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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *SALMONELLA SPP.* ISOLATES FROM FAECAL DROPPINGS OF COMMERCIAL AND FREE-RANGE CHICKENS IN SAMARU, NIGERIA.

INABO, HILDA*[1]; OLONITOLA, O. STEVE[2], YAKUBU, E. SABO[2]

[1]Department of Bioentrepreneurship and Extension Services,
National Biotechnology Development Agency, Abuja, Nigeria.
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Corresponding Author: hildainabo@gmail.com

ABSTRACT

Antibiotic susceptibility patterns of *Salmonella* spp. from faecal droppings of commercial and free-range chickens were determined. Six *Salmonella* spp. isolates were recovered from 200 samples of faecal droppings from live commercial and free- range chickens on *Salmonella-Shigella* Agar. The isolates were confirmed using standard biochemical tests. Susceptibility of the isolates was carried out by Disc Diffusion test: chloramphenicol, ciprofloxacin, cloxacillin, cotrimoxazole, gentamicin, nalixidic acid, streptomycin, and tetracycline. This study revealed that 100% of isolates from commercial chickens were susceptible to chloramphenicol, ciprofloxacin, gentamicin, nalixidic acid, streptomycin and resistant to cloxacillin, cotrimoxazole and tetracycline. However, the isolate obtained exhibited multi-antibiotic resistance to cloxacillin and tetracycline. Hundred percent of the isolates from free-range chickens were susceptible to ciprofloxacin, cotrimoxazole, gentamicin, nalixidic acid and streptomycin while 75% were susceptible to chloramphenicol. However, 100% were resistant to cloxacillin and tetracycline and all the isolates were observed to exhibit multi-drug resistance. The study has established the presence of antibiotic susceptible, resistant and multi-drug resistant *Salmonella* spp. isolates in commercial and free-range chickens to antibiotics in Samaru, Zaria. Multi-antibiotic resistance was higher in free-range chickens than in commercial chickens.

Keywords: Antibiotic susceptibility, *Salmonella* spp., fecal droppings

INTRODUCTION

Salmonella belongs to the Kingdom: Bacteria, Phylum: Proteobacteria, Class: Gamma Proteobacteria, Order: Enterobacteriales, Family: *Enterobacteriaceae* and Genus: *Salmonella*. (Fox, 2011). *Salmonella* is a genus of bacteria that can cause foodborne infections in humans and animals (Herkistad *et al*., 2000). The use of antibiotics in sub-therapeutic and therapeutic doses for poultry has helped in promoting growth, protection and cure from diseases (NOAH, 2009). Consequently, this has led to the emergence of antibiotic-resistant strains which are of great global concern. In developed countries, antimicrobial drug resistance in non-typhoidal *Salmonella* organisms is an almost inevitable consequence of the use of antimicrobial drugs in food producing animals. There has been an increase in the occurrence of resistance in both non-typhoidal and typhoidal *Salmonella* spp. in developing countries (Threlfall, 2002). However, the indiscriminate use of antibiotics has caused an increase in bacterial resistance, mainly in *Salmonella* strains (Berchieri *et al.*, 1989). The aim of this study was to determine the antibiotic susceptibility profile of *Salmonella* spp. isolates from the faecal droppings of commercial and free-range chickens in Samaru village of Zaria, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of Two hundred (200) faecal samples consisting of 100 samples each were randomly collected from commercial poultry houses and homes that rear free range chickens respectively. The faecal samples were collected in clean sterile glass containers. They were taken to the laboratory of the Department of Microbiology, Ahmadu Bello University, Nigeria at ambient conditions and processed immediately.

