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ISOLATION AND CHARACTERIZATION OF STRAINS OF SALMONELLA SP PREVALENT IN POULTRY FARM IN FEDERAL UNIVERSITY DUTSE JIGAWA STATE NIGERIA.

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Abstract

This research work was conducted for the isolation and characterization of strains of Salmonella sp. prevalent in poultry farm in (FUD). A total 60 samples consisting of 20 –cloacal swabs, 20-droppings and 20–water were collected from poultry section in (FUD) farm and brought to Microbiology laboratory. Samples were propagated in nutrient broth followed by culture on Salmonella-Shigella Agar and sub-cultured on Eosin Methylene Blue Agar and Xylose lysine Deoxycholate agar. Biochemical properties of the isolates were studied and reaction in TSI agar slant was observed. Gram's staining techniques were performed. The overall prevalence of Salmonella in the poultry farm was recorded 33.33% respectively. In cloacal sample the prevalence of Salmonella was 20% whereas in droppings is 45% and water sample it was 35%. The results suggest that poultry chickens can secrete Salmonella through their droppings, which can inter into their drinking water and transmited to the other chickens. The presence of Salmonella at the recorded rate in both the dropping and water pose significant alarming for the public health issue if not maintain proper hygienic steps in place.

KEYWORDS:
Poultry, Droppings, Water, Culture.

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INTRODUCTION

Salmonella is one of the most common causes of food-borne infectious disease in the world; the characteristics feature of these organisms is wide host range, which comprises most animal species including mammals, birds and cold-blooded animals in addition to humans (Orji *et al.*, 2005). The genus *Salmonella* contains two species, *Salmonella enterica*, which consists of six subspecies, and *Salmonella bongori.* The infectious dose, incubation period, symptoms and mode of transmissions of Salmonellosis caused by different serotypes is similar. Symptoms include diarrhea, fever and abdominal cramps with incubation periods ranging from 12 to 72 hours. The illness usually lasts from 4 to 7 days and most people recover without treatment. The elderly, infants and those with impaired immune systems are more likely to have a severe illness (Hans and Dean, 2006).

Salmonella spp are typically found in soil, water, food, and the gastro-intestinal tract of humans and other animals (Anderson and Ziprin, 2001). Most *Salmonella* are motile, with the exception of the poultry-specific serotypes of *S. gallinarium* and *S. pullorum* (Grimont *et al*., 2000). The organism is a facultative anaerobe that grows on food in the presence or absence of oxygen.

Despite the nutritional benefits of poultry derived products, the consumption of poultry derived product have become primarily source of Salmonellosis among the populace, Salmonellosis constitute the threat to the public health claiming the lives of millions, is caused by the variety species of the *Salmonella*. Many factors may be responsible for its widespread among the poultry. The research will be conducted to ascertain the primary source of it as well as highlight some precautionary measures that will break it transmission among the poultry, to save the life of the populace.

Poultry and the poultry derived products are nutritionally indispensable proteineous diet. Is important diet consumed by average populace on the daily basis. Unfortunately, it harbors pathogenic bacteria notably *Salmonella*. However, no available a research attempted to identify the

source of *Salmonella* from the poultry farm this create a vaccum for a research to identify the source of the Salmonellosis among the poultry.

Materials and Method

Study area

The study was conducted at the University Agricultural Farm, Federal University Dutse (FUD). The farm is located a few hundred meters behind the Faculty of Agriculture. It was established for practical teaching and Research purposes and for commercial agricultural production. The farm is divided into Livestock, Poultry and Fishponds sections.

Poultry section

The poultry section is sub-divided into two segments comprising of; Broilers and Layers.

Sample size

A total of 60 samples were collected from the Federal University Dutse poultry farm. These include 20 cloacal swabs, 20 droppings and 20 water samples.

Sample collection

Sterile swabs were used to collect cloacal swabs while sterile containers were used to collect poultry droppings and water samples. The samples were collected from the layer chickens and transported to the Microbiology Laboratory Federal University Dutse for bacteriological studies.

Preparation of culture media

Nutrient broth, peptone water broth, Salmonella-Shigella (SS) Agar, Eosin Methylene Blue (EMB) Agar, XLD Agar medium, Triple Sugar Iron (TSI) Agar slant were prepared according to the manufactured instruction.

Sample processing

Pre-enrichment

One (1) g of poultry droppings was added into 9ml peptone water for 30mins.One (1) ml of the water sample was added into 9ml nutrient broth

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and incubated 37°C overnight. The cloacal swabs were soaked in nutrient broth and incubated overnight at 37°C. 1ml of the incubated droppings was inoculated in to 9ml nutrient broth and incubated overnight 37°C as described by Shafiullah (2013).

