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Antibiotic Treatment and Antibiogram Profiles of *E.coli* Isolated from Selected Poultry Farms in Ido-Ekiti

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Authors' contributions

This work was carried out in collaboration between both authors. Author AOO designed the study managed the analyses of the study. Author KOO wrote the protocols, wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Antibiotic therapy in poultry is alleged to spread multiple antibiotic resistant bacteria**.** This study seeks to correlate antibiotic treatment in poultry with the occurrence of multiple bacterial resistances to critically important antibiotics and also determine the potential sources of acquisition of these bacteria.
Study Design: Experimental design.
Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti between February 2017 and December 2019.
Methodology: Data on antibiotic treatment, poultry management practices and use of natural water bodies in the area of study were collected from farm managers and residents using a questionnaire. The locations of the source area and their relative distances were determined using the Geographical System Information Software, Mapit. Fresh fecal droppings from poultry birds were randomly sampled with a sterile swab stick and transferred into a freshly-procured, sealed, factory-packed polythene bag. Farm feed, water, and soil from disposal sites were also collected in sterile universal containers. The suspension of the fecal droppings was streaked on Eosin Methylene Blue agar plates with sterile wire loop and incubated at 37 °C for 18-24 hours. Soil and water samples

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from the waste disposal sites were serially diluted and streaked as previously. The isolates were characterized using relevant biochemical tests. Modified Kirby Bauer method was used to determine the isolates' susceptibility to nine tested antibiotics, and the results were interpreted based on CLSI guidelines.

Results: Most of the examined birds, (92%) were exposed to antibiotics, of which 64% of the antibiotics were administered for therapeutic purposes, prophylaxis (27%) and enhancement of egg production in layers (9%). All the antibiotics were administered by mixing them in a specified quantity of water. The majority (46%) of the birds were first exposed to antibiotics at the age of 2 weeks. Routine charts were used by 25% of the farmers for the administration of antibiotics. Data from farm records show that eleven antibiotics were selectively used on the farms. Sulfonamide and diaveridine, an anti-coccidiostat, were administered in most of the farms (50%), while tylosine, metronidazole and chloramphenicol recorded the least (8.3%). Carbapenem, penicillin, and cephalosporin were not administered. From the data obtained on antibiotics-presence in two retail markets, tetracycline, neomycin, gentamycin and erythromycin were available in both retail markets, cephalosporins, meropenem, and metronidazole were not sold for poultry consumption. Both the percentage occurrences of *E. coli* from fresh poultry droppings (44.1%) and waste disposal sites (33.3%) were relatively low. Higher antibiotic percentage resistance to ciprofloxacin (87%), ofloxacin (83%), sulfonamide, and tetracycline (78%) were found in the isolates. Multiple antibiotic-resistant profiles occurred in patterns and different patterns were replicated across various farms. Also, 46 multiple antibiotic-resistant patterns were recorded, and two of these patterns (AMC, CIP, OFX, CN, SXT, TET and CRO, CAZ, AMC, CIP, OFX, CN, MEM, SXT) were spread across 50% of the farms. There was history of diarrhea in some respondents (12%), linked to human exposure to contaminated natural water bodies. From correlation studies, both data on antibiotic treatment and that from market survey were directly related to the antibiotic-resistant profiles of isolates. The Spearman correlation coefficient are (r_s (3) = 0.866, p = .333) and (r_s (3) = 0 .667, p = .500) respectively.

Conclusion: The development and spread of multiple antibiotic-resistant bacteria of poultry origin are primarily attributed to poor antibiotic formulation policy, crude antibiotic treatment on poultry farms, and indiscriminate supply of antibiotics to untrained poultry personnel. The effectiveness of the super drugs used for the treatment of superbugs in poultry birds may be undermined if policies on the antibiotic formulation, dispensing, and therapy are not reviewed.

Keywords: Poultry; antibiotic treatment; poultry droppings; resistance.

Abbreviations

M. A. R. B. - Multiple antibiotic resistant bacteria

1. INTRODUCTION

Poultry farming is practiced globally for dietary and economic purposes. Chicken meat is the most preferred and reared species of poultry birds [1]. Despite the huge economic prospect of the poultry industry, it is threatened by multiple antibiotic-resistant bacteria (MARB). These pathogens constitute a major challenge to the development of the poultry industry [2]. Culturally-based disease prevention and control measures comprising routine immunization, health monitoring, bio-security, farm hygiene, and sanitation are employed in poultry management; however, they were reported to achieve limited success warranting antibiotic treatment in the business of poultry production [3]. Antibiotics are used in poultry production sites at a therapeutic dose to treat infections and at a prophylactic dose to prevent diseases and reduce the burden of intensive medical care for deadly diseases in birds. They are also used as growth promoters or feed supplements [4]. There are assertions that the rates of illness and death from bacterial infection in poultry will be higher than at present if antibiotics are not administered [5]. Bacteria are known to cause infectious diseases like fowl cholera, salmonellosis, paratyphoid infection, pullorum disease, compylobacteriosis, cellulitis coliobacillosis, pasteurellosis, coliform, botulism, mycoplasmosis, staphlococcosis, and avian tuberculosis [6].

