Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.8, No.6, 2018

In Vivo Activities of Some Selected Antimicrobial Agents Against Enteric Bacteria Isolated from Chicken Feeds on Broiler Layers

Iheukwumere, Ikechukwu Harmony[1*] Chukwura, Edna Ifeoma[2] Chude, Charles[3]
1.Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
2.Department Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
3.Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria

Abstract
This study focused on in vivo activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds sold in Anambra State. A total of 1,536 different chicken feed samples (starter, growers, finisher and layers) were collected from open markets and shops and screened for the presence of enteric bacteria using pour plate technique. The isolates were characterized and identified using their colony descriptions, biochemical and molecular characteristics. The phytochemical constituents of the medicinal plants were carried out using spectrophotometric methods. The protective effects of commercially prepared probiotics (CP), oxy-tetracycline (OXY), ciprofloxacin (CPX) and *Zingiber officinale* (ZO) were investigated using *in vivo* method. The result of this study revealed that *Escherichia coli* O157:H7 SS52 (EC), *Salmonella* serovar Typhimurium U288 (ST), *Escherichia coli* SEC470 (ES), *Salmonella* serovar Enteritidis YU39 (SY) and *Salmonella* serovar Enteritidis FM366 (SE) were isolated from the feed samples. The results of *in vivo* activity showed that the CP, CPX, OXY and ZO extract were effective in reducing pathological changes in the experimental chickens, of which CPX and OXY were most effective, and ZO extract was also effective and most safe for protecting the broiler layers against the pathogenic isolates. Their effects were significant ($P<0.05$) when compared to the infected non treated chickens. Thus, this study has shown that EC, ST, ES, EY and SE were isolated from the studied feed samples. The tested antimicrobial agents have proved to be safe and effective in reducing pathological features associated with the studied isolates in broiler layers.
Keywords: In vivo study, Antimicrobial agents, Enteric bacteria, Chicken Feeds.

INTRODUCTION

High rate of chicken diseases and death have been traced to consumption of contaminated feed (Onyeze *et al.,* 2013). Some of the bacterial contaminants associated with commercially produced chicken feeds such as *Escherichia coli, Salmonella* species, *Campylobacter jejuni, Enterococcus fecalis, Listeria* species, *Bacillus cereus, Eriwinia* species have been reported (D' Mello, 2006; Arotupin *et al.,* 2007; Onyeze *et al.,* 2013). Various types of chicken diseases which also affect humans have been traced to the contamination of feeds and chicken products by microorganisms' mainly enteric bacteria (Onyeze *et al.,* 2013).

Chicken diseases have contributed significantly to increase in mortality rate and economic losses in the chicken industry. As a result, antibiotics, sometimes at sub-therapeutic concentrations, are often included in chicken feeds to prevent disease, enhance feed conversion efficiency and improve growth rates (Oguttu *et al.,* 2008). However, the use of antibiotics in chicken feeds is not totally safe. One of the main concerns is the development of antibiotic resistant bacteria (Oguttu *et al.,* 2008).

The WHO has estimated that about 80% of the population living in the developing countries relies on tradition medicine for their health care needs (WHO, 2002) and there is an estimation that about 80% of all South Africans use traditional medicine derived from plant species indigenous to the region. Medicinal plants are beneficial even in developed countries and have influenced pharmaceutical products. Extracts of plants and algae have been incorporated in the products and plants in particular are an indispensable source of pharmaceuticals (WHO, 2002). Recently, there has been a dramatic increase in the demand for "herbal medicine" According to one estimate; the world market for "herbal medicine" has reached 60 billion US dollars, with annual growth rates of between 5% and 15% (WHO, 2002).

Previous studies focused on physicochemical properties and microorganisms associated with poultry feeds (Arotupin *et al*., 2007; Chowdhuri *et al*., 2011; Habtamu *et al*., 2011; Onyeze *et al.,* 2013) and used of some antimicrobial substances to control these microorganisms (Wafaa *et al*., 2012; Ali *et al*., 2014). Hence, enteric bacteria majorly *E. coli* and *Salmonella* species remain the primary causes of reported periodic outbreak of poultry diseases and food poisoning in Nigeria (Ali *et al*., 2014). This shows that there is still paucity of information on the characterization of the actual strains of enteric bacteria causing these diseases, their pathogenicity studies and the use of naturally produced antimicrobial agents without any adverse health effects to control the menacing activities of these organisms.

