become a problem (Parvej *et al.,* 2016). They have been eradicated from commercial poultry in many developed countries of Western Europe, the United States of America serovar is referred to as *pullorum* (Hitchner, 2004), even though the strains are now considered to be the same serovar that is derived from *Salmonella enteritidis* by gene deletion events (Thomson *et al.,* 2008). 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 (Ivanics *et al.,* 2008). *Salmonella pullorum* remains as a constant reservoir in wild and game birds.

In food-producing animals and especially in poultry, *Salmonella* is one of the leading causes of infection, and this has a direct impact on the global marketing of the respective food-producing animals and animal-derived food products (Fagbamila *et al.,* 2017). Poultry salmonellosis related to host adapted serovars remains a major constraint on poultry production in all parts of Nigeria (Fagbamila *et al.,* 2017). Famers still experience great losses (due to mortality, morbidity, and drop in egg production) caused by host adapted *Salmonella* serovars despite huge amounts spent on vaccination and medication. In early life, *Salmonella pullorum* causes extremely high mortality of both broilers and commercial laying birds. Similarly, older birds succumb heavily to other

serovars of *Salmonella* and it is assumed that *Salmonella* infections of this category of birds are mainly due to *Salmonella gallinarum* (Fagbamila *et al.,* 2017). In addition to these host adapted *Salmonella* serovars causing systemic disease, poultry harbor the organism in their gastrointestinal tracts with no apparent signs of illness (commensal). Hence, these *Salmonella* serovars can be present in faeces excreted by healthy animals and may be transferred to raw foods of animal origin through contamination during slaughtering and processing. Generally, *Salmonella* in food producing animals, including poultry, manifests as long as period of latent carriage with occasional faecal shedding, which is the leading source of contamination of feed, water and environment (Fagbamila *et al.*, 2017).

Salmonellosis in poultry is endemic worldwide, causing morbidity and mortality and, thus, economic losses (Abiodun *et al.*, 2014; Ahmed *et al.,* 2017; Akter *et al.*, 2007; Kwon *et al.,* 2010). The disease is very significant by virtue of the fact that *Salmonella* can be transmitted vertically from parent to offspring. 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 (Abiodun *et al.*, 2014; Dawoud *et al.,* 2011; Hannah *et al.,* 2011). 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* (Ahmed *et al.,* 2017). They can also be transmitted by other animals, wild birds and personnel. *Salmonella* may contaminate young chicks directly through ovarian transmission or penetrate the egg shell after the egg has been laid (Maryam *et al.,* 2009). Poultry farms and poultry products are the major sources for Salmonella contamination (Ahmed *et al.,* 2017). Reports on various poultry diseases occurring in some parts of this country showed that salmonellosis is the major threat facing poultry production in Nigeria (Mamman *et al.,* 2014), and animal droppings have been shown to be a potential reservoir for many enteric organisms (Ahmed *et al.,* 2017). Hence, consumers of poultry and poultry products are at risk of contracting salmonellosis via consumption of contaminated products.

Although vaccination to prevent salmonellosis has been practised successfully on layer farms in several countries (Dawoud *et al.,* 2011; Kwon *et al.*, 2010), vaccines produced from local isolates are still not readily available on the market, especially in developing countries, for effective preventive measures. Hence, the control of salmonellosis predominantly lies on good sanitary practices and the use of antimicrobial drugs for prophylaxis and therapeutics (Abiodun *et al.*, 2014; Akter *et al.*, 2007), which subsequently leads to abuse of antimicrobial drugs in poultry settings, culminating in the development of resistance and the eventual limitation of the therapeutic outcome in the treatment of bacterial diseases (Ahmed *et al.*, 2017). 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 (Akinyemi *et al.,* 2010). This research will determine the phenotypic characters *Salmonella* from chickens presented for slaughter in some selected areas of Yobe state.

MATERIALS AND METHODS

3.1 Study area

Yobe is a state located in Northeast Nigeria Coordinates: 12°00′N 11°30′E / 12.000°N 11.500°E. A mainly agricultural state, it was created on August 27, 1991. Yobe state was carved out of Borno State. The capital of Yobe state is Damaturu. The state borders 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, conditions are hot and dry for most of the year, except in the southern part of the state which has a milder climate.

While Yobe state is an agrarian state it also has rich fishing grounds and mineral deposits of gypsum in Fune LGA, kaolin, and quartz. The state's agricultural products include gum arabic, groundnuts, beans, and cotton. The state is also said to have one of the largest cattle markets in West Africa, located in Potiskum. Yobe's terrain 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 predominantly of the Sudan savanna type, with scattered acacia trees. There is also an Nigeria 2007).

area of Sahel savanna, consisting of sandy soils and thorn scrub, which is located in the far north. The Kanuri are the principal 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 chief occupations. Damaturu is the state capital, and Nguru, Potiskum, and Gashua are sizable market towns. The state is served by trunk roads connecting Potiskum, Damaturu, and Maiduguri (Borno state). Pop. (2006) 2,321,591 (PHC 2006).

Yobe State consists of 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 (Yobe state online