Frequently used antibiotics for treatment in Nigerian poultry farms include erythromycin, tetracycline, ampicillin, chloramphenicol, neomycin, sulphonamides, enrofloxacin, furazolidone, nitrofurantoin, streptomycin [7]. Some of the critically-important antibiotics such

as the fluoroquinolones and newer generation of cephalosporins used in animal care are also considered to be critically-important in human. These two antibiotic-classes are not recommended as prophylaxis or for first-line treatment in poultry [8]. Poultry farming is increasingly practiced within residential areas in Nigeria and poultry droppings from the farm can contaminate food, water, soil and increase the burden of antibiotic-resistant bacteria in the immediate environment. Thus humans are at risk of contracting infection due to exposure to these bacteria.

There is an emerging threat of infections caused by multiple antibiotic-resistant bacteria in poultry. The potency of many antibiotics in veterinary medicine has been undermined, and the few available adequate reserves may be under a threat of extinction [9]. Bacterial pathogens can be resistant to many antibiotics, and they are often designated as superbugs [8]. Also, infections caused by multiple antibiotic-resistant bacteria can lead to treatment failure, worsened health conditions, and compromise both human and animal health [10].

Consequently, antibiotics are now endangered species with a threat of extinction due to the global emergence of antibiotic resistance [9]. Most generally-recommended antibiotic-classes for treatment, 80% are also administered in animal production sites. This can promote spread of antibiotic resistant bacteria from animal production facility to humans and endanger public health. Besides, statistics on antibiotic consumption in a poultry production facility is quite alarming [11]. Poultry and other farm animals are the critical reservoirs for multiple antibiotic-resistant *E. coli*. The indiscriminate use of antibiotics in animal production sites is considered the most important factor that promotes the emergence and spread of antibiotic resistant bacteria in poultry [12]. In contrast, some correlation studies have implicated other contributory factors to be responsible for the development and spread of multiple antibiotic-resistant bacteria in poultry [13,14]. It is necessary to determine if antibiotic treatment is the single cause of antibiotic resistance in poultry or if others exist.

2. MATERIALS AND METHODS

2.1 Research Tools

A brief questionnaire was administered to farm managers and residents, data on antibiotic treatment and poultry management practices were collected.

2.2 Determination of the Source Area

The coordinates of the poultry sites, natural water bodies, source area, and their distances were determined using the geographical system information software Mapit GIS as described [15].

2.3 Study Population and Site

The study population comprises layers, broilers, turkeys, and free-range birds from poultry farms in Ido-Ekiti and Usi-Ekiti in Ido-Osi local government area of Ekiti State. A total of 204 fecal droppings, 12 feed samples, 12 water samples, and 12 samples (soil/water) from disposal sites were collected.

2.4 Collection of Samples

Fresh fecal droppings from poultry birds were randomly sampled with a sterile swab stick and transferred into a freshly procured, sealed, factory-packed polythene bag. Farm feed, water, and soil from disposal sites were also collected in sterile universal containers and immediately transferred to the Microbiology Laboratory, Ekiti State University, Ado-Ekiti, for bacteriological analysis [16]. The samples were cultured within 2 hours of collection.

2.5 Isolation Techniques

Swab sticks containing the fecal droppings were suspended in 5 mL of sterile saline water, prepared as 10% suspension. The suspension was streaked on E.M.B plates with sterile wire loop and incubated at 37 °C for 18-24 hours. Distinct colonies with a green metallic sheen and dark centers from the primary culture were preliminarily identified as *E. coli* [7,16]. One gram of the collected soil sample was weighed and added to a test tube containing 9 mL of sterile distilled water. A volume of 1 mL of the stock water sample was serially diluted in series of test tubes containing 9 mL of sterile distilled water. About 0.1 mL of the tenth-fold dilution from the eighth tube was inoculated into plates, and pour plating was carried out with eosine methylene blue agar. The plates were incubated at 37 °C for 24 hours. A loopful of diluents from test tubes with a dilution factor of 10^{-5} and 10^{-6} were streaked on EMB agar. The plates were incubated as previously. Distinct colonies of *E.*

coli were sub-cultured on sterile eosin methylene blue agar to obtain a pure secondary culture. They were further preserved on nutrient agar slant for biochemical tests [7].

2.6 Antibiotic Susceptibility Test

Antibiotic susceptibility testing was carried out using the agar disc-diffusion method. Antibiotic discs (Oxoid) comprising ciprofloxacin (5 µg), tetracycline (30 µg), ofloxacin (5 µg), trimethroprim/sulfamethozazole (1.25/23.75 µg), gentamycin (10 µg), amoxicillin-clavulanic acid (20/ 10 µg), ceftaxidime (30 µg), meropenem (10 µg) and cefriazone (30 µg) were used. Mac Farland standard of 0.5 which gives an inoculum size of 1.5×10^{-8} CFU/mL was used to standardize the experiment. A sterile cotton swab was dipped into the standardized broth culture and excess inoculums were drained by pressing the cotton swab against the test tube above the broth suspension. The swab stick was evenly spread over the entire surface of the 15 mL Muella Hinton agar plate to obtain uniform distribution of inoculums. The inoculated plates were then allowed to dry for 3-5 minutes. Antibiotics impregnated discs were loaded and positioned on the surface of the inoculated plates with a multiple disc dispenser to ensure adequate spacing of the discs. A forceps was used to press the antibiotic discs slightly on the agar to ensure contact and diffusion of antibiotics into the agar medium [17]. The plates were inverted and incubated at 37 °C for 16-18 hours. The cultures were later examined and the diameters of the zones of inhibition were recorded and interpreted as susceptible, intermediate and resistant based on procedures of CLSI, 2013 [18].

