Isolation And Antimicrobial Susceptibility Studies Of Salmonellaspecies, From Chickens In Gwagwalada And Kwali Area Councils, Abuja, Federal Capital Territory, Nigeria

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Abstract: Isolation and antimicrobial susceptibility testing of Salmonella Species from live and dead chickens in Gwagwalada and Kwali area councils of Abuja, was studied to establish the prevalence and possible treatment regimen for Salmonella in the study area. Five hundred (500) samples of both faecal (180) and visceral organs (320) were collected from chickens in poultry farms and slaughter houses between May and August 2015. Salmonellae were isolated, identified and characterized using standard methods. Isolates were further subjected to antimicrobial susceptibility testing using disc diffusion method. The occurrence of Salmonellae species isolates revealed 8% (40) and these isolates were most susceptible to Ciprofloxacin and Gentamicin. Serotyping of isolates for effective control of outbreaks using vaccines is thus suggested, while farmers and poultry attendants should ensure strict hygienic practice.

Keywords: Salmonella, antimicrobial susceptibility testing, poultry farms and Slaughter houses.

I. Introduction

Avian Salmonella infections are important causes of clinical disease in poultry and a potential source of food borne transmission of Salmonella in humans (Shivaprasad, 2000). Salmonella organisms are classified under the family Enterobacteriaceae, Genus Salmonella which is a gram-negative, non-spore forming, aerobic or facultative anaerobic rods that are most motile with exception of S. gallinarum and S. pullorum which are non-motile, (Harris et al., 1997; Rao, 2000; Nwachukwu and Nwiyi, 2011). There are over 2,400 serologically different variants/serotypes of Salmonellae which inhabits the gastrointestinal tract of humans and animals (Faruk et al., 2005). These organisms are transmitted mainly through ingestion of feed or water contaminated by faeces of clinically infected birds or other animals and human carriers (Shivaprasad, 2000; Abdu, 2007). Avian Salmonella infections have been eradicated from commercial poultry in many developed countries of Western Europe, USA, Canada, Australia and Japan where intensive poultry industry operate (OIE, 2005). In Africa, fowl typhoid and pullorum disease caused by Salmonellagallinarum and Salmonellapullorum respectively, have been reported in many countries including Nigeria (Okoli et al., 2006; Ajayi and Egbebi, 2011). The disease has been reported in chickens with classical signs of septicaemia, diarrhoea, enteritis and is characterized by drop in egg production and increase Mortality (Jensen et al., 2003). A tentative diagnosis of Salmonellosis is based on flock history, clinical signs, mortality and lesions. However, a definite diagnosis requires the isolation and identification of Salmonella. In addition, various serological tests such as serum plate agglutination test, rapid agglutination test, tube and micro titre agglutination test and Polymerase Chain Reaction can be used to detect Salmonella infections (Shivaprasad, 2000). However, reports of increasing outbreaks associated with avian salmonellosis have recorded despite the use of vaccines (Barrow and Freitas, 2011). Therefore, this study employed the use of conventional methods for identification and characterization of Salmonellae isolates in order to establish the occurrence and burden of the disease as well as antimicrobial susceptibility testing of Avian Salmonella in poultry farms and slaughter houses in the study area.

II. Materials And Methods

Study area

Gwagwalada and Kwali are amongst the six area Councils in the Federal Capital Territory Abuja, Nigeria. Gwagwalada is located between latitude 8°45’N of the equator and longitude 6°45’ and east of the Greenwich meridian, with land mass of 1043Km² and ten wardshaving an annual rainfall of approximately 368mm, temperature of 25°C – 35°C yearly and a population of 157, 770 at 2006 census. Abuja is located in the North Central region of Nigeria and shares boundaries with Kogi State to the South East and South West, Niger State to the North West and Nasarawa States to the North (Anon, 2007).

Sample collection

Five hundred samples comprising of 180 faecal samples from 45 commercial layer flocks (n= 4 for each farm) and 320 visceral samples (intestine, liver and spleen) (n= 20) were collected from two poultry

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slaughter outlets in Gwagwalada and Kwali area council. This sample collection was conducted between May and August 2015 based on convenience random sampling method. Ten gram each of liver, spleen and intestine were aseptically collected from slaughtered chickens and placed in universal bottles containing 5mls of nutrient broth (Laboratorios Britania, Buenos Aires, Argentina), while deep cloacal and caecal tonsil swabs were also collected and placed in 5mls of nutrient broth. All samples were immediately transported on ice to the Veterinary Microbiology Laboratory of Faculty of Veterinary Medicine, University of Abuja and for processing and storage until use.