Isolation and identification of *Salmonella*

Fecal samples were inoculated into peptone water for enrichment at a ratio of 1gram to 9ml and incubated at 37^0C for 24hrs. The samples were inoculated on *Salmonella-Shigella* agar (SSA) and incubated at 37^oC for 24hrs. After incubation, the SSA plates were examined for bacterial growth. The suspected isolates were subjected to gram-staining and biochemically identified using triple sugar iron agar (TSI), Simmon citrate agar, Sulphur Indole Motility medium (SIM), and Methyl red-Voges proskauer broth (MR-VP) (Cheesebrough, 2006). Further confirmation was done using biochemical tests kits (Microgen^{TM} GnA- ID System) and accompanying computer software package (Microgen ID Version 1.2.5.26).

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing of the isolates were carried out using the disc diffusion technique (Kirby-Bauer disk diffusion method) according to the methods recommended by the Clinical Laboratory and Standards Institute (CLSI, 2011). About 3 discrete colonies of the isolates were inoculated into 5ml of nutrient broth and incubated overnight at 37^0C. The overnight culture was then standardized with 0.5 MacFarland standard. Sterile cotton swabs were used to inoculate the bacterial suspensions on freshly prepared pre-dried Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic discs were aseptically and spaciously placed on the inoculated Mueller-Hinton agar plates. The plates were inverted and incubated aerobically at 37^0C for 24hrs. The antibiotic discs used include: Ciprofloxacin (5 µg), Chloramphenicol (10 µg), Cloxacilin (5 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Nalixidic acid (5 µg), Streptomycin (10 µg), Tetracycline (10 µg). For each isolate, the zone of inhibition around each disk was measured, after incubation at 37^0C for 24hrs using the method of Cheesebrough, (2000).The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Olayinka *et al*., 2004).

RESULTS AND DISCUSSION

The biochemical characterization of *Salmonella* spp. is shown in Tables 1 and 2. Antibiotic susceptibility profile of *Salmonella* spp. isolated from faecal droppings of commercial and free range chickens is shown in Figure 1. Ciprofloxacin, chloramphenicol, gentamicin, nalixidic acid, and streptomycin had the highest antibiotic activities against the isolate (100%) and it was resistant to tetracycline and cloxacillin. In free-range chickens, ciprofloxacin, cotrimoxazole, gentamicin, nalixidic acid, streptomycin had the highest antibiotic activity against

Table 1. Conventional Biochemical tests for the characterisation of *Salmonella* spp.isolates from faeca droppings of commercial and free-range chickens

Isolates	Gram reaction	Triple Sugar Iron reaction	Indole test	Methyl red test	Voges Proskauer test
CC 44	Gram negative short rods	R/Y +H_2S	-ve	+ve	-ve
FR 23	Gram negative short rods	R/Y +H_2S	-ve	+ve	-ve
FR 32	Gram negative short rods	R/Y +H_2S+gas	-ve	+ve	-ve
FR 36	Gram negative short rods	R/Y +H_2S+gas	-ve	+ve	-ve
FR 67	Gram negative short rods	R/Y +H_2S	-ve	+ve	-ve

Table 2. Biochemical characterization of *Salmonella* spp. from the faecal droppings of commercial and free-range chickens using Microgen™ GnA- ID kit.

CODE	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal code	ID
CC 43	+	+	+	+	+	+	-	-	-	-	+	-	5702	*Salmonella* sp.
FR 23	+	+	+	+	+	+	-	-	-	-	+	-	5702	*Salmonella* sp.
FR 32	+	+	+	+	+	+	-	-	-	-	+	-	5702	*Salmonella* sp.
FR 34	+	+	+	+	+	+	-	-	-	-	+	-	5702	*Salmonella* sp.
FR 67	+	+	+	+	+	+	-	-	-	-	+	-	5702	*Salmonella* sp.