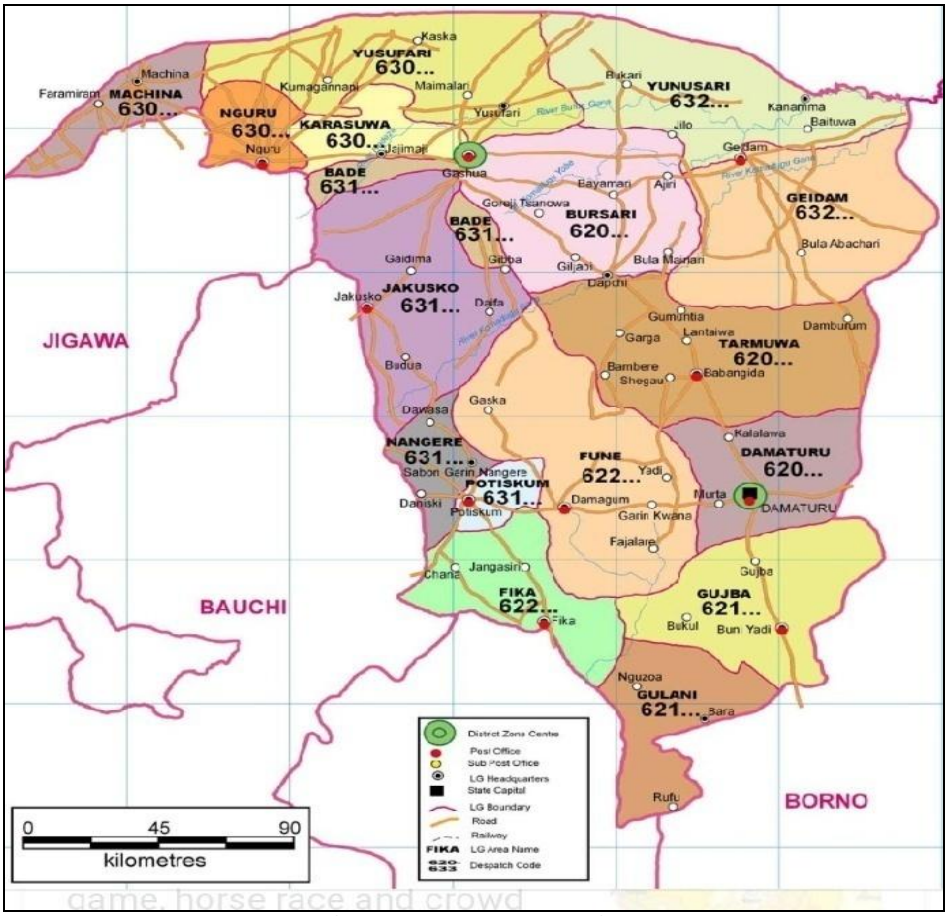

Figure 2: Map of Yobe showing study areas of sample collection
Cartography Laboratory, Yobe state University Damaturu (2019).

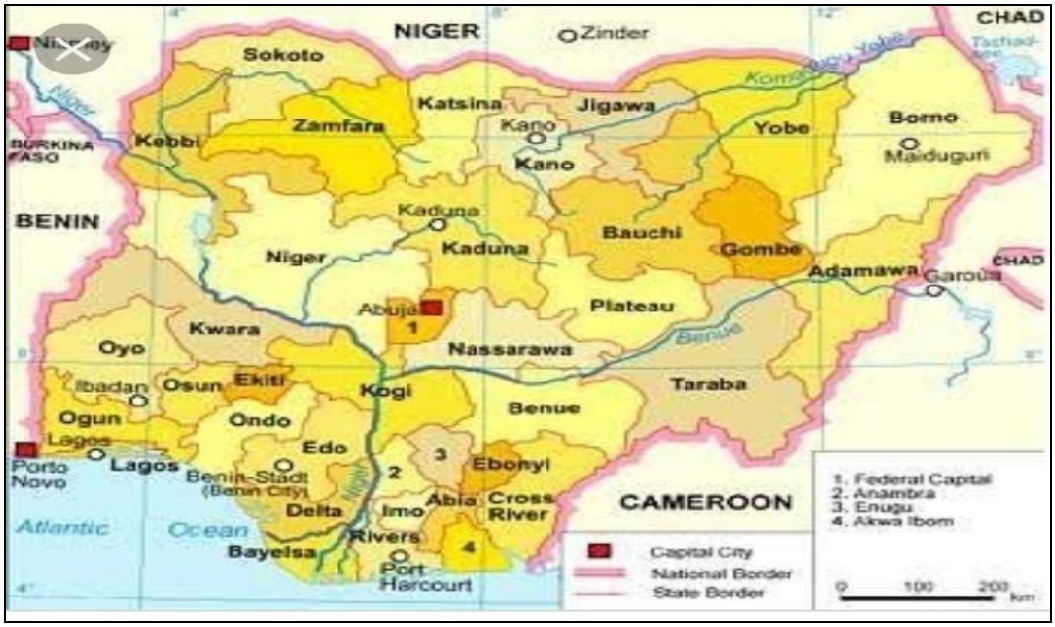

Figure 1: Map of Nigeria showing Yobe state

3.1 Sample Size Determination: The sample size is determined by using thrush field sample size calculation formulae

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

Where,

N=population size= unknown= 1000

e=0.05 at confidence level of 95%

Thus, N=1000/ (1+1000×0.0025) =399.99

Approximately =400 (Thrushfield 1997).

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

3.2 Experimental design

A total of 600 samples (300 Cloacal swab and 300 Blood samples) were obtained from chickens from four different locations in Yobe state. A sterile swab was inserted into the cloaca of the chicken and then turn slowly to take test fluid sample and the blood samples were collected from each chicken into EDTA and plain Vacutainer tube each. The swabs were kept in a sterile tube containing 10ml of buffered peptone water (transport/ pre enrichment medium),whereas, the blood samples were placed in EDTA container for culture and in plain container to obtain serum, arranged in Ice Park cooler and transported immediately to Microbiology Laboratory, University of Maiduguri for analysis. The samples were collected in a period of 12 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.

3.3 Sampling

Convenient sampling was conducted based on availability and willingness of the butchers, where, 75 chickens collected in four batches of Slaughtered chickens from four different chicken slaughterhouses (chicken abattoir). Each Butcher, before taking the samples was interviewed orally to obtain information on sex of each chicken, and was told the type of research that will be done.

3.4 Collection of cloacal sample

The swabs samples were carefully obtained, taking care to avoid contamination from the outside of the cloaca, a sterile swab was inserted into the cloaca of the chicken and then rotator moved to take test fluid sample. Until delivery to the laboratory, the swab samples were collected unto sterile tube containing 10ml of buffered peptone water (transport/ pre enrichment medium). The swab samples were kept refrigerated (in an Ice Park cooler) and transported to the laboratory (Mailafia *et al.,* 2017).

3.4.1 Collection of blood sample

Three milliliters of the blood sample were collected from each chicken into plain

container and EDTA container each, during slaughtering. Those in plain Vacutainer tubes were centrifuged at high speed for 5minutes in order to separate the serum from blood cells. The serum formed was separated from packed cell into clean container in each Pasteur pipette. The samples were processed immediately (Mailafia *et al.,* 2017).

3.5 Laboratory Culture and Identification

The laboratory identification in this study involves; enrichment, selective plating, preliminary identification and complete biochemical identification (Mailafia *et al.,* 2017).

3.6 Media Preparation

Standard media were prepared base on the manufacturer instruction. The specified Media used for this study is: Oxiod^{TM} Media, (Xylose Lysin Deoxycholate agar, Rappaport Vasiliadis, Tripple sugar ion agar, sulfur indole motility, Simon citrate agar, Urease, Nutrient agar and Nutrient Broth) manufactured by Thermo-Fisher Scientific, Walham, Massachusetts, USA (Mailafia *et al.,* 2017).