**Isolation and Identification of Salmonella species**

Isolation of *Salmonella* was conducted in accordance with standard methods as described by Mdegela et al. (2000) and Murugkar et al. (2005). Liver, spleen and intestine (10g) samples of the same chicken were homogenized by stomacher; both the homogenate and swabs were aseptically inoculated into 10ml of selenite-F broth for selective enrichment and incubated at 37°C for 24hrs. A loopful from each of the enriched broths were streaked onto plates of MacConkey(Oxoid Ltd, UK, without salt) and blood agar were incubated at 37°C for 24hrs. Selective plating was performed using *Salmonella-Shigella* Agar (SSA) and Deoxycholate Agar (DCA). All the plates were examined for presence of typical colonies with black centres on *Salmonella* Shigella Agar (SSA) and red colonies with black centres on Deoxycholate agar (DCA). Suspected colonies were confirmed positive using conventional biochemical methods (indole (I), methyl red (MR), Voges-proskauer (Vi), citrate (C)) triple sugar iron (TSI) and urease test) and the results obtained were recorded and interpreted as stated by Froux et al. (2002); Parmar and Davies, (2007).

**Antibiotic sensitivity testing**

An *in-vitro* antibiotics sensitivity test was conducted on positive *Salmonella* isolates using disc diffusion method as described by James (2009); Bauer et al. (1966) with a panel of eight (8) therapeutic antibiotics impregnated disc namely: Chloramphenicol, CH (30 µg), Gentamicin, GN (10 µg), Norfloxacin, NO (10 µg), Ciprofloxacin, CP (10 µg), Tetracycline TET (30 µg), Amoxicillin clavulanate, AU (30 µg), Ampicillin, AM (30 µg),NALIDIXIC acid, NA and Nitrofurantoin, NF (30 µg). Briefly, a MacFarland 0.5 standardized suspension of the bacteria in 0.8% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar (Oxoid) with a sterile swab loop. A ring of disks (Mast Diagnostics, UK) each containing single concentrations of antimicrobial agent was placed onto the inoculated lawn and incubated at 37°C for 24h. Clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler. The diameter of the zones was read using an interpreting chart for zone sizes in accordance with standard methods described by National Committee for Laboratory Standards (2004).

**Statistical analysis**

The numbers of positive *Salmonella* isolates were expressed using simple descriptive statistics such as percentages and frequencies.

### III. Results

Out of the 500 samples analysed 8% (40) were positive for *Salmonella*. Out of the forty (40) isolates, six (6) isolates were from 180 faecal samples, representing a percentage distribution of 1.2%, while 34 isolates were from 320 visceral samples with a percentage distribution of 6.8% as shown in Table I below. The biochemical characteristics of isolates to various chemicals and sugars are as shown in Table II.

The result of antimicrobial susceptibility testing showed isolates were only sensitive to Ciprofloxacin and Gentamicin, but were resistant to Neomycin, Ampicillin, Chloramphenicol, Tetracycline, Amoxicillin and Orfloxacin.

**Table 1: Distribution of Salmonellaspecies obtained from chicken fecal and visceral samples in Kwali and Gwagwalada Area Councils, Abuja-FCT**

<table>
<thead>
<tr>
<th>Type of samples Collected</th>
<th>No. of positive Samples (%)</th>
<th>No. of negative Samples (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeaces</td>
<td>6 (1.2)</td>
<td>174 (34.8)</td>
<td>180 (36)</td>
</tr>
<tr>
<td>Visceral</td>
<td>34 (6.8)</td>
<td>286 (57.2)</td>
<td>320 (64)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (8)</td>
<td>460 (92)</td>
<td>500 (100)</td>
</tr>
</tbody>
</table>

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Table II: Cultural and Biochemical Characteristics of *Salmonella* isolates obtained from visceral organs in the study area

<table>
<thead>
<tr>
<th>Sample</th>
<th>No of tested sample</th>
<th>No of positive</th>
<th>No of negative</th>
<th>Butt Color</th>
<th>TSI Slaat</th>
<th>H_{2}S prod.</th>
<th>TSI GLU</th>
<th>TSI Lact</th>
<th>Indole</th>
<th>catalase</th>
<th>Oxidase</th>
<th>MR</th>
<th>VP</th>
<th>Sucrose</th>
<th>Maltol</th>
<th>Detection(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC_{1}</td>
<td>20</td>
<td>4</td>
<td>16</td>
<td>(yellow)</td>
<td>+</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>LML_{2}</td>
<td>20</td>
<td>5</td>
<td>15</td>
<td>(yellow)</td>
<td>+</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>LMCT_{3}</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>(yellow)</td>
<td>+</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>LMS_{4}</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>(yellow)</td>
<td>+</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