MATERIALS AND METHODS

Collection of Samples: A total of 1536 commercially produced chicken feed samples (starter, growesr, finisher and layers) were aseptically collected from the wholesalers, retailers and consumers. The feed types which included X (756 samples), Y (756 samples) and Z (756 samples) were aseptically collected from twenty-one (21) major towns located within Anambra State. One cup of the feed sample was aseptically collected from each feed type by randomly collecting one Table spoon of the feed sample from each bag containing the feed type. The feed samples were mixed and homogenized to generate a representative sample for each feed type. The feed samples were collected from Broiler starter (128 samples), Grower mash (128 samples), Broiler finisher (128 samples) and Layer mash (128 samples) for each feed type (X, Y and Z) using aluminum foil. The samples were carefully labeled and classified based on the sources of collection. The feed samples were transported in cooler containing ice block for laboratory for analysis.

Culture and Isolation of Enteric Bacteria: This was carried out using the modified method of Cheesbrough (2000). One gram (1.0g) of each sample was dissolved in 5.0 ml of sterile distilled water, then make up the volume to 10.0 ml prior to serial dilution. One milliliter aliquot was aseptically transferred into a sterile test tube containing 9.0 ml of the diluent (distilled water) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the sample was plated on *Salmonella-Shigella* agar (SSA/Biotech) for *Salmonella* and *Shigella* species and MacConkey agar (MA/Biotech) for *E. coli*. All the plates in triplicates were incubated inverted at 44.5˚C for 24 h for *E. coli* and 37˚C for 24 h for other enteric bacteria.

Characterization and Identification of the Isolates: The isolates were subcultured on nutrient agar (Biotech), incubated invertedly at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2000).), biochemical reactions (Cheesbrough, 2000).) and molecular characteristics (Habtamu *et al.,* 2011; Gabriela *et al.,* 2014).

Preparation of plant materials: The fresh leaves of *Xylopia aethiopica, Piper guineense* and *Gongronema latifolium* and rhizomes of *Zingiber officinale* were collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The sample was authenticated appropriately. The fresh leaves of *Xylopia aethiopica, Piper guineense* and *Gongronema latifolium* and rhizomes of *Zingiber officinale* were dried under shade at room temperature for 14 days. The dried leaves were ground to powdered form using sterile electric grinder. Twenty gram of the ground leaves each was macerated with distilled water and ethanol respectively for 72 h. The mixture was filtered using Whatman No 1 filter paper. The extracts were concentrated by evaporating to dryness at room temperature in a steady air current (Iheukwumere *et al.,* 2012)

Phytochemical analysis of the plant extracts: The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Iheukwumere and Umedum (2013)

***In vivo* Activities of the Antimicrobial Substances against the Enteric Bacterial Isolates:** A total of 75 adult layers were purchased. The adult layers were grouped into five (5) groups which include: group A, B, C, D and E. Each group contained fifteen (15) adult layers each. The treatments to the groups were as follows: **Groups A and B:** Blank control (only distilled water) for for period of fourteen (14) days; **Group C:** Antibiotics (ciprofloxacin/oxytetracycline), 0.5g/L for the adult layers for a period of seven (7) days; **Group D:** Medicinal plant (*Zingiber officinale* aqueous extract), 1.0 ml (500 mg/L)/L of distilled water for period of fourteen (14) days; **Group E:** Probiotics, 2 g/ L for period of 14days. Groups B, C, D and E were then exposed to the isolates via oral route after 14 days. The chickens were carefully monitored for a period of 4 weeks (Anonymous, 2018).

Examination of experimented chickens: The administered chickens were carefully observed for the obvious pathological signs of the challenged organisms for period of 4 weeks, the protection rates of inhibitory substances were determined, and the chickens were sacrificed and gross examination of the morphologies of the internal organs and intestines were carried out. Also, the internal organs (Liver, Lung, Spleen and Heart) were harvested and some portions of these organs were cultured on MacConkey agar and *Salmonella-Shigella* agar, and incubated at 37˚C for 24 h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli.* Cloacae swabs were also taken from the adult layered chicken and plated on MacConkey agar and *Salmonella-Shigella* agar and incubated at 37˚C for 24 h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli* (Anonymous, 2018).

Examination of eggs laid by the experimented chickens: The harvested eggs from the adult layers were examined for the presence of the isolates by culturing the eggs on the MacConkey agar and *Salmonella-Shigella* agar and incubated at 37˚C for 24 h for *Salmonella* species and at 44.5˚C for 24 h for *E. coli* (Anonymous, 2018).

Statistical Analysis: The results of the data generated were expressed as mean ± standard deviation (SD). The statistical analysis of data generated from protective study was carried out using chi-square at 95% confidence limit (Wafaa *et al.,* 2012). The statistical analysis of the gross pathological changes, inhibitory activities, re-isolation, detection in the egg samples and other valuable data generated from this study were examined using SPSS package program version 20.0. Data were analyzed by one-way Analysis of Variance (ANOVA) to determine the significant difference of the mean values at 95% confidence limit. Pair wise comparison of mean was done by Least Significant Difference (LSD) (Wafaa *et al.,* 2012, Dashe *et al.,* 2013).