3.6.1 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).

3.6.2 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^{o}C for 24h. The *Salmonella* Isolates colonies, appears red with black centers on xylose lysine desoxycholate medium (Duerden *et al.,* 1998).

3.6.3 Preliminary Identification

The preliminary identification involved Gram staining, Oxidase test, and Catalase test.

3.6.3.1 Gram staining

Gram staining method is most frequently used in diagnostic bacteriology. 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, the slides were tilted slightly and gently rinsed with tap water or distilled water using a wash bottle, the smears were flooded with lugals/Gram's iodine and let stand for 1 minute, the slide were tilted slightly and gently rinsed with tap water or distilled water using a wash bottle. The smears were appeared as purple as circle on the slide, and decolorized using 95% ethyl alcohol. The slides were tilted and applied alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Careful not to over-decolorize, the slides were rinsed immediately and flooded gently with safranin to counterstain and let stand for 45seconds. The slide were 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 (Duerden *et al.,* 1998). *Salmonella* organisms are gram negative. Thus, they appeared pinkish/ red (Duerden *et al.,* 1998).

3.6.3.2 Catalase production test

The enzyme catalase mediates the 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).

3.6.3.3 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 putted on a piece of filter paper. The 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).

3.6.4 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, SIM test, indole test, Methyl red test, Voges-Proskauer test, and Hydrogen sulphide production test.

Urease test

Urease test is performed to check the capability of microbes to produce urease. The urea agar slant consists of urea and pH indicator phenol red which causes changing the medium color to yellow in an acidic environment and fuchsia in an alkaline environment. If urease is produced, it will hydrolyze urea to ammonia and carbon dioxide which creates alkaline environment. 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 (Magee *et al.,* 1993).

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 (Magee *et al.,* 1993).

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. The sugar fermentation products results in acidic environment turns both the butt and slant yellow. If hydrogen sulfide is produced, it will react with the iron in the agar to form ferrous sulfide, were observed as a black precipitate in the butt (Magee *et al.,* 1993). 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 (Magee *et al.,* 1993).

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^{o}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 lo pH) and the negative are yellow. If the test is negative after 2 days the tests were repeated after 5 days (Duerden *et al.,* 1998).

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^{o}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 (Duerden *et al.,* 1998). Kovac's reagent consist of; Paradimethylaminobenzaldehyde 10g, Amyl or isoamyl alcohol 150ml, and Conc. Hydrochloric acid 50ml (Duerden *et al.,* 1998).

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. This test is usually done in conjugation with the methyl red test. An organism of the family Enterobacteriaceae is usually either methyl red positive and Voges-Proskauer negative e.g *E. coli* or methyl red negative and Voges-Proskauer positive *Salmonella. Salmonella* isolates were inoculated in glucose phosphate broth and incubate at 37^{o}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).

Hydrogen sulphide production

Some organisms produce hydrogen sulphide from sulphur containing amino acids. It may be detected by suspending strips of filter paper impregnated with lead acetate between cotton plug and the tube. It has variable sensitivity. The cultured in media containing lead acetate or ferric ammonium citrate or ferrous acetate turned them black or brown (Duerden *et al.,* 1998).

3.6.5 Microbact^{TM} 24E GNB Identification System

The Microbact 24E system is a new 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. The specified Microbact system used for this study is: Oxiod^{TM} Microbact^{TM} GNB 24E System kit, manufactured by Thermo-Fisher Scientific, Walham, Massachusetts, USA. 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.

3.6.5.2 Sample Preparation of Microbact^{TM} 24E GNB Identification System

25g or 25mL of the samples were homogenised with 225mL of buffered peptone water in a stomacher bag. Using of a filter-fitted stomacher bag is recommended. The samples were incubated at 37°C for 16-20 hours and 0.1 mL of the pre-culture broth was homogenized and inoculated in 10mL of RVS. Then the samples were incubated for 18-24 hours at 41.5°C. However, 1 to 2 mL of the enrichment broth was heated in a tube in a water bath at 100°C (boiling water) for 20 minutes, and then cooled to room temperature. And kept the rest of the enrichment broth at 3 ± 2°C for confirmation of a positive result prove necessary later.

Preparation of inoculum

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.

Inoculation

The wells of 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, i.e. wells 1, 2 and 3. Whereas, kovac's reagent to well 8, VP1 and VP2 to well 10 and TDA to well 12.

Incubation

The inoculated rows were resealed with the adhesive seal and wrote the specimen identification number on the end tag with a marker pen. Followed by Incubation at 35° ± 2°C for 18-24 hours.

Reading the test strip

The 12A (12E) strips were read at 18-24 hours. The 12B/24E strips were read at 24 hours to identify *Salmonella* specie. The strips from the incubator, were peeled back the sealing tape. All positive results were recorded. The reactions are evaluated as positive or negative by comparing them with the colour chart. Record the results under the appropriate heading on the report form.

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.

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.

3.6.7 Serological Determination of antibodies of *Salmonella enterica*

Serological tests are blood tests that look for antibodies in patient's blood. They can involve a number of laboratory techniques. Different types of serologic test are used to diagnose various disease conditions (Krista 2018). Serologic tests have one thing in common. They all focus on protein made by patient's immune system. The process for having the test is the same regardless of which technique the laboratory uses during serologic testing (Krista 2018). Thus, in this research slide agglutination tests will be performed from the blood samples obtained from chickens (Krista 2018).

2.6.7.1 Slide agglutination test

One drop of positive control was placed on one reaction circles of the slide; another drop of isotonic saline was Pipette on the next reaction cirlcle. (-ve Control). Then, one drop of the patient serum was Pipette onto the remaining four reaction circles, one drop of Widal test antigen suspension 'H' was added to the first two reaction circles. Finally, one drop each of 'O', 'H', 'AH' and 'BH' antigens were added to the remaining four reaction circles. More so, the contents of each circle were mixed uniformly over the entire circle with separate mixing sticks and the slides were rocked, gently back and forth and observed for agglutination macroscopically within one minute (Krista 2018).

2.6.7.1.1 Interpretation of Slide Agglutination Test

Agglutination is a positive test result and if the positive reaction is observed with 20 uL of test sample, it indicates presence of clinically significant levels of the corresponding antibody in the patient serum. No agglutination is a negative test result and indicates absence of clinically significant levels of the corresponding antibody in the patient serum.

