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Short Communication

Prevalence and antimicrobial resistant patterns of Salmonella organisms isolated from commercial birds reared in Umuahia, South-eastern Nigeria

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ABSTRACT

The poultry industry plays an important role in the economy of every nation with poultry products contributing immensely to the protein needs of man. Infectious diseases pose a major challenge to the success of the poultry industry globally, especially in Africa. Salmonellosis is a major disease encountered in livestock production and has continued to be a major source of concern especially with the increasing incidence of antimicrobial resistance. This study was conducted to determine the occurrence and antimicrobial resistant patterns of Salmonella isolates from poultry reared in Umuahia. Cloacal swabs collected from 100 birds were screened for Salmonellae. Antibiogram was carried out using the disk diffusion method. A prevalence rate of 10% was recorded. All isolates were sensitive to Ofloxacin. Resistance to Augmentin, Amoxicillin and Co-trimoxazole was observed. This study reveals the presence of multidrug resistant organisms that possibly maintain the problem of antimicrobial resistance in the populace.

.**Keywords:** Antibiogram, birds multiple drug resistance, poultry, Salmonellae.

INTRODUCTION

Salmonellosis is one of the most important bacterial diseases in poultry causing heavy losses through mortality and reduced production (Muhammad *et. al.,* 2010) and one of the most common causes of food borne diarrhoeal diseases worldwide. In poultry, it causes pullorum disease and fowl typhoid, which are major diseases encountered in Nigerian poultry that give rise to heavy mortality in birds and hatching of poor quality chicks (Todar, 2005; Muhammad et. al., 2010; Agbaje *et. Al.,* 2010). Most of the infections are transmitted from healthy carrier animals to humans through contaminated food and water (Henrik *et al*., 2003). Poultry and poultry products such as meat and eggs have been recognised as major sources of *Salmonella*-related food borne infections and diseases (Folley *et al*., 2008; Carrique-Mas & Davies, 2008; Hassanein *et al.,* 2011; Okorie-Kanu *et al*., 2016). In humans, salmonellosis results in typhoid fever and gastroenteritis characterised by nausea, vomiting, diarrhoea and abdominal pain (Todar, 2005).

In Nigeria, the inclusion and administration of low doses of antibiotics through food and drinking water are common among poultry farmers. Despite its usefulness as growth promoters, the routine practice of giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria (Shah & Korejo, 2012). The increasing single and multidrug resistant Salmonella strains isolated from human cases of salmonellosis have been associated with widespread use of antimicrobial agents in food animals (Abuozeed *et al*., 2000). It is therefore imperative to assess the extent of this problem of drug resistance in our environment bearing in mind that the effective treatment of bacterial infections in any locality depends on the knowledge of the resistance profile of bacteria in that environment.

The purpose of this study was to isolate *Salmonella* organisms and determine the antimicrobial profile of isolates in some commercial birds in Umuahia, South-eastern Nigeria.

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MATERIALS AND METHODS

STUDY AREA AND STUDY POPULATION

This study was carried out in Umuahia, Abia State in the South-East of Nigeria. Umuahia is the capital city of Abia State, located along the railroad that lies between Rivers State to its south and Enugu State to its north. It lies within Latitudes 4.4˚ and 6.1˚ north of the Equator and Longitudes 7.0˚ and 8.0˚ east. By cluster sampling method (Thrushfield, 1997), six commercial poultry farms were selected from Umuahia and environs as sources of samples for this work. Samples were collected from laying birds only.

SAMPLE COLLECTION

A total of 100 cloaca swabs were collected from 100 apparently healthy birds. All faecal samples were collected aseptically with sterile swabs by placing the swabs into the cloacae of the birds, rotating gently against the lining of the cloacae and then withdrawing. Samples were transported within 20 minutes of collection to the Veterinary Microbiology Laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

LABORATORY PROCEDURES

CULTURE MEDIA PREPARATION

Culture media used were Salmonella-Shigella Agar, MacConkey Agar, Selenite F Broth base, Mueller-Hinton Agar, Triple-sugar Iron Agar, Simmons Citrate Agar, and Urea Agar. Commercial Polyvalent O and H antisera (Oxoid) were also used. All culture media were prepared aseptically and according to manufacturers' instructions.