RESULTS

The morphological characteristics of the isolates are shown in Table 1. Isolates 5, 7 and 11 were isolated from *Salmonella-Shigella* agar (SSA) and they exhibited similar morphological characteristics on SSA plates. In addition, isolates E and G exhibited similar morphological characteristics on MacConkey agar (MA) plates. The biochemical characteristics and identities of the enteric bacterial isolates are shown in Table 2. The results of the present study reveal that isolates 5, 7 and 11 exhibited similar biochemical characteristics; they showed positive results to hydrogen sulphide production, catalase, and methyl red, utilize citrate as carbon source and able to ferment glucose, dulcitol, arabinose and maltose. Isolate 5 fermented inositol, showed slight positive reaction to xylose and was negative to mucate unlike isolates 7 and 11 that fermented xylose but negative to inositol. Isolates E and G exhibited similar biochemical properties; they showed positive results to Indole reaction, methyl red, catalase and able to ferment glucose, maltose, arabinose and lactose.

The results of the sequencing of 16s rRNA using universal primer (16s) revealed the presence of *Escherichia coli* 0157:H7 strain SS52 (isolate E), *Escherichia coli* strain SEC 470 (isolate G), *Salmonella enterica* subspecies *enterica* serovar Typhimurium strain U288 (isolate 5), *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain FM366 (isolate7) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain YU39 (isolate11) (Table 3).

The quantitative phytochemical constituents of *Gongronema latifolium, Piper guineense, Xylopia aethiopica* and *Zingiber officinale* extracts are shown in Table 4. The results showed that *G. latifolium* extract contained significantly ($P<0.05$) higher alkaloids, tannins and saponins than other extracts. *Piper guineense* extract contained significantly ($P<0.05$) higher cardiac glycosides and non-significantly higher phenolics than other extracts. *Xylopia aethiopica* extract contained non-significantly higher steroids than other extracts. *Zingiber officinale* extract contained significantly ($P<0.05$) higher flavonoids than other extracts. The presence of these phytochemical constituents in the extracts could be responsible for the antibacterial activities of the extracts.

The total mean viable plate counts of challenge isolates from the cloaca of adult-layer chickens administered different antimicrobial substances is shown in Table 5. The results revealed that the total mean viable plate counts (TMPCs) of *E. coli* O157:H7 SS52 from the chickens administered *Z. officinale* extract and ciprofloxacin (CPX) were significantly ($P<0.05$) lower than the TMPCs from the infected and non-infected chickens, and the TMPC from the chickens administered commercially prepared probiotic (PRO) was significantly ($P<0.05$) lower than the TMPC from infected chickens but slightly had the same TMPC with non-infected chickens. The TMPC from chickens administered *Z. officinale* was significantly ($P<0.05$) greater than the TMPC from the chickens administered CPX and significantly ($P<0.05$) lower than the TMPC from the chickens administered PRO.

Also, the TMPCs of *S.* Typhimurium U288 from the cloaca of the chickens administered *Z. officinale* extract, PRO and Oxytetracycline (Oxy) were significantly ($P<0.05$) lower than the TMPC from infected chickens. The results also revealed that the TMPC from chickens administered *Z. officinale* extract was significantly ($P<0.05$) lower than the TMPC chickens administered PRO and non-significantly greater than the TMPC from chickens administered Oxy.

The present results also showed that the TMPC of *S.* Enteritidis FM366 from the cloaca of the chickens administered *Z. officinale* extract, PRO, and Oxy were significantly ($P<0.05$) lower than the TMPC from infected chickens, with the TMPC from the chickens administered Oxy significantly ($P<0.05$) lower than the TMPC from non-infected chickens. The TMPC from chickens administered *Z. officinale* extract was significantly ($P<0.05$) lower than the TMPC from the chickens administered PRO and significantly ($P<0.05$) greater than the TMPC from chickens administered Oxy. Generally, the chickens administered CPX and Oxy showed the lowest TMPC of the enteric bacteria, followed by those administered *Z. officinale* extract and then with PRO. Also, those chickens administered PRO against *E. coli* O157:H7 SS52 and *S.* Enteritidis FM 366 slightly had the same TMPCs.

The total mean viable plate counts of challenge isolates from the egg samples laid by the adult-layer chickens administered different antimicrobial substances is shown in Table 6. The result showed that *Z. officinale* extract, Ciprofloxacin (Cpx) and commercially prepared probiotic (PRO) were able to protect more than 50% of the eggs laid by adult-layer chickens against *E. coli.* The protection rate (PR) of *Z. officinale* extract against *E. coli* O157:H7 SS52 was significantly ($P<0.05$) lower than the PR of Cpx and significantly ($P<0.05$) greater that protection rate of PRO.