Key: Lys- Lysine, Orn- Ornithine, H2S- Hydrogen sulphide , Glu-Glucose, Man-Mannitol, Xyl- Xylose, ONPG- Ortho-nitrophenol- galactosidase, Ind - Indole, Ure- Urease, VP- Voges Proskauer, Cit- Citrate, TDA- Tryptophan deaminase acid , ID-Identification

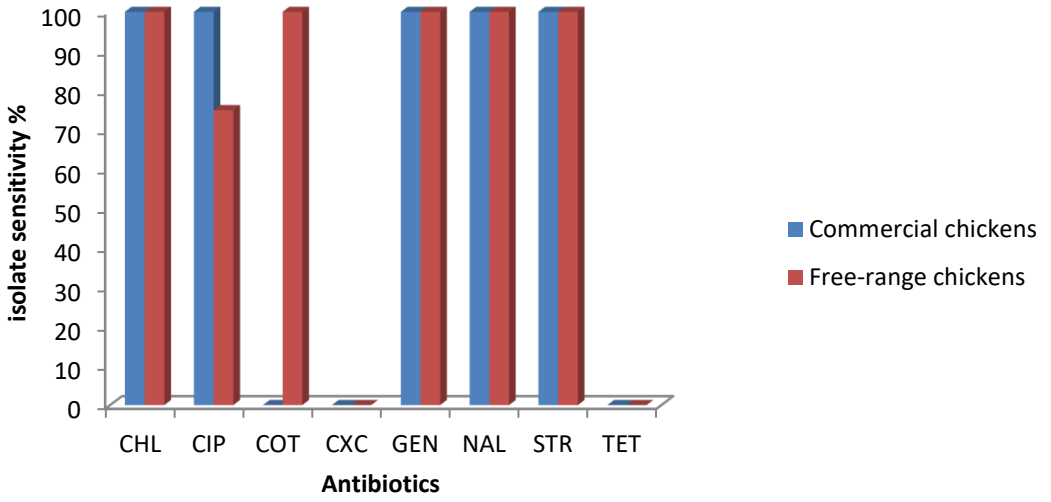

Figure 1. Antibiotic susceptibility profile of *Salmonella spp.* isolates from faecal droppings of commercial and free-range chickens

Key: CIP=Ciprofloxacin, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin, GEN=Gentamicin, NAL=Nalixidic acid, STR=Streptomycin, TET=Tetracycline

all the isolates (100%) while the isolates were susceptible (75%) to chloramphenicol. However all the isolates were resistant to tetracycline and cloxacillin.

Antibiotic susceptibility studies of *Salmonella* spp. isolates to the selected antibiotics showed that all the isolates obtained from commercial and free-range chickens were highly susceptible to chloramphenicol, ciprofloxacin, and gentamicin. This may be due to the difficulty encountered during injection and the cost of the drugs. This observation concur with report of Begum *et al*. (2010) but disagrees with the results obtained by Ojo *et al*. (2012) and Suh and Odeh (2008) where *Salmonella* spp. isolated from commercial and free-range chickens were susceptible to chloramphenicol, ciprofloxacin and gentamicin. The high susceptibility of *Salmonella* isolates to nalixidic acid and streptomycin in this study agrees with the result obtained by Enabulele *et al*. (2010). However, it contradicts the reports of Payne *et al*. (2006) and Siemon *et al*. (2007). The resistance of the isolates to both tetracycline and cloxacillin agrees with previous reports of Begum *et al*. (2010) and Payne *et al*. (2006) but disagrees with the observation of Ojo *et al*. (2012) and Alali *et al*. (2010) in commercial and free-range chickens. Resistance to tetracycline is very common. Tetracycline is one of the most widely used antibiotics especially in feed as feed additives for growth promotion and for the prevention of other diseases of bacterial origin (Muirhead, 1999; Rajashekara, 2000). Besides, studies have shown that tetracycline persists in the environment longer than any other antibiotic (Frost, 1991). In this study, *Salmonella* isolates from faecal droppings of free-range chickens were susceptible to cotrimoxazole, while that of commercial chickens was resistant to the drug. This result agrees with the reports of Alali *et al*. (2010) and Pan *et al*. (2010) and contradicts those of Begum *et al*. (2010) and Enabulele *et al*. (2010).