Selective enrichment

Using a sterile wire loop, a loopful of the enrichment culture were inoculated on SSA by streaking.

After 24hours incubation, the inoculated plates were removed from the incubator and presumptively identified. The colonies obtained were sub cultured on Eosin Methylene Blue (EMB) and Xylose-lysine Deoxycholate agar.

Microscopic Identification of Isolates.

Pure isolated colony on Salmonella shigella agar (SSA), Eosin Methylene Blue Agar (EMB) and Xylose-lysine Deoxycholate agar (XLD) was stained using the Gram staining method as described below:

A thin smear was made on a clean free-grease glass slide. Dried, heat-fixed and placed on a staining rack and covered with crystal violet for 1 minute and this was rinsed with distilled water, then Lugol's iodine was added for another 1 minute. The smear was then rinsed with distilled water. This was followed by decolarization using acetone. The smear was then rinsed with distilled water, this is followed by decolarization using acetone and rinsed with distilled water, and the smear was flooded with safranin for 1 minute and then rinsed with distilled water. Back of the slide was cleaned with a cotton wool and then placed on a draining rack. The Gram staining smear was examined microscopically using x100 oil immersion objective lens as described by Cheesbrough, 2000.

Biochemical Identification of the Isolates.

The following biochemical tests were carried out to further confirm the identify *Salmonella* isolated. This includes; IMVIC (Indole, Methyl-red, Voges-

Poskauer and Citrate utilization test) and TSI according to (Cheesbrough, 2002).

Indole test

Principle: Indole production test was used to determine the ability of microorganisms to degrade the amino acid tryptophan and produce indole.

Procedure: The isolated *Salmonella* was inoculated in to 5ml peptone water and incubated for 24hrs. After incubation, the reagent (Kovac's reagent) was added and shakes gently.

Result: A red color in the reagent layer above the broth within 1minute indicates a positive test while absence of the red layer indicates a negative test. (Cheesebrough, 2002).

Methyl-red test

Principle: Methyl-red test is the biochemical test that are perform on bacterial species to detect organism that have the ability to produce suitable acid end products (mixed acid fermentation) from supplied glucose. It is used as the part of the IMVIC test. Some bacteria have the ability to perform mixed acid fermentation of glucose in MR-VP medium. The products of mixed acid fermentation are the complex mixture of acid particularly lactate, acetate, succinate and formate as well as ethanol and equal amount of H_2 and CO_2.This causes the media to acquire an acidic pH.Methyl-red is a pH indicator, which remains red in color at pH of 4.4 or less.

Procedure: The isolate of *Salmonella* was inoculated in a MR-VP broth and incubated at 37^{o}C for 48hrs. After the incubation, the broth was divided into two equal parts. One for methyl red test and the other for Voges-Poskauer test as described by (Cheesbrough, 2002). For Methyl-red test, a drop of methyl-red reagents was added into the test tubes.

Result: Within a minutes the color was change from yellow to red respectively which indicated a positive result.

Voges-Poskauer test

Principle: is of the test used for identification of the members of *enterobacteriaceae*. It is usually performed alongside the methyl-red test

since both tests are performed on culture grown in MR-VP broth. Bacteria can metabolize glucose to key intermediate, pyruvic acid which can further metabolized to produce acetoin (i.eacetylmetylcarbinol or 3-hydroxybutanone) as an intermediate and can be further reduce to 2,3-butanetiol.

Procedure: The isolated *Salmonella* was inoculated in a MR-VP broth and incubated at 37°C for 48hrs. After the incubation, one drop of Barrett's reagent was added and the result was observed within a minute.

Result: Red color formation within 15-20 min indicated positive result.

Simmons citrate test

Principle: This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Procedure: The isolated *Salmonella* is inoculated in to bijou bottle containing slant Simmons citrate agar, stabbed and butt. It was incubated at 37°C for 48hrs.

Result: Bright blue color in the medium indicated positive result, no change in color indicated negative result.

Triple sugar iron test

Principle: TSI agar is use to determine whether a gram-negative rod utilizes glucose and lactose or sucrose fermentative and forms hydrogen sulphide (H_2S). TSI contains 10 parts lactose: 10 parts sucrose: 1part glucose and peptone. Phenol red and ferrous sulphate serves as indicators of acidification and H_2S formation, respectively. The formation of CO_2 and H_2 is indicated by the presence of bubbles or cracks in the agar or by separation of the agar from the sides or bottom of the tube. The production of H_2S requires an acidic environment is indicated by blackening of the butt of the medium in the tube.