Also *Z. officinale,* PRO and Oxytetracycline (Oxy) were able to protect the eggs laid by adult-layer chickens against *S.* Typhimurium U288 but only *Z. officinale* extract and Oxy were able to give more than 50% protection. *Z. officinale* extract and Oxy had the same PR against *S.* Typhimurium U288 and their PR was significantly ($P<0.05$) higher than the PR of PRO. The result also revealed that PRO, *Z. officinale* extract and Oxy protected more than 50% of the eggs laid by the adult-layer chickens against *S.* Enteritidis FM366. The protection rate of *Z. officinale* extract against *S.* Enteritidis FM366 was significantly ($P<0.05$) lower than the PR

of Oxy and non-significantly greater than the PR of PRO.

The total mean viable plate counts of challenge isolates from the internal organs of chickens administered different antimicrobial substances are shown in Tables 7 and 8. The results revealed that there was no visible growth observed in the internal organs of those chickens administered Ciprofloxacin (Cpx), Oxytetracycline (Oxy), Vaccination (Bacterin) and vaccination plus commercially prepared probiotic (PRO). Also, no visible growth was observed in the liver of those chicks administered *Z. officinale* extract (Table 7). The total mean viable plate counts (TMPCs) from the liver of the chicks administered *Z. officinale* extract and PRO against *S.* Typhimurium U288 and *S.* Enteritidis FM366 were significantly ($P<0.05$) lower than the TMPCs from the liver of the infected chicks (Table 8). The results of the present study also revealed that the TMPCs from the internal organs administered *Z. officinale* extract were significantly ($P<0.05$) lower than the TMPCs from the internal organs of those chicks administered PRO. There was no visible growth observed in the organs from non-infected (normal) chicks. The inhibitory substances showed more protection to the internal organs of the chicks against *E. coli* O157:H7 SS52 than *Salmonella* species.

Table 1: Morphological characteristics of the isolates from chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Appearance on agar plate	Red colony on MA	Red colony on MA	Colourless with black center on SSA	Colourless and dark at the center on SSA	Colourless and dark at the center on SSA
Edge	Entire	Entire	Entire	Entire	Entire
Size (mm)	1.00	1.20	2.20	1.40	1.60
Consistency	Soft	Soft	Soft	Soft	Soft
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Slightly raised	Convex	Slightly raised	Slightly raised	Slightly raised
Pigmentation	–	–	–	–	–
Gram Reaction	–	–	–	–	–
Shape	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+

SSA = *Salmonella-Shigella* Agar, MA = MacConkey Agar, + = Positive, – = Negative

Table 2: Characteristics and identities of the enteric isolates from the chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Indole production	+	+	–	–	–
Hydrogen Sulphide	–	–	+	+	+
Ornithine decarboxylase	–	–	–	–	–
Methyl Red	+	+	+	+	+
Voges-Proskauer	–	–	–	–	–
Citrate Utilization	–	–	+	+	+
Catalase	+	+	+	+	+
Urease	–	–	–	–	–
Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
Dulcitol	–	–	+	+	+
Lactose	+	+	–	–	–
Xylose	+	+/–	+/–	+	+
Arabinose	+	+	+	+	–
Inositol	–	–	+	–	–
Mucate	–	–	–	+	+

E – *Escherichia coli*, G – *Escherichia coli*, 5 – *Salmonella* species, 7 –*Salmonella* species
11 – *Salmonella* species, + = Positive, – = Negative

Table 3: Molecular identities of the isolates

Isolate	Max score	Total score	Query Cover	Gap	Identity	Accession Number	Description
E	2856	2967	100%	0%	100%	CO010304.1	*Escherichia coli* strain 0157:H7 str SS52 Complete genome
G	1297	1297	100%	0%	96%	CP007594.1	*Escherichia coli* strain SEC470 Complete genome
5	2193	4386	100%	0%	98%	CP003836.1	*Salmonella enterica* subsp. enterica serovar Typhimurium str U288 Complete genome
7	660	660	100%	0%	96%	NG03836.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str FM366 Complete genome
11	2844	2844	100%	0%	100%	CP011428.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str YU39 Complete genome

Table 4: Phytochemical constituents of various extracts studied

Phytochemical constituent	*G. latifolium* (g/100g)	*P. guineese* (g/100g)	*X. aethiopica* (g/100g)	*Z. officinale* (g/100g)
Alkaloids	10.19	1.86	1.92	10.12
Tannins	7.62	2.81	0.62	4.38
Saponins	3.14	0.18	0.22	0.81
Flavonoids	1.06	0.10	0.44	5.62
Phenolics	1.25	1.81	1.51	1.32
Steroids	0.04	0.06	0.11	0.02
Cardiac glycosides	0.32	1.64	0.35	1.08