Multi-antibiotic resistance patterns of *Salmonella* spp. isolated from fecal droppings of commercial and free-

range chickens is shown in Table 3. No antibiotic resistance phenotype was observed with the single antibiotic resistance type in the isolates from the commercial and free-range chickens. One multiple resistance phenotypes was obtained with varying combinations of 2 antibiotics while in free-range chickens, two multiple resistance phenotypes were obtained with varying combinations. The highest frequency of 3 was observed in the combinations of 2 antibiotics.

The multi-antibiotic resistance of *Salmonella* spp. to several antibiotics observed in this study agrees with the results of Ojo *et al*. (2012) but disagrees with reports of Alali *et al*. (2010) and Siemon *et al*. (2007). Furthermore, the isolates from the study exhibited one and two patterns of resistance phenotypes from commercial and free-range chickens respectively. Both patterns contradict the observations of Payne *et al*. (2006), Ojo *et al*. (2012) and Pan *et al*. (2010). This variation in the pattern of resistant phenotypes may be due to differences in the choice and concentrations of the antibiotics administered.

Table 3.Multidrug-resistance pattern of *Salmonella* spp. isolates from the faecal droppings of commercial and free-range chickens

Single antibiotic resistance		Multiple antibiotic resistance		
No. of isolates	Resistance Phenotype	No. of antibiotic Combination	No. of isolates(%) with pattern	Resistance phenotype
CC 0(0)	Nil	2	1(100)	CXC, TET
FR 0(0)	Nil	2	3(100)	CXC, TET
	Nil	3	1(100)	CXC,CHL,TET

Key: CIP=Ciprofloxacin, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin, GEN=Gentamicin, NAL=Nalixidic acid, STR=Streptomycin, TET=Tetracycline CC= Commercial chickens, FRC= Free-range chickens

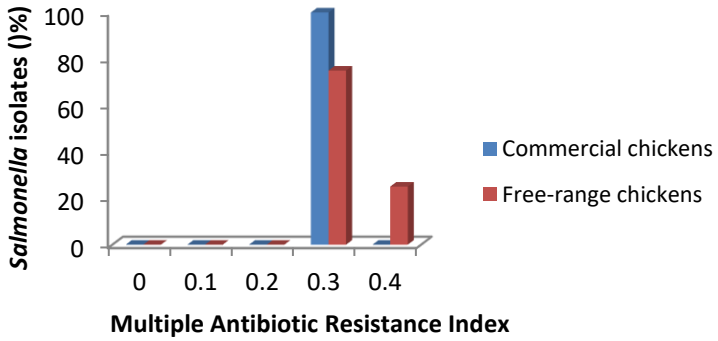

Figure 2: Multiple antibiotic resistance indices of *Salmonella spp.* isolates from faecal droppings of commercial and free-range chickens

All isolates from both commercial and free-range chickens exhibited multi-antibiotic resistance. The multiple antibiotic resistance index (MARI) of the isolates is presented in Figure 2. All isolates from commercial chickens and free-range chickens had a MAR index greater than 0.2.

The frequency of isolation of *Salmonella* strains resistant to one or more antimicrobial agents has risen in countries like Saudi Arabia (Al-Tawfiq, 2007), the UK (Snow *et al*., 2007), the USA (Frye and Fedorka-Cray, 2007), Nigeria (Muhammad *et al*., 2010). An understanding of the epidemiology of emerging *Salmonella serovars* is dependent on the investigation of multi-drug-resistant *Salmonella*. Multi-antibiotic resistance in *Salmonella* renders treatment with antibiotics ineffective, thereby resulting in grave zoonotic implication. In addition, the high MARI observed in this study is suggestive of the fact that the probable source of the isolates was the environment where these antibiotics are frequently used.

CONCLUSION

This study has established the presence of resistant strains of *Salmonella* spp. isolated from both commercial and free-range chickens to some antibiotics used both in humans and animals. The presence of high MARI in *Salmonella* spp. isolates from faecal droppings of commercial and free-range chickens connotes the frequent use of these antibiotics in the environment. Resistance was common to antibiotics frequently and commonly used by both humans and in the poultry industry in Nigeria.

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