**KEY:** PF_{A}– PF_{D} = Faecal Samples, MR = Methyl Red, VP = Vogo’s Proskuer, Cit = Citrate, Glu = Glucose, Lact = Lactose, LM = Local Market, C_{1} = Caecal, L_{2} = Liver, CT_{3} = Caecal and S_{4} = Spleen, A = Fermented, + Positive, = Negative, Yellow butt = Acidic (colour changes to yellow due to acid formation), red = Alkaline (colour changes to red due to Alkalization) H_{2}S = + (blackening due to H_{2}S)

Table III: Cultural and Biochemical Characteristics of *Salmonella* from chicken faecal samples in Gwagwalada and Kwali Area Council.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No of tested sample</th>
<th>No of positive</th>
<th>No of negative</th>
<th>Butt Color</th>
<th>TSI Slaat</th>
<th>H_{2}S prod.</th>
<th>TSI GLU</th>
<th>TSI Lact</th>
<th>Indole</th>
<th>catalase</th>
<th>Oxidase</th>
<th>MR</th>
<th>VP</th>
<th>Sucrose</th>
<th>Maltol</th>
<th>Detection(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF_{A}</td>
<td>45</td>
<td>2</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>PF_{B}</td>
<td>45</td>
<td>1</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>PF_{C}</td>
<td>45</td>
<td>2</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>PF_{D}</td>
<td>45</td>
<td>1</td>
<td>44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>+</td>
<td>-</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

**KEY:** PF_{A}– PF_{D} = Faecal Samples, MR = Methyl Red, VP = Vogo’s Proskuer, Cit = Citrate, Mortility, Glu = Glucose, *Sal.* Spp. = *Salmonella* species

Table IV: *In vitro* antibiotics sensitivity testing of *Salmonella spp*. Isolates from chicken faecal and visceral organs. No of isolates 40

<table>
<thead>
<tr>
<th>Drug</th>
<th>% sensitivity to <em>Salmonella</em> spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>95.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>87.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>5.0</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>7.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7.5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>5.0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>0.0</td>
</tr>
</tbody>
</table>

IV. Discussion

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The overall 8% prevalence of Salmonella in this study is relatively similar to 11% reported by Fashae et al., (2010). However, Mike et al. (2004) and Raufu et al. (2010) reported 12.5% and 15% respectively, which was a slightly higher than that obtained in this present study. The variation in the isolation rate might be due to variation in climatic conditions, difference in the management system or better still due to difference in the number of samples in each study, since large sample size can influence the chances of obtaining more isolates on culture as previously stated (Mollenhorst et al., 2005; Raufu et al., 2010). In addition, the difference in study location may play a role in the proliferation and occurrence of the organism, as Salmonella species are known to thrive differently in various environmental conditions and seasons (Okoli et al., 2006).

In this study, the percentage occurrence of Salmonella isolates obtained from chicken fecal samples is lower than that obtained from chicken visceral samples, this finding is in agreement with the report of OIE, (2010) and this is because chickens can become chronic carriers of Salmonella organism and thus excretes the organism in their faces intermittently (Raufu, et al., 2010). It is important to note that the visceral samples in this study were obtained from chicken slaughter outlets in the market, therefore the high isolation rates in the visceral organs could be due to environmental contamination. Also, sampling was in various markets with chickens brought in from different locations within the study area that were raised under different management systems such as free range or backyard system with little or no biosecurity practice. Hence, a potential probability of increase occurrence of the organism, as unhygienic and poor sanitary practices predisposes to infection (Mike et al., 2004; OIE, 2010). All the isolates in this study post biochemical characterization showed typical reactions of Salmonella which is indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, triple ion sugar positive and oxidase negative as previously described by OIE, (2010).

Antimicrobial susceptibility testing in this study showed sensitivity of isolates to Ciprofloxacain and Gentamicin with an associated high rate of multiple antibiotic resistances. This is in agreement with previous works of Fashae et al. (2010), Ayajii and Egbebi. (2011); Ifeanyi et al. (2013), which reported high rates of Salmonella species multiple antibiotic resistances to commonly used antimicrobial agents. The resistance may be associated with indiscriminate use of antibiotics by clinicians, poultry farmers and local birds in Ado Ekiti, Ekiti State, Nigeria. Annals of Biological Research, 2 (3): 431 – 437.

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References

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