Table 5: Total mean viable plate counts of challenge isolates from the cloaca of adult-layer chickens administered different antimicrobial substances

Protection	*E. coli* O157:H7 SS52($x10^8$CFU/g)	*S.* Typhimurium U288 ($x10^8$CFU/g)	*S.* Enteritidis FM366 ($x10^8$CFU/g)
ZO	16.00 ± 2.00	5.00 ± 1.00	9.00 ± 1.00
Pro	25.00 ± 3.61	8.00 ± 0.00	13.00 ± 2.31
Cpx/Oxy	11.00 ± 1.73	2.00 ± 0.00	6.00 ± 1.00
C_1	67.00 ± 7.00	13.00 ± 1.58	29.00 ± 4.00
C_2	24.00 ± 0.00	3.00 ± 0.00	14.00 ± 0.00

ZO — *Zingiber officinale,* Pro — commercially produced probiotic, Cpx — Ciprofloxacin, Oxy — Oxytetracycline, C_1 — Infected chicken without protection, C_2 — Normal chicken

Table 6: Total mean viable plate counts of challenge isolates from the eggs laid by the adult-layer chickens administered different antimicrobial substances

Protection	x	*E. coli O157:H7 SS52*			*S.* ser. Typhimurium U288			*S.* ser. Enteritidis		
		P (%)	N (%)	PR (%)	P (%)	N (%)	PR (%)	P (%)	N (%)	PR (%)
ZO	30	3 (10)	27 (90)	88.46	1 (3.33)	29 (96.67)	75	8 (26.67)	22 (73.33)	57.89
Pro	30	11 (36.67)	19 (63.33)	57.69	3 (10.00)	27 (90.00)	25	9 (30)	21 (70)	52.63
Cpx/Oxy	30	0	30 (100)	100	1 (3.33)	29 (96.67)	75	6 (20)	24 (80)	68.42
C	30	26 (86.67)	4 (13.33)	0^a	4 (13.33)	26 (86.67)	0^a	23 (76.67)	7 (23.33)	0^a

ZO — *Zingiber officinale,* Pro — commercially produced probiotic, Cpx — Ciprofloxacin, Oxy — Oxytetracycline,
C — Control, 0^a — No protection, x — Number of eggs, T — Total number of eggs, P — Positive to enteric bacteria, N — Negative to enteric bacteria, PR — Protection Rate

Table 7: Total mean viable plate counts of *E. coli* O157:H7 SS52 re-isolated from the internal organs of the chicks administered different antimicrobial substances

Protection	Liver ($x10^8$CFU/g)	Lungs ($x10^8$CFU/g)	Heart ($x10^8$CFU/g)
ZO	0.00 ± 0.00	4.00 ± 1.73	6.00 ± 1.53
Pro	0.00 ± 0.00	7.00 ± 1.53	11.00 ± 1.73
Cpx	0.00 ± 0.00	1.00 ± 0.00	0.0 ± 0.00
C_1	28.00 ± 3.61	24.00 ± 3.61	39.00 ± 4.58
C_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

ZO — *Zingiber officinale,* Pro — commercially prepared probiotic,
Cpx — Ciprofloxacin, Vac — Vaccination, C_1 — Infected chicken without protection,
C_2 — Normal chicken

Table 8: Total mean viable plate counts of *Salmonella* species re-isolated from the liver of the chickens administered different antimicrobial substances

Protection	ST ($x10^8$CFU/g)	SE ($x10^8$CFU/g)
ZO	7.00 ± 1.00	4.00 ± 1.73
Pro	11.00 ± 2.65	14.00 ± 2.00
Oxy	0.00 ± 0.00	0.00 ± 0.00
C_1	36.00 ± 4.63	43.00 ± 1.73
C_2	0.00 ± 0.00	0.00 ± 0.00

ZO — *Zingiber officinale,* Pro — commercially prepared probiotic,
Cpx — Ciprofloxacin, Vac — Vaccination, C_1 — Infected chicken without protection,
C_2 — Normal chicken
ST------- *S.* ser. Typhimurium U288 SE---------- *S.* ser. Enteritidis FM366

DISCUSSION

The presence of *Escherichia coli* O157:H7 SS52, *Escherichia coli* SEC470, *Salmonella enterica* subspecies *enterica* serovar Typhimurium U288, *Salmonella enterica* subspecies *enterica* serovar Enteritidis FM366 and *Salmonella enterica* subspecies *enterica* serovar Enteritidis YU39 from studied feed samples supported the occurrence enteric bacteria in the samples (Davies and Wales, 2010; Chowdhuri *et al.,* 2011; Fredrick and Huda, 2011).

