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Occurrence and Determination of Antibiogram of Salmonella Isolates from Nigerian Indigenous Breeds of Chicken in Umudike and Environs.

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Abstract: Salmonellosis is a major disease of poultry that can be contracted by ingestion of contaminated poultry and poultry products. It has remained a major disease of global health significance and the situation especially in Nigeria has been aggravated by the increasing rate of antimicrobial resistance. Despite the many studies on prevalence and antimicrobial susceptibility of Salmonella in birds, little or nothing has been said on the situation in Nigerian indigenous birds. Hence, this study was conducted to determine the occurrence and antimicrobial resistance pattern of salmonella isolates from indigenous Nigerian breeds of chicken in Umudike environs. Cloacae swabs from 50 local birds were screened for Salmonella. A prevalence rate of 30% from the local birds was recorded The Antibiogram was determined using disk diffusion technique. Organisms showed multiple drug resistance. All isolates were sensitive to Ofloxacin but resistance to Augmentin, Amoxicillin and Co-trimoxazole were the most common.

Key words: Antibiogram · Indigenous · Multiple Drug Resistance · Nigerian · Salmonellae · Umudike

INTRODUCTION

Salmonella is a major pathogenic bacterium in humans and animals and is a leading cause of food-borne illness in man and animals and remains a problem of great public health significance [1]. Poultry and its products have been incriminated as a source of human infection [2-4]. People get infected when they eat contaminated foods of animal origin such as meat or eggs or by ingesting the faeces indirectly through contaminated food and water.

In Nigeria, local chickens also called village chickens or free range chickens constitute about 60% of the poultry population [5] and represent a significant part of the rural economy and hitherto the national economy [6]. They are kept by over 90% of households, by especially the women [6], providing an important source of income and high quality protein for these families [7]. Local chickens are usually managed under extensive or semi-intensive system [8, 9]. This exposes them to a number of infectious agents and even drug resistant strains in the environment as they share common environments with animals and humans that may be carriers of *Salmonellae* as the use of antimicrobials in any environment creates selection pressures that favour the survival of antibiotic resistant organisms [10]. Though, these local birds are widely believed to act as reservoirs of important poultry diseases including fowl typhoid [11, 12], most folks in Nigeria, especially, the Eastern Nigeria still prefer them to the exotic birds because they are cheaper, said to taste better [13] and are assumed to be free from disease since they are rarely morbid.

Since there is paucity of information regarding salmonellosis in these local birds, especially in Abia State, this study intends to test this local myth that local birds in Nigeria are somewhat free from disease organisms.

The purpose of this study was to isolate *Salmonella* organisms and determine the antimicrobial profile of isolates in some local chickens in Umudike area.

MATERIALS AND METHODS

Study Area and Study Population: This study was carried out in Umudike and neighbouring village of Ndooru, all in Ikwuano Local Government area of Abia State in the South-East of Nigeria. Samples were collected from local

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birds from community compounds at Ndooru and Umudike and local chicken markets at Umuahia and Ndooru.

Samples Collection: A total of 50 cloacae swabs were collected from apparently healthy local `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 30 minutes of collection to the Veterinary Microbiology Laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Laboratory Procedures

Culture Media Preparation: Culture media used were Salmonella-Shigella Agar (Fluka Biochemika, Germany), MacConkey Agar(Fluka Biochemika, Germany), Selenite F broth base (biotec Laboratories Ltd, Martleharm Health, Uk), Mueller-Hinton Agar (Lab M, UK), Triple-sugar Iron Agar (Scharlau, Spain), Simmons Citrate Agar (Britannia Labs) and Urea Agar (Himedia Labs, India). All culture media were prepared aseptically and according to manufacturer's 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. The plates were examined for typical colonies of *Salmonella*, that is, 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 [14].

Characterization and Identification: Colonies suggestive of Salmonella were gram stained and morphologically studied [15]. 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 [14].

Serology: This was carried out by using polyvalent O and H antigens. 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 antigen 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 antigen.