3.7 Statistical findings

The findings of this research were analyzed using descriptive statistics such as

charts, plates, graphs, figures, percentages and tables.

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 were presented in tables (i-x) below.

Table I shows the overall isolation of *Salmonella enterica* isolated from faeces and blood of poultry in Yobe state. The overall number of samples examined during the study were, 600 with isolation rate of 80 (13.33%). 72 (24.00%) from cloacal swab and 8 (2.66%) from blood samples.

Table II shows monthly distribution of *Salmonella enterica* Collected from poultry in Yobe state, the samples were collected from July to October, the highest isolation rate was observed in July where 34 (22.67%) of 150 samples examined found to be positive, followed by October with 17 (11.33%), then September with 16 (10.67%). The lowest isolation rate month was observed in August with only 13 (11.33%) out 150 samples examined.

Table III shows the distribution of *Salmonella enterica* isolated from chickens (cloacal swab and blood) presented for slaughter based on sex. The result show that 22 (24.48%) *S. enterica* was isolated from female chickens, 50 (24.75%) were isolated from male chickens (cloacal swab).

Table IV shows the distribution of *Salmonella enterica* isolated from chickens presented for slaughter based on chicken's type. The result show that 64 (42.66%) were isolated from local chickens while 16 (10.66%) was isolated from exotic chickens.

Table V shows the distribution of *Salmonella enterica* isolated from chickens (Cloacal swab and blood) presented for slaughter based on chickens Sample sites. The samples were collected from 4 different local governments of Yobe state, the highest isolation rate was observed in Damaturu where 28 (38.89%) of 75 cloacal samples examined found to be positive, followed by Potiskum with 16 (21.33%), then Bade with 15 (20.00%). The lowest isolation rate was observed in Fune with only 13 (17.33%) out 75 cloacal samples examined.

Table VI shows the distribution of *Salmonella enterica* isolated from chickens (blood) presented for slaughter based on chickens Sample sites. The samples were collected from 4 different local governments of Yobe state, the highest isolation rate was observed in Damaturu where 6 (8.00%) of 75 cloacal samples examined found to be positive, followed by Potiskum and Bade with 1 (1.33%) each. The lowest isolation rate was observed in Fune with 0 (0.00%) out 75 blood samples examined.

Table VII shows the Biochemical reactions of presumptive *Salmonella enterica* from chickens presented for slaughter in Yobe state using conventional method. All the 80 presumptive positive isolates showed 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.

Table VIII shows Biochemical reactions of presumptive *Salmonella enterica* isolated from chickens Using Microbact 24E GNB Computer Identification system. Where, of the 40 selected presumptive isolates (36 from blood and 4 from cloacal swab) tested using Microbact 24E GNB Computerize system 10 (9 from cloacal swab and 1 from blood) samples were found to be positive for *Salmonella* organism.

Table IX shows other species, isolated from chickens Using Microbact 24E GNB Computer Identification system. Where *K.pneumonae* has high rate of isolation with 7 (23.33%) of the 30 (100%) other species, isolated, followed by *E. agglomerance* with 6 (20.00%), then followed by *S. rubideae* with 5 (16.67%), then *S. liquefaciens* , *E. cloacae* and *P. mirabilis* with 3 (10.00%) each. The lowest isolated rate was observed in *S. marcescens, C. braakii* and *M. morganii* with only 1 (3.33%) each.

Table X shows Slide agglutination test of *Salmonella enterica* from chickens in some selected areas of Yobe state**.** Where of the 300 blood samples tested for agglutination using slide method, 255 were found to be positive, showing a wide range with positives after blood culture 8, local chickens shows high prevalence with 131 (87.33%) positives and 19 (12.67%). Whereas, in exotic chickens shows 124 (82.67) positives and 24 (17.33%).

Table I: Isolation of *Salmonella* species isolated from faeces and blood of poultry in Yobe state on XLD Agar.

S/n	Sample	No. +ve isolated	No.-ve isolates	% of +ve
1	Blood	8	292	2.66%
2	Feces	72	228	24.00%
3	**Total**	**80**	**520**	**13.33%**

Table II: Monthly distribution of *Salmonella* species from poultry in Yobe state on XLD Agar.

Month	Frequency	% of +ve isolates
July	34	22.67%
August	13	8.67%
September	16	10.67%
October	17	11.33%
Total	**80**	**13.33%**

The chart showed monthly distribution of *Salmonella* isolated where, the highest isolation rate was observed in July with 34 (22.67%) of 150 samples examined found to be positive, followed by October with 17 (11.33%), then September with 16 (10.67%). The lowest isolation rate month was observed in August with only 13 (11.33%) out 150 samples examined.

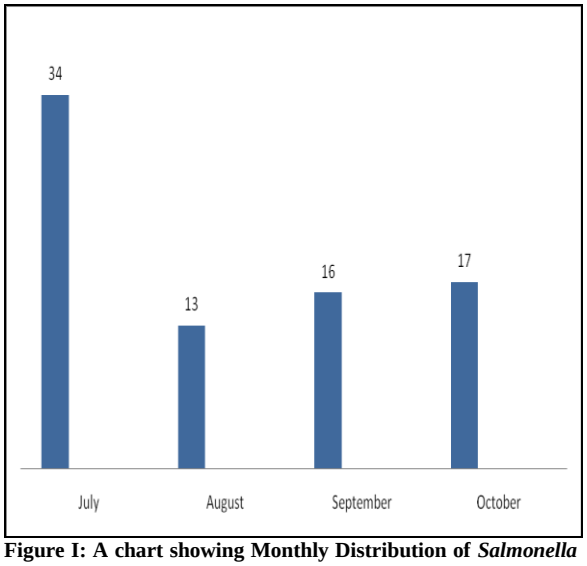

Figure I: A chart showing Monthly Distribution of *Salmonella* in poultry from some selected areas of Yobe state.

Table III: Distribution of *Salmonella* species isolated from chickens (cloacal swab) presented for slaughter based on sex.

Sex	No. sampled	No. +ve isolated	% of +ve Isolates
Male	202	50	24.75%
Female	98	22	24.48%
Total	**300**	**72**	**24.00%**

Table IV: Distribution of *Salmonella* isolated from chickens (blood) presented for slaughter based on sex.

Sex	No. sampled	No. +ve isolated	% of +ve isolates
Male	202	6	2.97%
Female	98	2	2.04%
Total	**300**	**8**	**2.66%**

Table V: Distribution of *Salmonella* isolated from chickens presented for slaughter based on chicken's breed.

