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ANTIMICROBIAL RESISTANCE IN *ENTEROBACTERIACEAE* FROM INTENSIVELY-REARED APPARENTLY HEALTHY AND DISEASED POULTRY IN ABEOKUTA, NIGERIA

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ABSTRACT

The emergence and wide-spread dissemination of antimicrobial resistant bacteria strains is a global phenomenon of great public health and economic implications. Antimicrobial resistance was investigated in enterobacteriaceae isolated from apparently healthy and diseased poultry birds using the broth micro-dilution method to determine antimicrobial minimum inhibitory concentration (MIC). In all, 504 bacterial isolates including *Escherichia coli* (471)*, Klebsiella spp* (28) and *Salmonella enterica* isolates (5) were studied. The isolates were resistant to ampicillin (88.5%), chloramphenicol (62.3%), ciprofloxacin (74.8%), enrofloxacin (81.0%), neomycin (83.9%), norfloxacin (78.8%), streptomycin (91.3%) and tetracycline (83.3%). The geometric mean MIC (µg/µL) of tested antimicrobials for enterobacteriaceae is as follows: ampicillin (102.5), chloramphenicol (48.4), ciprofloxacin (19.1), enrofloxacin (34.5), neomycin (47.7), norfloxacin (24.5), streptomycin (142.2) and tetracycline (62.5). Although rates of resistance to ampillin, streptomycin and tetracycline were similar among isolates from apparently healthy and diseases birds, resistance to chloramphenicol, ciprofloxacin, enrofloxacin, neomycin and norfloxacin were significantly higher ($p<0.05$) in isolates from diseased chickens than in those from apparently healthy chickens. The high rates of antimicrobial resistance in bacteria may contribute to the persistence of pathogens in poultry flock and ineffectiveness of antimicrobial chemotherapy during disease outbreaks.

Keywords: Antimicrobial resistance, apparently healthy chickens, diseased chickens, diseased turkeys, *enterobacteriaceae*

INTRODUCTION

Antimicrobials are important drugs for the prevention and treatment of bacterial infections in humans and animals (Schwarz and Chaslus-Dancla 2001; DANMAP, 2011). The introduction and use of antimicrobials have contributed remarkably to the sustenance and growth of the livestock industry (Schwarz and Chaslus-Dancla 2001). As a result of the benefits derivable from antimicrobials usage, these drugs have been used without restriction in livestock production.

Many livestock producers depend on antimicrobials to cover-up for unhygienic and inadequate management practices that expose animals to potential pathogens and increase their susceptibility to infections (Soulsby, 2007; Silbergeld *et al.,* 2008). Oftentimes, antimicrobials are administered without due consideration for the possible deleterious effects they exert on the micro- and macro- ecosystem (WHO, 2007).

Over the years, the continuous use of antimicrobials has boomerang into a situation where the continued efficacy of these drugs is under threat due to the occurrence of highly resistant bacteria strains which are refractory to antimicrobial therapy (Barbosa and Levy, 2000). The increasing widespread emergence and dissemination of these multi -drug resistant bacteria is a result of the combined effects of overdependence on antimicrobials, inadequate management practices, climate change, globalization and international trade (Harbarth and Samore, 2005; MacPherson *et al.,* 2009). Globally, there is an increase in reports of resistant bacteria of human and animal origins. The socio-economic consequences associated with increased morbidity and mortality from refractory infections have reached such a magnitude that calls for concerted efforts by all local and international stakeholders in tackling the problem of antimicrobial resistance in bacteria (WHO, 2001).