The phytochemical constituents present in the plant extracts could be responsible for the antibacterial activity of the various sample extracts. Similar findings were made by different researchers (Parekh *et al.,* 2005; Iheukwumere *et al.,* 2012).

In this research, an *in vivo* study was carried out to determine the protection rate of *Zingiber officinale*

extract, oxytetracycline, ciprofloxacin and commercially prepared Probiotics. The significant decrease in TMPCs of the protected–infected chickens administered *Z. officinale* extract, commercially prepared probiotics and antibiotics (ciprofloxacin/Oxytetracycline) as compared to the infected chickens is in consonance with the findings of other researchers (Liu *et al.,* 2001; Clifton-Hadley *et al.,* 2002; Davis and Breslin, 2003; Radwan, 2007). The significant decrease of the TMPCs of those chickens administered antibiotics below the TMPCs of non-infected chickens (normal control) shows the loss of protective normal intestinal flora ecology within the cloacae (Rofail and Germin, 2003).

The non-significant decrease in TMPCs of those chickens administered commercially prepared probiotics as compared to non-infected chickens (normal control) shows the roles of probiotics in re-establishment of the normal flora ecology and reduction of the colonization of the pathogenic enteric bacteria in the cloacae. These findings are in parallel with the findings of other researchers (Tellez *et al.,* 2001; Rahimi *et al.,* 2007). The protective efficacy of the probiotics which contained *Lactobacillus* spp. against *S.* Enteritidis infection was evaluated by Samanta and Biswas (2005); Somoro *et al.* (2002); Timmerman *et al.* (2006); Wafaa *et al.* (2006) who detected significant decreased in mortality of *S.* Enteritidis infected chickens. Higgins *et al.* (2007) and Vicente *et al.* (2007) concluded that effective probiotics may accelerate the development of normal microflora in chicks and increase the resistance to infection by some enteric bacterial pathogens. *Lactobacillus* spp. have been widely reported to produce antibacterial compounds called bacteriocins and the effect of bacteriocins have been hypothesized to be the mechanism by which *Lactobacillus* spp. exert cytotoxic effects *in vivo* (Bogovic–Matijastic *et al.,* 2008; Ocan *et al.,* 2009)

The total mean viable plate counts of challenge isolates from the eggs laid by infected adult-layer chickens administered *Z. officinale* extract, antibiotics (ciprofloxacin/Oxytetracycline) and commercially prepared probiotics showed that these substances were able to give reasonable protections to eggs against *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 of which antibiotics showed most pronounced protection. It was documented that the frequency of enteric bacteria re-isolated from the eggs (shells or contents) was significantly reduced in protected adult-layer chickens (Okamura *et al.,* 2005; Radwan *et al.,* 2007)

The absence of growth observed in the internal organs administered ciprofloxacin, Oxytetracycline in this study supports the findings of Wafaa *et al.* (2012). Several researchers have documented that the frequency of enteric bacteria re-isolation from the internal organs was significantly reduced in protected chickens (Khan *et al.,* 2003; Okamura *et al.,* 2005; Radwan *et al.,* 2007). The significant decrease in TMPCs of the internal organs of those chickens administered *Z. officinale* extract and commercially prepared probiotics as compared to the TMPCs from infected organs supported the findings of many researchers (Barbour *et al.,* 2003; Nisbet *et al.,* 2006; Wafaa *et al.,* 2012). The reducing effect of probiotics on the colonization of enteric bacteria was studied comprehensively by several researchers. It was reported that probiotics maintained or increased the normal intestinal flora which are normally found in the intestinal tract of hatched chicken and these flora can exclude enteric bacteria colonization (Mead, 2000; Seo *et al.,* 2000; Wafaa *et al.,* 2012).

The protection achieved by those chickens fed with diet supplemented with commercially prepared probiotics could be attributed to the possible protective roles of probiotics in nature. The probiotics produced lactic acid that created unfavourable P^H for the growth of the enteric bacteria pathogens (Alkoms *et al.,* 2000; Johansen *et al.,* 2004). The probiotics also compete with the pathogens (Wafaa *et al.,* 2012) and produced bacteriocin that was toxic to the enteric bacteria (Pascual *et al.,* 2009). The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on an acquired immune response exerted by T and B lymphocytes. The direct effect might be related to the stimulation of lymphatic tissue (Kabir *et al.,* 2004), whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract or through the reduction of enteric bacteria pathogen colonization. Shoeib *et al.* (2007) reported that the bursa of probiotic treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. Christensen *et al.* (2002) suggested that some of these effects were mediated by cytokines secreted by immune cells stimulated by probiotic bacteria.