Animal species	No. sampled	No. +ve isolated	% of +ve isolates
Exotic chicken	150	16	10.67%
Local Chicken	150	64	42.67%
Total	**300**	**80**	**26.67%**

Table VI: Distribution of *Salmonella* isolated from cloacal swab of Chickens presented for slaughter based on chickens Sample sites.

Sample sites	No. sampled	No. +ve isolated	% of +ve isolates
Damaturu	150	34	22.67%
Potiskum	150	17	11.33%
Bade	150	16	10.67%
Fune	150	13	8.67%
Total	**600**	**80**	**13.33%**

The chart shows that the highest isolation rate was observed in Damaturu where 34 (26.67%) of 150 cloacal and blood samples examined found to be positive, followed by Potiskum with 17 (11.33%) and Bade with 16 (10.67%). The lowest isolation rate was observed in Fune with 16 (8.67%) out of 150 samples examined.

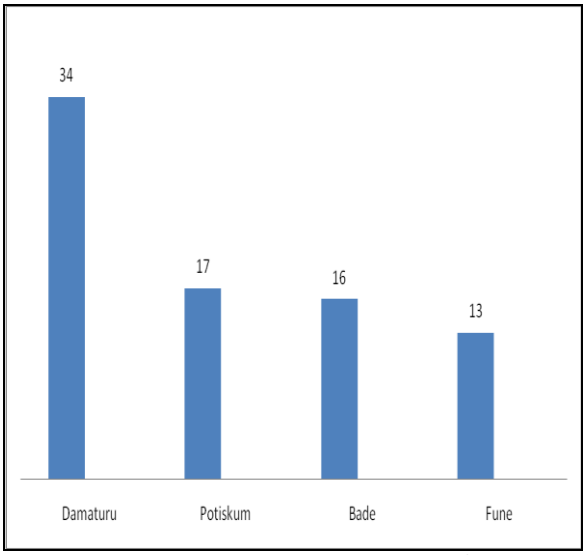

Figure II: A chart showing overall prevalence of *Salmonella* enterica in the study area

Table VII. Biochemical reactions of presumptive *Salmonella* species from chickens presented for slaughter

Biochemical tests	No. sampled	No. of +ve	No. of –ve	% of +ve
Catalase	80	80	0	100%
Oxidase	80	0	80	0.00%
Indole	80	0	80	0.00%
Urease	80	0	80	0.00%
Methyl-red	80	80	0	100%
Citrate utilization	80	80	0	100%
Triple sugar ion	80	80	0	100%
H_2S Production	80	80	0	100%
Voges Proskuer	80	0	80	0.00%
Motility	80	80	0	100%
	70	**10**		

Table VIII: *Salmonella enterica* isolated from chickens Using Microbact 24E GNB Computer Identification system.

Salmonella species	No. +ve isolates	No. -ve isolates	% of +ve isolates
S. typhi	3	7	30.00%
S. paratyphi A	2	8	20.00%
S. gallinarum	2	8	20.00%
S. pullorum	2	8	20.00%
Salmonella subs. 3B	1	9	10.00%
Total	**10**	**30**	**25.00%**

Table IX: Other specie isolated from chickens Using Microbact 24E GNB Computer Identification system.

S/No	Species	No. +ve isolates	% of +ve isolates
1	*S. liquefaciens*	3	10.00%
2	*S. rubideae*	5	16.67%
3	*S. marcescens*	1	3.33%
4	*E. agglomerance*	6	20.00%
5	*K. pneumonae*	7	23.33%
6	*C. braakii*	1	3.33%
7	*E. cloacae*	3	10.00%
8	*M.morganii*	1	3.33%
9	*P. mirabilis*	3	10.00%
10	**Total**	**30**	**75.00%**

Table X: Slide agglutination test of *Salmonella* from chickens in some selected areas of Yobe state.

Source	No. of Samples tests	No.-ve tests	No. +ve tests% of	%+ve
Exotic	150	26	124	82.67%
Local	150	19	131	87.33%
Total	**300**	**45**	**255**	**85.00%**

DISCUSSION

The present study was conducted to determine phenotypic characters, antimicrobial susceptibility patterns of *Salmonella* isolates from chickens presented for slaughter in some selected local governments of Yobe state. The *Salmonella* serovars that 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 possess a continuous threat to man (Muragkar *et al.*, 2005). The isolation rate of *Salmonella* from this study corroborated a similar study from Maiduguri, northeastern Nigeria, with a rate of 7% (Raufu *et al.*, 2013), and Ibadan, south western Nigeria, with a rate of 10% (Fashae *et al.*, 2010). A higher rate (37%) of *Salmonella* contamination on broiler farms had been reported from Algeria (Elgroud *et al.*, 2009), thus suggesting chickens and poultry environments as important reservoirs of *Salmonella* in Nigeria. This is the first comprehensive study on the occurrence and distribution 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 surviving and proliferating in diverse environments (Winfield and Groisman, 2003). The 13.33% prevalence of *Salmonella* obtained in this study is however lower than the 40% isolation rate by (Ndiaye *et al.*, 2011) in Dakar, Senegal. 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 (Kupriyanov *et al.,* 2010). The *Salmonella* isolates obtained from local (42.66%) layer chicken were high than the 10.66% isolates obtained in the work and other the current work could be attributed to management and husbandry practices as well as the climatic conditions of the study areas. (Mills *et al.,* 2010), reported that the emergence or resurgence of numerous infectious diseases is strongly influenced by environmental factors such as climate. Climate, weather, topology and hydrology influence the magnitude and frequency of pathogenic microorganisms transfer from environmental sources (World Health Organization, 2011). 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 distribution of *Salmonella* show that *Salmonella enterica* isolated from cloacal swab 24.00% is higher than 2.66% isolated from chickens blood. This is because *Salmonella* can persist in human and livestock excrement, than blood (Kupriyanov *et al.,* 2010). The difference in prevalence might partly be due to use of sero-screening in the present study compared to directly taking cloacal swab sample and processing in others. Moreover, *S. Pullorum* and *S. Gallinarum* are not excreted extensively in the faeces (Berchieri *et al., 1995*; Proux *et al.,* 2002). The seropositivity and isolation of *Salmonella* in this study serum samples and cloacal swab samples in local chickens indicates the existence of fowl typhoid and Pullorum disease in local breeds of different eco-type and exotic breed in the project farm.