2.7 Statistical Analysis

Statistical analysis was carried out using IBM SPSS statistics, version 20.0 to analyze the Spearman correlation coefficient [19].

3. RESULTS AND DISCUSSION

The types of antibiotics administered on the farms are shown in Table 1. The administered antibiotics were enrofloxacin, erythromycin, tetracycline, gentamycin, streptomycin, neomy cin, colistin, cotrimoxazole, metronidazole, and chloramphenicol. Cotrimoxazole was adminis tered in 5 farms. Anticoccidistats were also administered in some farms. Antibiotics were not administered on the free range birds.

The antibiotic treatment profile of the birds is shown in Table 2. Ninety two percent of the birds were exposed to antibiotics of which 64% were administered for therapeutic purpose, prophylaxis (27%) and enhancement of egg production in layers (9%). All the antibiotics were administered by mixing them in specified quantity of water. Majority of the birds (46%) were first exposed to antibiotics at age of 2 weeks. Routine charts were used by 25% of the farmers for administration of antibiotics. Farm records show that eleven antibiotics; enrofloxacin, erythromycin, tetracycline, gentamycin, streptomycin, neomycin, colistin, cotrimoxazole, metronidazole, tylosine and chloramphenicol were selectively used on the farms. The birds were not exposed to cephalosporin, penicillin and meropenem. Sulfonamide (cotrimoxazole) and diaveridine an anticoccidiostat were administered in most of the farms (50%), Based on the antibiotic-classes administered, sulfonamides were administered most (50%), while nitroimidazole and amphenicol were the least administered (8%). Carbapenem, penicillin and cephalosporin were not administered. From the survey of two retail markets, tetracycline, neomycin, gentamycin and erythromycin were available in both markets. Cephalosporins, meropenem and metronidazole were not sold for poultry consumption in the market.

The isolation rate of *E. coli* from poultry in Ido-Ekiti is shown in Table 3. A total of 204 poultry droppings, 12 feed samples, 12 water samples and 12 samples from disposal sites were examined. A total of 94 isolates consisting of 90 samples from fecal droppings and 4 from waste disposal sites were obtained. The percentage occurrence of *E. coli* from all the examined birds was 44.1%. Farm K comprising free-range birds recorded the highest isolation rate (82%), while the lowest isolation rate, (18%), was recorded in farms B and C comprising layers and broilers. Besides, *E. coli* was also isolated from waste-water collected from waste disposal site of a farm and soil samples from waste disposal sites of four farms. The isolation rate of samples obtained from poultry waste disposal sites was 33.3%.

The antibiotics percentage resistant-profile of *E. coli* isolated is shown in Table 4. The average resistance of the isolates to ciprofloxacin was the highest (87%), followed by ofloxacin (83%), cotrimoxazole and tetracycline (78%), gentam ycin and amoxicillin clavulanate (72%), cefriazone (64%), ceftaxidime (60%). The

average resistance of the isolates to meropenem was the lowest (40%). Besides, resistance to multiple classes of antibiotics was recorded in 95.7% of the isolates

The multiple antibiotic resistant patterns of *E. coli* isolated are shown in Table 5. There was replication of many antibiotic resistant patterns in different farms. A total of three different multiple antibiotic resistant-patterns, the lowest occurred in 4 farms and a disposal farm while a total of 9 patterns, the highest were recorded in one farm. A total of Forty-six different multiple antibiotic-resistant patterns were recorded in all the farms.

The natural water sources located within the area of study and their uses are shown in Table 6. The water bodies consist of spring and streams in Ido and Usi-Ekiti. They were used for religious, domestic, recreational, fish farming and construction activities. There was previous history of diarrhea attributable to exposure to contaminated natural water bodies in 14% of the respondents in the area of study.

The Spearman's statistical correlation of antibiotics treatment and resistant profile is shown in Table 7. There was a strong, positive correlation between antibiotics used for treatment and resistant profile of the isolates, which was statistically significant ($r_s(3) = 0.866$, $p = .333$). Also, a strong, positive correlation was recorded between level of antibiotics-presence from market survey and resistant profile of the isolates, which was statistically significant ($r_s(3) = 0.667$, $p = .500$).

4. DISCUSSION

Many of the administered therapeutic agents were combinations of various classes of substances with different mechanisms of action. This formulation strategy is intended to enhance synergy of the multiple components. The high level of antibiotic resistance observed in poultry may be associated with the crude use of antimicrobial substances administered on the birds. Besides, the development and spread of multiple antibiotic-resistant bacteria of poultry origin is largely attributable to policies on antibiotic production involving combination of different antibiotics as a single formulation, and indiscriminate supply of antibiotics to untrained poultry personnel. The effectiveness of super drugs used for treatment of super bugs in poultry birds may be undermined if policies on antibiotic formulation, dispensing and treatment are not reviewed.