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). [16]

RESULTS

Biochemical Identification: All isolates that produces 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 50 samples collected from local birds, 15(30%) were positive.

Serology: From isolates from the local birds, somatic (O) antigen was detected from 15(100%) of them and the H antigen was detected in 11 (73%) of them.

Antimicrobial Susceptibility Test: Results of the resistance pattern of the *Salmonella* isolates from the birds reveal that all isolates tested were resistant to one or more of the antibiotics used (Table 3). The resistance of isolates to the tested antimicrobial drugs ranged from 20% to 100%. Resistance to Augmentin and Amoxicillin (100%) in appeared 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 results of the biochemical tests and percentage resistance and susceptibility patterns of isolates from these birds to the various tested antimicrobial drugs are represented in Table 1 and 2 respectively.

Table 1: Results of Biochemical tests of isolates

Isolate	Indole	Citrate	Urease	TSI	H2S Production	Motility
a	-	+	-	+	+	+
b	-	+	-	+	+	+
c	-	-	-	+	-	-
d	-	+	-	+	+	+
e	-	+	-	+	+	+
F	-	-	-	+	-	-
g	-	+	-	+	+	+
h	-	-	-	+	-	-
I	-	-	-	+	+	+
J	-	+	-	+	-	-
k	-	-	-	+	+	+
L	-	-	-	+	-	-
m	-	+	-	+	+	-
n	-	+	-	+	-	-
o	-	-	-	+	+	-

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

Table 2: Antimicrobial sensitivity profile of Salmonella isolates from tested local birds

Antimicrobial agents	Conc (µg)	No. of sensitive isolates (%)	No. of isolates with intermediate sensitivity (%)	No. Of resistant isolates (%)
Amoxicillin	25	0 (0)	0(0)	15 (100)
Augmentin	30	0 (0)	0(0)	15 (100)
Ofloxacin	5	11(73.3)	4 (26.7)	0 (0)
Gentamycin	10	10 (66.7)	2 (13.3)	3 (20)
Nalidixic acid	30	8(53.3)	1 (6.7)	6 (40)
Nitrofurantoin	200	6 (40)	5 (33.3)	4 (26.7)
Co-trimoxazole	25	3 (20)	0 (0)	12 (80)
Tetracycline	25	2 (13.3)	3 (20)	10 (66.7)

DISCUSSION

The isolation of *Salmonella* from the birds in this work is an indication of contamination and agrees with the fact that bacteria are part of enteric flora of birds. It also nullifies the myth that local or indigenous birds are free from disease agents. According to Obi and Ike [12], these indigenous birds may be infected with *Salmonella* through contact with wild animals, domestic animals or even commercial poultry that are carriers of *Salmonellae*. The relatively high occurrence of isolates from the local birds is worrisome as most people in the rural areas prefer these local breeds to exotic poultry.

The high incidence of resistance against tested antibiotics is also worrisome. Resistance to tetracycline, amoxicillin and Augmentin which are commonly used drugs in the study area and the susceptibility to Ofloxacin agrees with Ajayi and Egbegbi [14]. Ofloxacin is not a commonly used drug and this may be the reason for the susceptibility of the isolates to it. This antibiotic resisance profile in these indigenous birds gives cause for more research in this area. These birds neither receive treatment nor sub therapeutic dosings of antibiotics but yet show a high rate of resistance. This may be as a result of contact with animals that do. Okoli [15], reports that the use of antibiotics in a host in an environment may increase the risk of infection with resistant organisms in other hosts who have not received the same antibiotics but are sharing a common environment with the treated ones.

The multiple resistances to antibiotics observed in this work is a source of concern, as these birds do not receive antibiotic dosings as poultry birds and is of serious public health significance because the consumption of meat and products from these local birds are on the increase especially among rural dwellers who may not be able to afford or access good medical care. The fact that indigenous birds are sturdy and do not come down with diseases does not mean they are free from infection. They are and can serve as reservoirs and thus, sources of diseases. Wise use of antimicrobials in our environment must be practiced to curb 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.

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