The distribution of *Salmonella enterica* isolated from chickens (cloacal swab) presented for slaughter observed in Damaturu 28 (38.89%) is higher than that of Potiskum with 16 (21.33%), Bade with 15 (20.00%) and the lowest isolation rate was observed in Fune with only 13 (17.33%) out 75 cloacal samples examined. This may be attributed to fact that Damaturu being the state capital and centre where chickens are transported to from all the local governments or sample collection month. The differences in the results could also be due to the differences in the management practice that were used on the farm (Kupriyanov *et al.,* 2010). The feeds and water available for these birds could be a major source of *Salmonella* contamination. The farm attendant and the history of contact with other avian species may also serve major sources of contamination. These factors, collectively, appear to have contributed to differences in the results as the aforementioned factors could lead to the prevalence of the organism as reported by (Kupriyanov *et al.,* 2010).

Further investigations could be useful to clarify the sources of infections and factors leading to the wide spread isolation of *Salmonella* in poultry in the study area. Nevertheless, the high prevalence rate found in all the sampled matrices is in agreement with the primitive level of the infrastructure and bio-security measures observed on some of the farms involved in the study. Data collected demonstrated that there is an urgent need to improve personnel awareness about the importance of implementing good practices and sanitary measures in order to curtail *Salmonella* infections in Nigerian poultry. Aside from resource constraints, several measures are suggested to limit vertical and horizontal transmissions of *Salmonella* on farms and make the birds less vulnerable to *Salmonella*. Namely, to ensure *Salmonella*-

free feed and water, implementing effective cleaning and disinfection of the farms, applying appropriate measures against animate and inanimate vectors, and improving the sanitary status of animals should be implemented.

The Biochemical reactions for pathogenic identification and confirmation of presumptive *Salmonella enterica* (Jacobsen and Bech, 2012) 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, H_2S, Motility test, Methyl red and Tripple sugar ion tests. While negative for Gram stain, Oxidase, VP, Indole and Urease tests. For indole test, all the 80 positive isolates were negative when 2-3 drops of Kovac's reagent was added to the medium. Citrate utilization test showed that 80 positive isolates produces positive result and was evidenced by the formation of a bright blue colouration within the medium. The methyl red test was conducted for 80 positive isolates by the formation of a pink-red product within 5-15 minutes when 5-7 drops of methyl-red solution were added. Catalase test was prepared for all the 80 positive isolates and evidenced by production of a gas bubbles. Urease test showed that all the 80 positive isolates produces negative result by the red-pink colour change within the media. Triple sugar ion test was showed that 80 positive isolates produces Hydrogen sulfur at the butt of the test tubes and yellow colouration at the surface of the slant. SIM showed that all the 80 prositive isolates produces sulfur by blackening of the media, indole negative by absence of pinkish coloration, and motility positive. Oxidase was prepared for the all the 80 positive isolates and showed negative result by absence of deep blue colouration after addition of drop of oxidase reagent. Gram staining was conducted where all the 80 isolates showed gram negative bacilli.

Microbact^{TM} GNB 24E System kit proves to be accurate and convenient in the identification of microorganisms. The specified Microbact system used for this study is: Oxiod^{TM} Microbact^{TM} GNB 24E System kit, manufactured by Thermo-Fisher Scientific, Walham, Massachusetts, USA. This system assists in final identification of fresh isolates from cloacal swabs. The distribution of serotypes of *Salmonella* in the studied using Microcat 24E GNB Computerize system, comprised *S. typhi* 4.0%, *S. paratyphi* A 2.66%, *S. gallinarum* 2.66%, *S. pullorum* 2.66% and *Salmonella subs. 3A* 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 (Braden, 2006). 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 (Famurewa and Moro, 1989; Uzeh and Agbonlahor, 2001). Other species, isolated from chickens Using Microbact 24E GNB Computer Identification system show that *K.pneumonae* has high rate of isolation with 7 (23.33%) isolated, followed by *E. agglomerance* with 6 (20.00%), then followed by *S. rubideae* with 5 (16.67%), then *S. liquefaciens, E. cloacae* and *P. mirabilis* with 3 (10.00%) each. The lowest isolated rate was observed in *S. marcescens, C. braakii* and *M. morganii* with only 1 (3.33%) each hence, More research should be conducted in other other organism in the study area.

The blood samples were tested for slide agglutination for antibody-antigen reaction of H- flagella and O- somatic antigens, where of the 300 blood samples tested for slide agglutination, 255 (85%) were found to be positive, showing a wide range with positives after blood culture 120, local chickens shows high prevalence with 131 (87.33%) positives and 19 (12.67%). Whereas, in exotic chickens shows 124 (82.67) positives and 24 (17.33%). The rate of positives results from this study is higher than that of (Raufu *et al.,* 2013) with a rate

of 70%. The slide agglutination test in this research was done for diagnostic identification of antibodies present in the serum typically formed in response to an infection against *Salmonella* organism.

The result of this study may indicate that challenge of free ranging village poultry production and intensive poultry production may face in future the country from fowl typhoid and /or Pullorum disease unless due attention is given to the prevention and control of these diseases. Therefore, organized national regulatory survey programs should be established for both free ranging village chicken and intensively produced chickens to reduce the losses and to control the diseases, Farmers should be advised and educated on the use of *Salmonella* free parents.

CONCLUSION

In the present study, it was ascertained the phenotypic characters of *Salmonella* organism in blood and cloacal samples from Chickens in some selected areas of Yobe state Nigeria. The objectives of the study were greatly achieved. The results showed that *Salmonella* organism were prevalent in some selected Local governments of Yobe state. To my knowledge this is the first study that has involved used of Microbact in the detection of *Salmonella* from these regions and in chickens, most of the researches are restricted to human Salmonellosis. The overall percentage of isolates was 13.33%. Whereas, percentage of isolates from local and exotic chickens is 42.66% and 10.66% respectively, while 32.67% from males and 24.48% from female chickens.