Also, administering antibiotics in water was a common practice and they were administered majorly for therapeutic purposes. Antibiotics were also administered for disease prevention and enhancement of egg production in layers. The administration of metronidazole, an antimicrobial, primarily used in humans and canine for treatment of poultry birds may constitute an abuse of the substance. Antibiotics were routinely administered on newly acquired birds on some farms. This disease preventive measure was presumed to prevent the spread of specific diseases believed to be endemic or prevalent with birds from specific hatcheries. The fluoroquinolones and cephalosporins are critically essential antibiotics in human medicine [8]. The indiscriminate use of these antibiotics together with penicillin in poultry production can select for normal microbial population that are resistant to antibiotics.

Comparatively, the observed isolation rates of *E. coli* in both fecal and waste samples were low. These relatively low isolation rates might be attributed to the sample size. The higher level of occurrence of the bacteria in poultry droppings might be associated with a lower die-off rate of *E. coli* in fresh fecal samples than in stale samples with prolonged exposure to harsh environmental conditions. Besides, the abiotic factors may lead to loss of genes that are responsible for the metabolism of certain nutrients and promotion of bacterial growth outside their primary. However, these isolation rates contrast with [20], who recorded an isolation rate of 83% in fecal droppings from poultry sources. The presence of multiple-antibiotic resistant *E. coli* in soil and water samples from disposal sites shows that contaminated soil and water in the environment can be a secondary reservoir of multiple antibiotic-resistant bacteria. Improper poultry waste disposal and wandering activity of free-range birds could constitute a mobile source of reach of the bacteria from fecal materials to the environment.

Table 1. Types of antibiotics/antimicrobials administered on each farm

Farm code	ENR	ERY	CN	SXT	TET	NEO	STR	COL	MET	CHL	TYL	DIA	DIC	Total
A	+	-	-	+	-	-	-	-	-	-	-	+	-	3
B	-	+	+	+	+	+	+	+	-	-	-	+	-	8
C	-	-	+	-	-	-	-	-	-	-	-	-	-	1
D	+	-	-	-	-	-	-	-	-	-	-	-	+	2
E	-	+	-	+	+	-	+	+	-	-	-	+	+	7
F	-	-	-	+	-	-	-	-	-	-	-	+	-	2
G	-	-	-	+	-	-	-	-	-	-	-	+	-	2
H	-	-	-	-	+	-	-	-	+	-	-	-	-	2
I	-	-	-	-	-	+	-	-	-	+	-	-	-	3
J	-	-	-	-	-	-	-	-	-	-	-	-	-	0
K	-	-	-	-	+	-	-	-	-	-	-	-	-	1
L	-	-	+	+	+	-	-	-	-	-	+	+	-	5
Total	2	2	3	6	5	2	2	2	1	1	1	6	2	

Key: ENR- Enrofloxacin, ERY- Erythromycin, CN-Gentamycin, SXT-Trimethroprim/ sulfamethoxazole, TET- Tetracycline, NEO- Neomycin, STR- Streptomycin, COL- Colistin, MET-Metronidazole, CHL-Chloramphenicol, DIA-Diaveridine , TYL- Tylosine, DIC- Diclazuril

Table 2. Antibiotic consumption profile of poultry birds examined

Characteristics		Frequency (%)
Exposure to antibiotics	Yes	11 (91.7)
	No	1(8.3)
Purpose of usage	Disease prevention	3 (27.3)
	Treatment	7 (63.6)
	Enhance egg production	1 (9.1)
Method of administration	Water	11 (100)
	Feed	0 (0)
	Injection	0 (0)
Age of birds when first exposed	2 weeks	5 (45.5)
	1 month	3 (27.3)
	2 months	2 (18.2)
	3 months plus	1 (9.1)
Routine chart for antibiotic use	Yes	3 (25)
	No	9 (75)
Frequency of administration of antibiotics	Cotrimoxazole	6 (50)
	Tetracycline	5 (41.7)
	Gentamycin	3 (25)

Characteristics		Frequency (%)
	Enrofloxacin	2 (16.7)
	Erythromycin	2 (16.7)
	Streptomycin	2 (16.7)
	Neomycin	2 (16.7)
	Colistin	2 (16.7)
	Metronidazole	1 (8.3)
	Chloramphenicol	1 (8.3)
	Tylosine	1 (8.3)
Frequency of administration of classes of antibiotics	Sulphonamide	6 (50)
	Tetracycline	5 (41.7)
	Aminoglycoside	5 (41.7)
	Fluoroquinolone	2 (16.7)
	Macrolide	2 (25)
	Polymyxin	2 (16.7)
	Nitroimidazole	1 (8.3)
	Amphenicol	1 (8.3)
Product availability from market survey	Tetracycline	2 (100)
	Cotrimoxazole	1 (50)
	Neomycin	2 (100)
	Gentamycin	2 (100)
	Erythromycin	2 (100)
	Penicillin	1 (50)
	Enrofloxacin	1 (50)
	Streptomycin	1 (50)
	Colistin	1 (50)
	Chloramphenicol	1 (50)
	Tylosine	1 (50)