CULTURAL PROCEDURES

The samples were directly inoculated onto Salmonella-Shigella agar (SSA), MacConkey (MCA) agar and into Selenite F broth. The plates and tubes were incubated at 37˚C for 24-48 hours. Samples that showed no growth were sub-cultured from Selenite F broth. The plates were examined for typical colonies of *Salmonella*, that are usually colourless colonies with irregular edges on MCA and with black centres on SSA. Typical colonies were further streaked on SSA plates to obtain pure cultures. Presumptive *Salmonella* colonies were transferred to nutrient agar slopes and stored at 4˚C in the refrigerator after incubation at 37˚C for 24 hours as stock culture.

CHARACTERIZATION AND IDENTIFICATION

Colonies suggestive of Salmonella were gram stained and morphologically studied (Merchant & Packer, 1967). Gram negative rods were tested for motility. Suspected colonies of *Salmonella* were taken for further biochemical tests which included: citrate, oxidase, indole, and triple-sugar iron and urease tests.

Serology was carried out by using the polyvalent O and H antisera according to Martins and Washington, (1980). Two separate drops of normal saline were placed on two sides of clean glass slides. Test colonies were picked with a sterile wire loop and mixed with the normal saline. A drop of the O antisera was placed on one of the suspension and observed for clumping, the other served as control. The presence of clumping shows a positive reaction, while its absence shows a negative reaction. This was repeated for the H antisera.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

This was carried out by the disk-diffusion method using antibiotic disks (Abtek Biologicals, UK) on Mueller-Hinton Agar. Overnight broth culture of each isolate was used to flood the surface of the whole plate. Excess was drained off and the agar was allowed to dry with the lid of the petri-dish in place. The antibiotic disks were applied aseptically to the surface of the plates and pressed gently to ensure contact with the medium. The plates were then transferred immediately to the incubator for 24 hours at 37˚C. Antibiotics tested include Augmentin (25µg), Ofloxacin (5µg), Gentamycin (10µg), Co-trimoxazole (25µg), Nalidixic acid (30µg), Nitofurantoin (200µg), Tetracycline (25µg) and Amoxicillin (25µg).

RESULTS

All isolates that produced characteristic reaction on TSI (glucose fermentation with or without hydrogen sulphide production) and were oxidase negative, indole negative, citrate variable and urease negative were considered to be *Salmonella* organisms. Consequently, of the 100 samples collected from commercial layers, 10 (10%) were positive for *Salmonella*. The results of the biochemical tests are shown below (Table I).

SEROLOGY

For the isolates, somatic (O) antigen was detected from 10 (100%) of them while the flagellar (H) antigen was detected in 8 (80%).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Results of the resistance pattern of the *Salmonella* isolates from commercial birds reveal that all isolates tested were resistant to one or more of the antibiotics used in the test (Table II). The resistance of isolates to the tested antimicrobial drugs ranged from 10% to 50% in these birds. Resistance to Augmentin and Amoxicillin (60%) in these birds appears to be highest, followed by Co-trimoxazole (80%) and Tetracycline (60%). Nitrofurantoin, Gentamycin and Nalidixic acid recorded least resistance. All (100%) isolates were susceptible to Ofloxacin. The percentage resistance and susceptibility patterns of isolates both group of birds to the various tested antimicrobial drugs are represented in Table II.

DISCUSSION

The isolation of *Salmonella* from commercial birds in this work is an indication of contamination and should be of concern to health authorities since poultry farms, poultry products, vegetables, feed, soil, litter have been incriminated as an important source of Salmonella infections (Bryan & Doyle, 1995; Muhammad *et al*., 2010). These sources have been identified as important contributors of horizontal transmission of salmonellosis (Grimont *et al.,* 2000; Helmuth, 2000). Soil can be contaminated with animal faeces, faeces from wild birds and rodents (Rodriguez *et al*., 2006), consequently, the organisms can be spread to chickens through water and feed and the birds become potential carriers of *Salmonella*. *Salmonella* can also be carried by insects on their legs and bodies; this can result in human diseases like diarrhoea and food poisoning (Skov *et al*., 2004; Obi and Ike, 2015). According to Obi and Ike, (2015), birds may be infected with *Salmonella* through contact with wild animals, domestic animals or even other poultry that are carriers of *Salmonellae*.