REFERENCES

1. Abiodun, A., Lloyd, W., Lisa, M., Bowen, L., George, J., Alva, S.J. et al., (2014). 'Resistance to antimicrobial agents among Salmonella isolates recovered from layer farms and eggs in the Caribbean region', Journal of Food Protection 77(12), 2153-2160. https://doi.org/10.4315/0362-028X. JFP-14-162 4(4), 462-479. https://doi.org/10.9734/BMRJ/2014/5872
2. Ahmed, O.A., Raufu, I.A., Odetokun, I.A., Oladunni, F.S., Akorede, G.J., Ghali, I.M. (2016). 'Isolation and antimicrobial susceptibility profiles of Salmonella species from patients attending hospitals and diagnostic laboratories in Maiduguri, Northeastern Nigeria', Alexandria Journal of Veterinary Science 48(1), 7-11. https://doi.org/10.5455/ajvs.199165
3. Akinyemi, K.O., Philipp, W., Beyer, W. & Bohm, R., (2010). 'Application of phage typing and pulsed-f ield gel electrophoresis to analyse Salmonella enterica isolates from a suspected outbreak in Lagos, Nigeria', Journal of Infectious Developed Countries 4, 828-834. https://doi.org/10.3855/jidc.744
4. Akter, M.R., Choudhury, K.A., Rahman, M.M & Islam, M.S., (2007). 'Seroprevalence of salmonellosis in layer chickens with isolation, identification and antibiogram study of their causal agents', Bangladesh Journal of Veterinary Medicine 5, 32-42. https://doi.org/10.3329/bjvm.v5i1.1307
5. Baggesen, D.L., Sandvang, D., Aarestrup, F.M., (2000). Characterization of Salmonella enterica serovar typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J. Clin. Microbiol. 38, 1581–1586.
6. Barbour EK, Ayyash DB, Alturkistni W, Alyahiby A, Yaghmoor S, Iyer A, (2015). Impact of sporadic reporting of poultry Salmonella serovars from selected developing countries. J Infect Dev Ctries. 2015;9(1):1–7. Epub 2015/01/18. doi: 10.3855/jidc.5065 pmid:25596565.
7. Berchieri Jr. A and Freitas Neto O.C. (2009). Salmonella aviarias. Doeca das aves. 2. Ed. Compinas: Facta; p.435-451.
8. Braden, C.R., (2006). *Salmonella enterica serotype Enteritidis* and eggs: a national epidemic in the United States. Clin. Infect. Dis. 43, 512–517.
9. Callaway, T. R., T. S. Edrington, R. C. Anderson, J. A. Byrd and D. J. (2008). Nisbet. *Gastrointestinal microbial ecology and the safety of our food supply as related to Salmonella. Journal of Animal Science*. Volume 86 P. 163-172.
10. Centers for Disease Control and Prevention, *Salmonella*. (2011). Web. 16 Mar 2011.

Division of Foodborne, Waterborne, and Environmental Diseases.
11. Chopra, A.K., Huang, J.H., Xu, X.J., Bruden, K., Niesel, D.W., Rosenbaum, M.W.,Popov, V.L., Peterson, J.W., (1999). Role of Salmonella enterotoxin in overall virulence of the organism. Microb. Pathogen. 27, 155–171.
12. Clinical Laboratory Standard Institute (CLSI). (2014). A model for laboratory services. GP 26-A4. Lab medicine. 1(46) 26
13. Cummings, K.J., Warnick, L.D., Davis, M.A., Eckmann, K., Grohn, Y.T., Hoelzer, K.,MacDonald, K., Root, T.P., Siler, J.D., McGuire, S.M., Weidmann, M., Wright,E.M., Zansky, S.M., Besser, TE, (2012). Farm animal contact as risk factor fortransmission of bovine associated Salmonella subtypes. Emerging Infect. Dis.18 (12), 1929-1936.
14. Dawoud, T.M., Hererra, P., Hanning, I., Kwon, Y.M. & Ricke, S.C., (2011). 'In vitro invasion of laying hen ovarian follicles by Salmonella Enteritidis strains', Poultry Science 90, 1134-1137. https://doi.org/10.3382/ps.2010-01182
15. Duerden B.I., Towner K.J., Margee J.T. (1998). Isolation, description and identification of bacteria. Topley and Wilson's *Microbiology and Microbial Infections*, eds Balows A, Duerden BI vol 2, ninth edn, Arnold, London, Sydney, Auckland: 65-84.
16. Elgroud, R., Zerdoumi, F, Benazzouz, M, Bouzitouna, C., Granier, S.A., Fremy S. (2009) 'Characteristics of Salmonella contamination of broilers and slaughterhouses in the region of Constantine (Algeria)', Zoonoses and Public Health 56, 84–93. https://doi.org/10.1111/j.1863-2378.2008.01164.
17. Fagbamila I.O., Barco L., Mancin M., Kwaga J., Ngulukun S.S., Zavagin P. (2017). *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. PloS ONE 12(3):e0173097. doi:10.1371/journal.pone.0173097.
18. Famurewa, A., Moro, D.D., (1989). Incidence of *Salmonella* and other intestinal worms in food handlers in Ado-Ekiti, Ondo State, Nigeria. Acta Mediterranea Patol Infett E Tropic 8, 5-9.
19. Fashae, K., F. Ogunsola, F.M. Aarestrup and R.S. Hendriksen, (2010). Antimicrobial susceptibility and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. J. Infect. Dev. Countries, 4: 484-494.
20. Gordon, M.A., Feasey, N.A., Gordon, D., Robert, K.A., Heyderman, R.S., (2012). Invasive non typhoidal *Salmonella* disease: an emerging and neglected tropicaldisease in Africa. Lancet 379 (9835), 2489-2499.
21. Hannah, J.F., Wilson, J.L., Cox, N.A., Richardson, L.J., Cason, J.A., Bourassa, D.V. (2011). 'Horizontal transmission of Salmonella and Campylobacter among caged and cage-free laying hens', Avian Diseases 55, 580–587. https://doi.org/10.1637/9717-031511-Reg.1
22. Hitchner S.B. (2004). History of biological control of poultry diseases in the U.S.A. Avian Dis., 48, 1-8
23. Ivanics E., kaszanyitzky E., Glavits R., Szeredi L., Szakall S., Imre A., Kardos G.and Nagy B. (2008). Acute epidemic disease in laying hen flocks, caused by *Salmonella gallinarum*. Magyar Allatorvosok Lapja, 130, 611-617.
24. Jacobsen, C.S., Bech, T.B., (2012). *Salmonella* and transfer in freshwater and fresh produce? Food Res. Int. 45 (2), 557–566. Krista O. (2018). Antibody-antigen complex. Microbiology online.org. uk/about-microbiology/microbes-and-the-human-body/antibody-antigen-complex
25. Krista O. (2018). Antibody-antigen complex.Microbiologyonline.org.uk/about-microbiology/microbes- and-the-human-body/antibody-antigen-complex
26. Kwon, Y.K., Kim, A., Kang, M.S., Her, M., Jung, B.Y., Lee, K.M. (2010). 'Prevalence and characterization of Salmonella Gallinarum in the chicken in Korea during 2000 to 2008', Poultry Science 89, 236–242. https://doi.org/10.3382/ps.2009-00420
27. Lepage P, Bogaerts J, Van Goethem C, Hitimana DG, Nsengumuremyi F. Multiresistant (1990). *Salmonella Typhimurium* systemic infection in Rwanda: clinical features and treatment with cefotaxime, J Antimicrobe Chemother , vol. 26 Supply A(pg. 53-7).
28. Liljebjelke, K.A., Hofrace, C., Liu, T., White, D., Ayers, S., Young, S., Maurer, J., (2005). Vertical and horizontal