** Numbers in parenthesis are percentage value*

Table 3. Isolation rate of *E. coli* from poultry in Ido-Ekiti

							Waste sources/samples examined		
Poultry site	**No of Poultry droppings examined**	**No positive (%)**	**Poultry feed examined**	**No positive (%)**	**Poultry Water examined**	**No Positive (%)**	**Waste water samples examined**	**Soil samples examined**	**No. positive (%)**	**Total**
A (Pullet)	17	5 (29.4)	1	-	1	-	-	1	-	20
B(Layer)	17	3 (17.7)	1	-	1	-	-	1	-	20
C(Broiler)	17	3 (17.7)	1	-	1	-	-	1	-	20
D(Broiler)	17	5 (29.4)	1	-	1	-	-	1	-	20
E(Turkey)	17	6 (35.3)	1	-	1	-	-	1	-	20
F(Turkey)	17	5 (29.4)	1	-	1	-	-	1	-	20
G(Layer)	17	8 (47.1)	1	-	1	-	-	1	1	20
H(Layer)	17	11 (64.7)	1	-	1	-	-	1	-	20
I(Layer)	17	13 (76.5)	1	-	1	-	-	1	1	20
J(Cockerel)	17	12 (70.6)	1	-	1	-	-	1	-	20
K(Local)	17	14 (82.4)	1	-	1	-	1	-	1	20
L(Layer)	17	5 (29.4)	1	-	1	-	-	1	1	20
Total (%)	204	90 (44.1)	12	-	12	-	1	11	4 (33.3)	240 (100)

** Numbers in parenthesis are percentage val*

Table 4. Antibiotics percentage resistant profile of *E. coli* isolated

		Cephalosporin		Penicillin	Fluoroquinolone		Aminoglycoside	Caberpenem	Sulfonamide	Tetracycline	
Source code	**N**	**CRO (%)**	**CAZ (%)**	**AMC (%)**	**CIP (%)**	**OFX (%)**	**CN (%)**	**MEM (%)**	**SXT (%)**	**TET (%)**	**n (%)**
A	5	0	0	5 (100)	4 (80)	3 (60)	4 (80)	0	3 (60)	4 (80)	4 (80)
B	3	1 (33.3)	1 (33.3)	3 (100)	1 (33.3)	1 (33.3)	1 (33.3)	0	1 (33.3)	3 (100)	3 (100)
C	3	1 (33.3)	1 (33.3)	3 (100)	1 (33.3)	2 (66.7)	3 (100)	0	2 (66.7)	2 (66.7)	3 (100)
D	5	1 (20)	1 (20)	5 (100)	4 (80)	5 (100)	5 (100)	0	2 (40)	3 (60)	5 (100)
E	6	0	0	5 (83.3)	5 (83.3)	5 (83.3)	3 (50)	2 (33.3)	4 (66.7)	5 (83.3)	6 (100)
F	5	1 (20)	1 (20)	5 (100)	5 (100)	5 (100)	1 (20)	3 (60)	4 (80)	4 (80)	5 (100)
G	8	5 (62.5)	5 (62.5)	2 (25)	8 (100)	6 (75)	3 (37.5)	3 (37.5)	6 (75)	7 (87.5)	8 (100)
H	11	9 (81.9)	8 (72.7)	7 (63.6)	11 (100)	9 (81.9)	8 (72.7)	1 (8.7)	11 (100)	11 (100)	11 (100)
I	13	13 (100)	12 (92.3)	9 (69.2)	11 (84.6)	12 (92.3)	13 (100)	9 (69.2)	10 (76.9)	10 (76.9)	12 (92)
J	12	11 (91.7)	10 (83.3)	7 (58.3)	12 (100)	10 (83.3)	7 (58.3)	7 (58.3)	10 (83.3)	11 (91.7)	12 (100)
K	14	9 (64.3)	10 (71.4)	10 (71.4)	12 (85.7)	12 (85.7)	12 (85.7)	7 (50)	11 (78.6)	10 (71.4)	12 (86)
L	5	5 (100)	4 (80)	4 (80)	5 (100)	5 (100)	4 (80)	4 (80)	5 (100)	1 (20)	5 (100)
F/W	-	-	-	-		-	-	-	-	-	-
DS	4	4 (100)	3 (75)	3 (75)	3 (75)	3 (75)	4 (100)	2 (50)	4 (100)	2 (50)	4 (100)
Total	94	60 (63.8)	56 (60)	68 (72.3)	82 (87.2)	78 (83)	68 (72.3)	38 (40.4)	73 (77.7)	73 (77.7)	90 (95.7)

Keys: n=number of isolates, N- Number of isolates showing multiple antibiotic resistance, F/W- isolates from feed and water, DS-number of isolates from disposal site, OFX-Ofloxacin; CIP-Ciprofloxacin, GN-Gentamycin; AMC-Amoxycillin-clavulanate, CRO-Cefriaxone; MEM-Meropenem, CAZ= Ceftaxidime TET= Tetracycline, SXT= Trimethroprim/Sulfamethoxazole. Source A- pullets, B- layers, C- broilers, D- broilers, E-,turkeys, F-,turkey, G-layers, H-layers, I- layers, J-cockerels, K-local birds, L-layer