CONCLUSION

This study has revealed the presence of *Escherichia coli* O157:H7 SS52*, E. coli* SEC470 *Salmonella* serovar Typhimurium U288, *Salmonella* serovar Enteritidis FM366 and *Salmonella* Enteritidis YU39 in the chicken feed samples randomly collected from major towns in Anambra State, of which the occurrences of E. coli SEC470 and S. serovar Enteritidis YU39 were negligible due to very low counts of the isolates from the studied samples. The *in vivo* study of the susceptibility patterns of these organisms to both natural and synthetic antibiotics showed the safety and pronounced activities of *Zingiber officinale* extracts, ciprofloxacin, oxytetracycline and commercially prepared probiotics. From this study, it could be concluded that the use of the studied antimicrobial agents in the right doses could be an effective and safe method of preventing *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 infections in chickens. It should be taken

into consideration that this practice must go in parallel with bio-security measures and good management practices to eradicate *E. coli* O157:H7 SS52*, S.* serovar Typhimurium U288 and *S.* serovar Enteritidis FM366 infections in chicken flocks.

REFERENCES

Ali, A., Uzma, S., Shabir, A. K., Imran, A., Muhammed, I. K., Tanrawee, P. and Anil, K. A. (2014). Presence of *Escherichia coli* in poultry meat: A potential food safety threat. *International Food Research Journal* **21**(3):941 – 945.

Alkoms, H. L., Skytta, E., Saaela, M., Maltila, S. T., Latra, K, K. and Helender, I. M. (2000). Lactic acid permeabilizes Gram-negative bacteria by disturbing the outer membrane. *Applied Environmental Microbiology* **66**:2001 – 2005.

Anonymous (2018).

Arotupin, D.J., Kayode, R.M.O. and Awojobi, K.O. (2007). Microbioilogical and physicochemical qualities of Selected commercial poultry feeds in Akure, Nigeria. *Journal of Biological Sciences* **7**(6): 981 – 984.

Barbour, E. K., Frerichs, W. M., Nabbut, N. H., Poss, P. E. and Brinton, M. K. (2003).Evaluation of bacterins containing three predominant phage types of *Salmonella* Enteritidis for prevention of infection in egg-laying chickens. *American Journal of Veterinary Resource* **54**:1306 – 1309.

Bogovic-Matijasic, B., Rogelji, I., Nes, I. F. and Holo, H. (2008).Isolation and characterization of two bacteriocins of *Latobacillus acidophilus* LF221. *Applied Microbiology Biotechnology* **49**:606 – 612.

Cheesbrough, M. (2000).District Laboratory Practice in Tropical African Countries, First Edition .Cambridge University Press, Cambridge, UK, pp. 157–159.

Chowdhuri, A., Iqbal, A., Giasuddin, M. and Bhuiyan, A. A. (2011). Study on isolation and identification of *Salmonella* and *Escherichia coli* from different poultry feed of savar region of Dhaka, Bangladesh. *Journal of Science Resources* **3**(2):403–411.

Christensen, H. R., Frokraer, H. and Pestka, J. J. (2002).*Lcatobacillus* differently modulate expression of cytokines and maturation surface markers in murine dendritic cells. *Journal of Immunology* **186**:171–178

Clifton-Hadley, F. A., Breslin, M., Venables, L. M., Sprigings, K. A., Cobles, S. W., Houghton, S. and Woodward, M. J. (2002). A laboratory study of an in activated bivalent iron restricted *Salmonella* Enteritidis and Typhimurium dual vaccine against *S.* Typhimurium challenge in chickens. *Veterinary Microbiology* **89**(2-3):167–179.

Dashe, Y. G., Raji, M. A., Abdu, P. A. and Oladele, B. S. (2013).Distribution of aerobic bacteria in visceral organs of sick and apparently healthy chickens in Jos, Nigeria. *International Research Journal of Microbiology* **4** (3):79–83.

Davies, R. and Breslin, M. (2003).Effects of vaccination and other preventive methods from *S.* Enteritidis on commercial laying chickens farms. *Veterinary Resource* **153**(22):673–677.

Davies, R. H. and Wales, A. D. (2010). Investigation into *Salmonella* contamination in poultry feed mills in the United Kingdom. *Journal of Applied Microbiology* **109**:1430 –1440.

D'Mello, J.P.F. (2006).Microbiology of animal feed. *Journal of Biological Sciences* **7**(6): 981– 984.

Frederick, A. and Huda, N. (2011).*Salmonellas,* poultry house environments and feeds: A review. *Journal of Animal and Veterinary Advances* **10**(5):679– 685.