Table I: Biochemical characteristics of the 10 isolates in the commercial birds

Isolates	Indole	Citrate	Urease	TSI	H_2S production	Motility
A	-	+	-	+	-	-
B	-	-	-	+	+	+
C	-	+	-	+	-	-
D	-	-	-	+	+	+
E	-	-	-	+	-	-
F	-	+	-	+	+	+
G	-	-	-	+	+	+
H	-	+	-	+	-	-
I	-	+	-	+	-	-
J	-	+	-	+	+	+

KEYS: (+) = POSITIVE; (-) = NEGATIVE; (TSI +) = Yellow (acid) butt, Red (alkaline) slant.

Table II: Antimicrobial sensitivity profile of Salmonella isolates from tested birds (n =10)

Antimicrobial agents	Conc. (µg)	No.of sensitive isolates (%)	No. of isolates with intermediate sensitivity (%)	No. of resistant isolates (%)
Amoxicillin	25	3 (30)	2(20)	5 (50)
Augmentin	30	3 (30)	1(10)	6 (60)
Ofloxacin	5	10(100)	0 (0)	0 (0)
Gentamycin	10	5 (50)	2 (20)	3 (30)
Nalidixic acid	30	3(30)	1 (10)	6 (60)
Nitrofurantoin	200	8 (80)	1 (10)	1 (10)
Co-trimoxazole	25	1 (10)	1 (10)	8 (80)
Tetracycline	25	1 (10)	3 (30)	6(60)

The prevalence rate recorded in this work (10%) is lower than 24% recorded by Ekundayo & Ezeoke, (2011) and 81.7% reported by Salihu *et al*. (2018) but seems to agree with the 8.2% reported by Muhammad *et al,* (2010) and higher than 5.6% reported by Nwachukwu & Nwiyi (2011). Recent works have reported the high prevalence of species other than *S. pullorum* and *S. gallinarum.* The information on serotypes present in an environment can be used to detect the prevalence pattern (epidemiology) of the disease and helps in propagating efficient control measures (Muhammad *et al.,* 2010). Therefore, there is a need for further work to be carried out to ascertain serotypes of this organism in commercial birds from this environment.

The level of antibiotic resistance in this study is disturbing. Isolates were resistant to most of the drugs tested. There was high resistance to Augmentin (100%) and Amoxicillin (60%). This could be attributed to the fact that these drugs are on the general sale list (GSL) drugs and so, are very common veterinary drugs in Nigeria and are sold over the counter, most often without prescription. This encourages indiscriminate use of drugs thus, increasing problems of resistance. Resistant strains can be picked up from the environment, as it is possible that a host that has not received an antibiotic but shares an environment with a host that has received such antibiotic may be infected with resistant organism from the treated host (Lipstitch & Samore, 2002). More of the isolates from local birds were resistant to the test drugs than from the commercial birds.

Most isolates showed resistance to more than one of the antimicrobial drugs used. This indicates the presence of multiple drug resistant (MDR) strains of *Salmonellae* amongst the isolates. This may be as a result of the wide use of these antibiotics. Resistance to tetracycline has been common and may be because of its use as a growth promoter (Roberts, 1996). Extra-label use of drugs has also contributed to drug residue and resistance problems (Aliyu, 2007). These highly multiple drug resistant strains of bacteria can be transmitted from animals to humans directly or through the food chain. There is therefore the need for stronger public health campaign in regulating and monitoring the use of antimicrobial drugs (Helmuth, 2000). This will ensure proper use of antimicrobials and reduce resistance problems. Best overall potency was seen with Ofloxacin (a fluoroquinolone) and thus may be called the antibiotic of choicein salmonella infections. This may be because they are new group of drugs but relatively expensive and so not so available to the public. Quinolones have been

shown to be highly effective in the treatment of salmonellosis, including infections caused by multiple drug resistant strains (Barnes *et al*., 1990).

Control of salmonella depends on controlling sources of contamination by maintenance of strict hygiene in poultry farms and in homes and by vaccination. Negligence of vaccination against fowl typhoid by most farmers seems to be a contributing factor to the increasing prevalence of salmonellosis.

CONCLUSION

High level of antimicrobial resistance to the commonly prescribed antimicrobials may be a hindrance to effective treatment of clinical cases. So, wise use of antimicrobials must be practiced to combat the antimicrobial resistance pattern. This study has revealed a need for wider studies to be conducted to determine the predominant *Salmonella* serotypes in the study area so as to proffer means of efficient treatment of salmonellosis.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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