Table 5. Multiple antibiotic resistant patterns of *E. coli* isolated

		Source code													
S/No.	**Patterns of antibiotic resistance**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**D/S**	**Total**
1	AMC, CIP,CN, SXT, TET	1													1
2	AMC, CIP, OFX, CN, SXT, TET	2	1	1	2	1			1						6
3	AMC, CIP, OFX, CN	1													1
4	CAZ, AMC, TET		1	1	2	1			1						6
5	CRO, AMC, TET														1
6	CRO, CAZ, AMC, CN		1												1
7	AMC, OFX, CN, SXT, TET		1												1
8	CRO, CAZ, AMC, OFX, CN			1											1
9	AMC, OFX, CN			1											1
10	CN, MEM, SXT, TET				1										1
11	AMC, CIP, OFX, SXT				2										1
12	AMC, CIP, OFX, CN, MEM, TET					1									1
13	AMC, CIP, OFX, TE					1									1
14	AMC, CIP, OFX, SXT, TET					1									1
15	CRO, AMC, CIP, OFX, MEM, SXT, TET					1									1
16	CAZ, AMC, CIP, OFX, CN					1	1								1
17	AMC, CIP, OFX, MEM, SXT, TET						1				1				2
18	CRO, CIP, SXT, TET						1								1
19	CAZ, CIP, OFX, CN, SXT, TET				2										1
20	CRO, CAZ, CIP, MEM							1							1
21	CIP, OFX, SXT, TET							1				1			2
22	CRO, CAZ ,AMC, CIP, OFX, CN, SXT, TET							1	1						2
23	CRO, CAZ, AMC, CIP, OFX, CN, MEM, SXT, TET							1							1
24	CIP, OFX, MEM, SXT, TET							1	2			1			2
25	CRO, CAZ, CIP, OFX, CN, SXT, TET							1	1	7		5	1	1	6
26	CRO, CAZ, CIP, OFX, SXT, TET							1							1
27	CRO, CIP, CN, SXT, TET.								1						1
28	AMC, CIP, SXT, TET								4						1
29	CRO, CAZ, OFX, CN								1						1
30	CRO, CAZ, AMC, CIP, OFX, CN, MEM								1						1
31	CRO, CAZ, CIP, OFX, CN, MEM									1					1
32	CRO, CAZ, CIP, OFX, CN, SXT, TET									1					1
33	CRO, CAZ, AMC, CIP, OFX, SXT, TET									1					1
34	CRO, CAZ, AMC, CIP, OFX, MEM, TET									2	1			1	3
35	CRO, CAZ, AMC, CIP, MEM, SXT, TET										2				1

		Source code													
S/No.	**Patterns of antibiotic resistance**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**D/S**	**Total**
36	CRO,CAZ,AMC, CIP,CN,TET										1				1
37	CRO, CAZ, AMC, CIP, OFX, CN, MEM, SXT										1				1
38	CRO, CAZ, CIP, OFX, CN, MEM, SXT, TET									1					1
39	CAZ, AMC, CIP, OFX, CN, SXT, TET										2				1
40	CRO, CIP, OFX, CN, SXT, TET										1				1
41	CRO, CAZ, AMC, CIP, OFX, CN, MEM, SXT										1				1
42	CRO, AMC, CIP, OFX, CN, SXT, TET										1				1
43	CIP, OFX, CN, SXT											2	3	1	3
44	CRO, CAZ, AMC, MEM, TET											1			1
45	CRO, CIP, OFX, SXT											1			1
46	CRO, AMC, CN, SXT												1		1
	Total number of patterns in each farm	3	3	3	4	6	3	7	8	6	9	6	4	4	

Key: OFX=Ofloxacin; CIP=Ciprofloxacin; GN=Gentamycin; AMC= Amoxycillin-Clavulanic Acid, CRO = Cefriaxone; MEM=Meropenem; CAZ= Ceftaxidime, TET= Tetracycline, SXT= Trimethroprim/Sulfamethoxazole

Table 6. Natural water sources located within the area of study and their uses

No	**Water bodies**	**Types**	**Percentage of use of the water bodies (%)**							**Previous history of diarrhea attributable to water use**
			Fishing	**Domestic**	**Farming**	**Recreational**	**Construction**	**Religious**	**None**	
1	Ogudu	Stream	-	1 (10)	3 (30)	2 (20)	2 (20)	2 (20)	-	2 (20)
2	Igemo	Spring	-	1 (10)	-	-	-	-	9 (90)	-
3	Apalogbo I	Stream	2 (20)	1 (10)	3 (30)	-	3 (30)	-	1 (10)	1 (10)
4	Apalogbo II	Stream	1 (10)	3 (30)	1 (10)	5 (50)	-	-	-	3 (30)
5	Ijokole	Stream	-	3 (30)	5 (50)	-	-	-	2 (20)	-
	Total		3 (6)	9 (18)	12 (24)	7 (14)	5 (10)	2 (4)	12 (24)	6 (12)

Key: A-L (farm code), 1-Ogudu stream, 2-Igemo spring, 3-Apalogbo (I stream), 4-Apalogbo (ii) stream, 5-Ijokole stream
** Total number of respondents is 50*

Table 7. Spearman's statistical correlation of antibiotics consumption and resistant profile