Gabriela, I. F., Cecilia, L. E., Teresa, I. C. and Maria, E. E. (2014).Detection and characterization of shiga toxin producing *Escherichia coli, Salmonella* species and *Yersinia* strains from human, animal and food samples in San Luis, Argentina. *International Journal of Microbiology* **2014**:1–11.

Habtamu, T. M., Rajesh, R., Kulip, D. and Rajesh, K. A. (2011). Isolation, identification and polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. *International Journal of Microbiological Research* **2**:135–142.

Higgins, J. P., Higgins, S. E., Salvador, V., Wolfenden, A. D., Tellez, G. and Hargis, B. M. (2007). Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in poultry products. *Poultry Science* **84**:1141–1145.

Iheukwumere, I., Uba, B. O. and Ubajekwe, C. C. (2012). Antibacterial activity of *Annona murricata* and *Persea americana* leaves extracts against ampicillin- resistant *Staphylococcus aureus. Journal of Science Engineering and Technology* **19**:10786–10798.

Iheukwumere, I. H. and Umedum, C. U. (2013). Effect of *Gossypium hisutum* leaf extracts on Gram negative bacteria isolated from cervix of females with unexplained infertility. *African Journal of Science* **14**:3261–3270.

Johanseen, S. A., Griffith, R. W., Wesley, I. V. and Scanes, C. G. (2004).*Salmonella enteric* serovar Typhimurium colonization of the crop in the domestic turkeys: Influence of probiotic and probiotic treatment (*Lactobacillus acidophilus). Avian Discovery* **48**:279–286.

Kabir, S. M. L., Rahman, M. M., Rahman, M. B. and Ahmed, S. U. (2004).The dynamics of probiotics on

growth performance and immune response in broilers. *International Journal of Poultry Science* **3**:361–365.

Khan, A. A., Nwaz, M. S., Khan, S. and Sernigella, C. E. (2009).Detection of multidrug resistant *Salmonella* Typhimurium DT104 by multiplex polymerase chain reaction. *FEEMS Microbiology Letter* **182**:355 – 360.

Liu, W., Yang, Y., Chung, N. and Kwang, J. (2001).Induction of humoral immune response and protective immunity in chickens against *S.* Enteritidis after a single dose of killed bacterium-loaded microspheres. *Avian Discovery* **45**:497–806.

Mead, G.C. (2000). Prospects of competitive exclusion treatment to control *Salmonella* and other food borne pathogens in poultry. *The Veterinary Journal* **59**:111–123.

Nisbet, D. J., Corrier, D. E., Ricke, S. C., Hume, M. E., Byrdii, J. A. and deLoach, J. R. (2006). Maintenance of the biological efficacy in chicks of a caecal competitive exclusion culture against *Salmonella* by continous-flow fermentation. *Journal of Food Protection* **59**:1279–1283.

Ocan, V. S., deRuiz, H. A. A. and Nader-Macias, M. E. (2009).Characterization of bacteriocin-like substance produced by vaginal *Lactobacillus salivarius* strain. *Applied Environmental Microbiology* **65**:5631–5635.

Oguttu, J.W., Veary, M. and Picard, J.A. (2008). Antimicrobial drug resistance of Escherichia coli isolated from poultry abattoir workers at risk and broilers on antimicrobials. *Journal of the South African Veterinary Association* **79**(4): 161–166.

Okamura, H., Lillehoj, S., Rayburne, R. B., Babu, U. and Heckert, R. (2005). Antigen-specific lymphocyte proliferation and interleukin production in chickens immunized with killed *S.* Enteritidis vaccine experimental subunit vaccines. *Avian Discovery* **47**(4):1331–1338.

Onyeze, R.C., Onah, G.T. and Eluke, O. (2013). Bacterial contaminants associated with commercial poultry feeds in Enugu, Nigeria. *International Journal of Life Sciences Biotechnology and Pharmaceutical Research* **2**(3):432–433.

Parekh, J., Nair, R. and Chanda, S. (2005). Preliminary screening of some forklore medicinal plants from western indica for potential antimicrobial activity. *Indian Journal of Pharmacology* **37**:408–409.

Pascual, M., Hugas, M., Badiola, J. I. Monfort, J. M and Garriga, M. (2009).*Lactobacillus salivarius* CC2197 prevents *Salmonella* Enteritidis colonization in chickens. *Applied Environmental Microbiology* **65**:4981–4986.

Radwan, H. M. (2007). Studies on *Salmonella* infection in chicken flocks. Masters in Vetinary Science, Thesis in.Faculty of Vetinary Medicine, Department of Avian and Aquatic, Animal Medicine, Alexander University, Egypt, pp. 89–103.