transmission of Salmonella within integrated broiler production system. J. Foodborne Pathogen Dis. 2, 90–102.
29. Magee J.T., Goodfellow M., and O'Donnell A.G (1993). Whole organism fingerprinting *Handbook of New Bacterial Systematics* Academic press, London: 383-427.
30. Mamman, P.H., Kazeem, H.M., Raji, M.A., Nok, A.J. & Kwaga, J.K., (2014). 'Isolation and characterization of Salmonella Gallinarum from outbreaks of fowl typhoid in Kaduna State, Nigeria', International Journal of Public Health and Epidemiology 3(10), 082=088.
31. Marrero-Ortiz, R., Han, J., Lynne, A.M., David, D.E., Stemper, M.E., Farmer, D., Bukhardt, W., Nayak, R., Foley, S.L., (2012). Genetic characterization of antimicrobial resistance in *Salmonella enterica* serovars isolated from dairy cattle in Wisconsin. Food Res. Int. 45, 962–967.
32. Maryam, M., Lawal, U.M., Abdul-Ganiyu, A., Aliyu, U.M., Samuel, A. & Lisa, B., (2009). 'Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents', Veterinary Microbiology 140, 131-135. https://doi.org/10.1016/j.vetmic.2009.07.009
33. Mills J.N., Gage K.L., Khan A.S. (2010). Potential influence of climate change on vectorborne and zoonotic diseases: A review and proposed research plan. Int J Health Geogr; 9:50.
34. Muragkar, H.V., Rahman, H., Ashok, K. & Bhattacharyya, D., (2005), 'Isolation, phage typing and antibiogram of Salmonella from man and animals in northeastern India', Indian Journal of Medicine Research 122, 237–242. PMID:16251781.
35. Ndiaye, M.L., Dieng, Y., Niang, S., Pfeifer, H.R., Tonolla, M., Peduzzi, R., (2011). Effect of irrigation water on the incidence of *Salmonella spp.* on lettuce produced bu urban agriculture and sold in markets in Dakar Senegal. Afr. J. Microbiol. Res. 5 (19), 2885–2890.
36. Parvej M.S., Nazir K.H., Rahman M.B., Jahan M., Khan M.F. and Rahman M. (2016). Prevalence and characterization of multi-drug resistant *Salmonella Enterica serovar Gallinarum biovar Pullorum* and *Gallinarum* from chicken. Vet. World, 9, 65–70.
37. Proux K., Jouy E., Haudayer C., Protais J., Dibb-Fuller M., Boscher E., Gillard A., Gracieux P., Gilbert E.(2002). Relaible ELISAs showing differences between resistant ans susceptible lines in hen orally inoculated with Salmonella Enteritidis. Vet. Res; 33:23-33.
38. Raufu I, Bortolaia V, Svendsen CA, Ameh JA, Ambali AG, Aarestrup FM, (2013). The first attempt of an active integrated laboratory-based *Salmonella* surveillance programme in the north-eastern region of Nigeria. J App Microbiol. 2013; 115, 1059–1067. [PubMed] [Google Scholar]
39. Salehi, T.Z., Mahzounieh, M., Saeedzadeh, A., (2005). Detection of invA gene inisolated Salmonella from broilers by PCR method? Int. J. Poult. Sci. 4 (8), 557–559.
40. Thomson N.T., Clayton D.J., Windhorst D., vernikos G., Davidson S., Churcher C., Quail M.A., Stevens M., Jones M.A., Watson M., Barron a., Layton A., Pickard D., Kingsley R.A., Bignell A., Clark L., Harris B.,Ormond D., Abdellah Z., Brooks K., Cherevach I., Chillingworth T., Woodward J., Norberczak H., Lord A.,Arrowsmith C., Jagels k., Moule S., Mungall K., Sanders M., Whitehead S., Chabalgoity J.A., Maskell D.,Humphrey T., Roberts M., Barrow P.A., Dougan G. & Parkhill J. (2008). Comparative genome analysis of *Salmonella enteritidis* pt4 and *Salmonella gallinarum* provides insights into evolutionary and host adaptation pathways. Genome Res., 18, 1624-1637.
41. Thrush field (1997). Organizational Research: Determining Appropriate Sample Size in Survey Research. Retrieved January 15, 2018.
42. Vazquez, N. J., Cordoba, B.C., Lopez N.Y and Mancera, M.A. (2005): identification del gene da la integrasa tipo 1 y perfil da Resistencia antimicrobiana em *Salmonella entertidis.* Cuajimalpa (cited 2005 Nov 24). Available from: www. Vet-uy.com/articulos/artic_nicro/001/micro001.htm.
43. Wegener, H.C., Bager, F. and Aarestrup, F.M. (1997): Vigilancia da Resistencia aos antimicrobianos no homem, nos produtos alimentares e no gado na Dinamarca Euro surveillance 3(2):17-19.
44. Wei, S.L., Wee, W., Siong, F.Y.J., Syamsumir, F.D., 2011. Characterization of

antimicrobial, antioxidant, anticancer properties and chemical composition of Malaysian Andrographis paniculata leaf extract. Pharmacologyonline 2,996

45. Winfield, M.D., Groisman, E.A., (2003). Role of non host environments in the lifestyles of *Salmonella* and *Escherichia coli.* Appl. Environ. Microb. 69, 3687-3694.
46. World Health Organization (WHO) (2011). Food Safety Report. Available at: http://www.who.int/foodsafety/en/, accessed January 25, 2014
47. "Yobe state" Online Nigeria, (2007). Conback Global Income Distribution Database. Retrieved 2008-08-20.

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