Antibiotics	Frequency of use (%)	r_s	Resistant profile (%)	r_s	Market survey (%)
Cotrimoxazole	6 (50)		73 (77.7)		1 (100)
Tetracycline	5 (41.7)		73 (77.7)		2 (100)
Gentamycin	3 (25)		68 (72.3)		2 (100)
Enrofloxacin	2 (16.7)		NT		2 (100)
Erythromycin	2 (16.7)		NT		2 (100)
Streptomycin	2 (16.7)		NT		1 (50)
Neomycin	2 (16.7)		NT		2 (100)
Colistin	2 (16.7)	r_s (0.866)	NT	r_s (0.500)	1 (50)
Metronidazole	1 (8.3)		NT		NS
Chloramphenicol	1 (8.3)		NT		1 (50)
Ceftriazone	NA		60 (63.8)		NS
Ceftazidime	NA		56 (60)		NS
Amoxycillin-clavulanate	NA		68 (72.3)		NS
Ciprofloxacin	NA		82 (87.2)		NS
Meropenem	NA		38 (40.4)		NS
Ofloxacin	NA		78 (83)		NS
Penicillin	NA		NT		1 (50)
Tylosine	1 (8.3)		NT		1 (50)

Keys: NA – not administered, NS not sold, NT- not tested, r_s- Spearman's correlation coefficient

There was a predominance of multiple antibiotic-resistant bacteria on the poultry farms than resistance to single antibiotics. This finding agrees with the assertions of [21], that antibiotic use eliminates susceptible bacterial populations and selects wild strains that continue to grow in its presence through a Darwinian selection process. The resistant variants multiply and become the predominant bacterial population. Infection with this multiple antibiotic-resistant bacteria that evade treatment may threaten humans and health institutions if the spread-sources are not checked.

The isolates were resistant to some antibiotics used for treatment, including meropenem, amoxycillin-clavulanate, and the cephalosporins that were not administered on the farms. The observed resistance might be caused by treatment with antimicrobial substances that are chemical analogs with similar modes of action or class to the administered antibiotics. Resistance of the isolates to the fluoroquinolones (ciprofloxacin and ofloxacin) was the highest. The high level of resistance might be associated with the availability and use of enrofloxacin and its use for therapy. In contrast, resistance to meropenem was the lowest (40.4%), and this may be due to the non-availability of the antibiotic in tablet form on the counter. Conversely, [22] reported that the percentage resistance of *E. coli* from human origin to carbapenemase in most European countries was less than 1% except Belaraus, with a higher range of 10% - 25%. Also, the level of resistance of *E. coli* from poultry and human sources in Iran to meropenem was reported to be 20% and 60%, respectively [23].

The observed multiple antibiotic resistant profiles were pattern oriented with duplication of similar patterns in different farms. This result is consistent with the statistical findings of [24] on the predominance of multiple antibiotic-resistant profiles than resistance to a single antibiotic. It was observed that antibiotic resistance was pattern-oriented and similar patterns were replicated in different farms. Treatment failure may arise in poultry if a narrow spectrum of antibiotic is administered for therapy without an antibiotic susceptibility test that details the antibiotic-resistant patterns. Patterns 2 (AMC.CIP, OFX, CN, SXT, TET) and 23 (CRO, CAZ, AMC, CIP, OFX, CN, MEM, SXT, TET) had the highest frequency with a spread in six different farms. Fluoroquinolone, sulfonamide, and tetracycline were common to both patterns. These antibiotic classes may become in-efficient both in human and veterinary medicine when used to treat infections caused by the bacteria above.

The documented incidence of diarrhea in some respondents may be linked to human exposure to contaminated water. Human activities involving water use for religious, domestic, recreational, fish farming, and construction works, together with poor poultry waste disposal options can expose man to soil, and water contaminated with multiple antibiotic-resistant bacteria from poultry. This finding is consistent with [25] that antibiotic-resistant bacteria may reach humans indirectly along the food chain through consumption of contaminated food and direct contact with infected animals.

The strong positive Spearman correlation between antibiotic treatment and the resistant profile of bacteria suggests a directly proportional relationship between antibiotic treatment on the farm and bacterial resistance to antibiotics. Besides, there was also a strong positive relationship between level of antibiotics-presence from market survey and resistant profile of the isolates. Though both data on antibiotic treatment and that from market survey are positively related to antibiotic resistant profile of isolates, there is an indication that data on antibiotic treatment on the farm are more related to resistance of bacteria than those obtained from market survey. This result contrasts the finding of [19], who recorded a negative Spearman correlation coefficient, r_s (8) = - 0.243 between antibiotic treatment and antibiotic-resistant profile of isolates. The difference may be due to the sample size of the antibiotics used for both therapy and antibiotic susceptibility test.