Procedure: The isolated *Salmonella* was first inoculated in to TSI slant by stabbing through center of the medium to the bottom of the tube and then streaked the surface of the agar slant. It was incubated at 37°c for 24hrs.

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Result: Presence of yellow color on the butt, red on slant and H_2S production indicate positive.

Result

A total number of 60 samples from the poultry farm (cloacal swab, droppings and water) were examined out of which 20 (33.33%) were found to be positive for *Salmonella*.

Prevalence of positive for *Salmonella* from various poultry sample, the cultural characteristics, gram reaction and biochemical identification of isolates obtained from the site in the study are determined and shown in Table 1.0, Table 2.0 and 3.0 respectively.

Table 1.0: Cultural prevalence of *Salmonella* from various sample collected.

Type of sample	Number of sample Analyzed	Number Positive	Percentage (%) Prevalence
Cloacal swab	20	4	20
Droppings	20	9	45
Water	20	7	35
Total	60	20	33.3

Table 2. Cultural characteristics and Gram's reaction of the Isolates.

Media Used	Colony Characteristics	Gram's Reaction
NB	Turbidity of the broth	
SSA	Translucent, black, smooth, small round colonies	Gram negative, pink colored short rod shaped bacteria, arranged in single and paired
EMB	Pink colored colonies	
XLD	Pink colored colonies with black center	

KEY;
NB= Nutrient broth
SSA= Salmonella shigella agar

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EMB= Eosine methylene blue agar
XLD= Xylos-lysine Deoxycholate agar

Table 3. Biochemical Profile of the Isolates.

Biochemical Tests		Results
MR		+
VP		-
CIT		+
IND		-
TSI	Butt	Y
	Slant	R
	H_2S	+

KEY;
MR = Methyl-red
VP = Voges-proskeaur
CIT = Citrate utilization
IND = Indole
TSI = Triple sugar iron

DISCUSSION

Poultry and poultry derived product can harbor a diverse group of microorganisms especially enteric organisms such as *Salmonella* and *E. coli* which may be due to contaminated drinking water and feed which can serves as their food . Out of 60 samples analyzed 20 (33.3%) were found positive for *Salmonella* from both the samples analyzed (Both Poultry droppings, Cloacal swab and water), which was higher than with those recorded by Ahmed *et al.* (2014). This high result can be attributed to the sample size and geographical area of which the poultry farm is located.

In this study, specific enriched media were used for the isolation and characterization of strain of *Salmonella,* which was also used by a number of researchers such as Hyeon *et al.* (2012), Muktaruzzaman *et al.* (2010), Habrun, and Mitak. (2003). The colony characteristics of *Salmonella* found in

this study was translucent, black, smooth, small round colonies on SS agar, Pink colored colony on EMB agar and pink colored colony with black centre on XLD agar, were similar to the findings of other authors (Muktaruzzaman *et al.* (2010) and Sujatha *et al*. (2003).

This study showed that the percentage distribution of *Salmonella* from cloacal swabs were relatively lower compared to that of water and poultry droppings, this result is in agreement with result obtained by Kotton *et al*., (2006). This confirmed that cloacal swabs are oftenly less colonized in individual birds because low number of organisms excreted by infected birds in many cases.

Also in the present study, the result obtained from water sample for *Salmonella* is 7 (35%) which is in agreement with result obtained by Nayak *et al.* (2003). *Salmonella* were isolated from poultry's drinking water, which shows that either the poultry chickens may secrete their droppings in to their drinking water or when the poultry dropping air-dried, air may take up the droppings in to the water, because *Salmonella* can survive for a long period of time in a dry dropping.

In addition, the high prevalence of *Salmonella* obtained from poultry droppings in this study was higher to that obtained by Ahmed *et al.,* (2014). Out of 18 samples analyzed 2(11.1%) were found to be positive for *Salmonella* in Khartoum North Locality, Sudan Poultry Farms.

Salmonellae might originate either from faeces and secretions of sick birds in the same flock or from water already contaminated by pathogenic organisms. Outbreak originated from poultry or poultry derived products continue to be a major worldwide problem. Substantial economic losses manifested through mortality and poor growth of infected animals as well as the risk of transmission to humans either through food borne chain or through a zoonotic means.

CONCLUSION

Based on the finding from this study, it was concluded that poultry droppings have the highest occurrence of *Salmonella* than the water samples and cloacal swabs. The results also suggest that poultry chickens

can secrete *Salmonella* through their droppings, which can inter into their drinking water and transmit it to the other poultry chickens.

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