CONCLUSION

The development and spread of multiple antibiotic-resistant bacteria of poultry origin are primarily attributed to poor antibiotic formulation policy, crude antibiotic treatment on poultry farms, and indiscriminate supply of antibiotics to untrained poultry personnel. The effectiveness of the super drugs used for the treatment of superbugs in poultry birds may be undermined if policies on the antibiotic formulation, dispensing, and therapy are not reviewed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO.Gateway to poultry production and products.www.fao.org/poultry production product/production/poultry-species; 2017.
2. Ewers C, Bethe A, Semmler T, Guenther T, Wieler LH. Extended spectrum beta-lactamase-producing and AmpC producing *Escherichia coli*from livestock and companion animals: And their putative impact on public health: A global perspective. Clinical Microbiology and Infection. 2012;18:646-655.
3. Omer MM, Abusalab SM, Gumma SA, Mulla SA, Omer EA, Jeddah IE, Al-Hassan AM, Hussein MA, Ahmed AM. Outbreak of Cocillobacillosis among broiler and layer flocks in intensive and semi-intensive poultry farms in Kassala state, Eastern Sudan. Asian Journal of Poultry Science. 2010;4:173-181.
4. Nhung TN, Niwat CN, and Carrique-Mas JJ. Antimicrobial resistance in bacterial poultry pathogens: A Review. Front. Veterinary Science. 2017;4:126.
5. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. British Journal of Pharmacology. 2008; 153(1):347–357.
6. Agyare C, Boamah EV, Zumbi CN. Osei FB. Antibiotic use in poultry production and its effects on bacterial resistance, antimicrobial resistance– A global threat. 2018. Available:https://www.intechopen.com/books/ antimicrobial-resistance-a-globalthreat/ antibiotic-use-in-poultry production and-its-effects-on-bacterial-resistance.
7. Adelowo OO, Fagade OE, Agerso Y. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. Journal of Infection in Developing Countries. 2014;8(9):1103-1112.
8. WHO. Antimicrobial resistance fact. Available:http://www.who.int/mediacentre/factsheets/fs194/en/2017 a.
9. Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: A developing country-perspective. Frontiers in Microbiology. 2016;7:1881.
10. Nsofor AC, Ukwandu NCD. Transfer of resistance plasmids between *Escherichia coli* isolates from domestic livestock. ECronicon Microbiology. 2016;3(1):402-408.
11. Spellberg BG, Hansen GR, Kar A, Cardova CD, Price BL, James RJ. Discussion paper: Antibiotic resistance in humans and animals. National academy for medicine, Washington DC; 2016. Available:http://www.nam.edu/antibiotic-resistance in humans and animals.
12. Yassin AK, Gong J, Kelly P, Lu G, Guardabassi L, Wei L. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. PLoS ONE. 2017;12(9):e0185326
13. Lentz SA, de Lima-Morales D, Cuppertino VM, Nunes LS, da Motta AS, Zavascki AP, Barth AL, Martins AF. Letter to the editor: *Escherichia coli*harbouring mcr-1 gene isolated from poultry not exposed to polymyxins in Brazil. European Surveillance. 2016;21(26):30267.
14. Solveig SM, Jannice SS., Einar SB, Norstrom M, Marianne S. Plasmid and Host Strain Characteristics of *Escherichia coli* resistant to extended spectrum cephalosporins in the Norwegian broiler production. PLoS ONE. 2016;11(4): e0154019.
15. Wampler PJ, Rediske RR, Molla AR. Using Arc Map, Google Earth, and global positioning systems to select and locate random households in rural Haiti. International Journal of Health Geogra phics. 2013;12(3):12-13
16. Ieven ME, Vercauteren P, Descheemaeker F, Van Laer F, Goossens H. Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide-resistant Enterococciamong hospitalized patients. Journal of Clinical Microbiology. 1999;37 (5): 1436–1440.
17. Muhammad AA, Hassan SMR, Saidul A, Momena S. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. American Journal of Environmental Sciences. 2009;5(1):47-52.

18. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard—Eleventh Edn.CLSI document M02-A11. Wayne, PA, USA; 2013.
19. Awogbemi J, Adeyeye M, Akinkunmi EO. A survey of antimicrobial agents usage in poultry farms and antibiotic resistance in *Escherichia coli* and Staphylococci isolates from the poultry in Ile-Ife, Nigeria. Journal of Infectious Disease and Epidemiology. 2018;4(1):047.
20. Mahmud S, Nazir NH, Rahman MT. Prevalence and molecular detection of fluoroquinolone-resistant genes (*qnrA and qnrS*) in *Escherichia coli* isolated from healthy broiler chickens. Veterinary World. 2018;11(12):1720-1724.
21. Holmes AH, Moore LS, Sundsfjord A, Steinbakk M, Regmi S, Karley A. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016;387:176–187.
22. WHO. Carbapenem-resistant *Escherichia coli*; Percentage of invasive isolates of *Escherichia coli* with resistance to carbapenems.Division of information, evidence, research and innovation, European health information gateway. Available:https://gateway.euro.who.int/en/indicators/amr_19-carbapenem-resistant-*Escherichia coli*/visualizations/#id=32511&tab=table.2017 b.
23. Doregiraee F, Alebouyeh M, Fasaei BN, Charkhkar S, Tajeddin E, Zali MR. Changes in antimicrobial resistance patterns and dominance of extended spectrum β-lactamase genes among faecal *Escherichia coli* isolates from broilers and workers during two rearing periods. Italian Journal of Animal Science. 2018;17(3): 815-824.
24. Nsofor AC, Iroegbu CU. Antibiotic resistance profile of *Escherichia coli* isolated from apparently healthy domestic livestock in South-East Nigeria. Journal of Cell and Animal Biology. 2012;6(8):129-135.
25. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP. Antibiotics in agriculture and the risk to human health: how worried should we be? Evolutionary Application. 2015;8:240–245.

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