Escherichia coli is an important pathogen in humans and animals. Pathogenic *E. coli* is capable of causing devastating intestinal and extra-intestinal diseases in infected hosts (Nataro and Kaper, 1998). *Escherichia coli* is a major cause of morbidity and mortality in poultry and can be transmitted to humans through the consumption of contaminated poultry products (van den Bograad *et al.,* 2001; Stordeur *et al.,* 2002; Kabir, 2010). The organism is also used as an indicator bacterium for the surveillance of antimicrobial resistance in the ecosystem and also for tracing faecal contamination of food products, hence, the possible presence of other pathogenic bacteria (Momtaz *et al.*, 2012; Bergeron *et al.,* 2012). Antimicrobial resistance in commensal *E. coli* plays important roles in the maintenance and dissemination of resistant traits in the community (Kijima-Tanaka *et al.,* 2003). Drug-resistant *E. coli* may serve as important reservoirs of resistant genes for pathogenic and non-pathogenic recipient bacterial species (Osterloh, 2004; Sunde and Norström 2006). Surveillance programmes for monitoring antimicrobial resistance in bacteria are important in the development of strategies for the prevention and control of antimicrobial resistance. However, in the developing countries, scarcity of data complicates attempts to assess the magnitude of threat to the livestock industry public health by resistant bacteria. Inadequate documentation of observable trends in antimicrobial resistance hampers risk assessment and development of suitable interventions to mitigate the menace of antimicrobial resistance in developing countries.

The present study investigates the incidence of antimicrobial resistance in *E. coli*, *Klebsiella* spp and *Salmonella* serotypes bacteria isolated from apparently healthy and diseased intensively-reared chickens and turkeys in Abeokuta, Nigeria.

MATERIALS AND METHODS

Sampling information

Between March 2008 and December 2011, samples were collected from seven poultry farms for bacteria isolation and determination of antimicrobial susceptibility.

Sampling from apparently healthy chickens:

Faecal samples were collected from apparently healthy, intensively raised commercial layer chickens from five farms. The birds were in battery cages and had history of previous vaccination against Infectious Bursal Disease, Newcastle Disease, Marek's Disease and Fowl Pox. In addition, regular prophylactic antimicrobial and booster Newcastle Disease vaccine administrations were common practices in all the farms. Pooled cloacal swabs were collected from live birds on the farms. Five cloacal swabs were pooled as one sample. Sixty pooled samples (300 cloacal swabs) were collected from each farm. Pooled faecal sampling was used because it increases the chance of inclusion of faecal materials from infected birds which may contain high numbers of organisms and thus compensate for the possible low level present in other birds (Carrique-Mas and Davies, 2008; Varga *et al.,* 2008). Chickens sampled were randomly selected among the flock. A total of 300 pooled faecal samples were thus collected from five farms.

Sampling from sick birds:

Clinical samples (diarrhoeic faeces and tissue samples) from two farms with history of diarrhoea were examined. One of the farms was a commercial layer farm with adult laying chickens. The other farm was a broiler farm with young chicks and turkey poults of four to six weeks old. Bacterial infections were suspected in both cases (after ruling out viral and protozoan involvement) and samples submitted for bacteriology. Cloacal swabs were collected from individual live sick birds. Post mortem tissue samples from liver, lung and spleen samples were aseptically collected for bacteriological examination. From the commercial layer farms, 78 cloacal swabs and 60 tissue samples (20 each of liver, lung and spleen) were examined. From the broiler farm, 62 cloacal swabs and 33 tissue samples (11 each of liver, lung and spleen) were collected from diarrhoeic and dead chicks while 96 cloacal swabs and 60 tissue samples (20 each of liver, lung and spleen) were collected from diarrhoeic and dead poults.

Bacteria isolation and identification:

Faecal samples were each inoculated directly onto MacConkey agar (CM 0115 Oxoid® Basingstoke, UK) while tissue samples were first inoculated into Tryptic Soy Broth (TSB) for enrichment before being transferred onto MacConkey agar and 5% blood agar. Cultures were incubated at 37°C for 18 to 24 hours. After incubation, agar plates were examined for bacterial growth. Discrete colonies of bacteria were identified and selected. Selected colonies were purified on MacConkey agar and blood agar, Gram-stained for microscopy and tested for catalase and cytochrome oxidase production. Colonies that yielded oxidase negative, catalase positive, Gram-negative rods were subjected to further identification using biochemical tests kits (Oxoid Microbact GNB 24E®) and reactions interpreted by using accompanying computer software package (Oxoid Microbact® 2000 version 2.03).

Antimicrobial susceptibility testing

The bacteria isolated from samples were tested for susceptibility to antimicrobial agents. Susceptibility to ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), enrofloxacin (Enr), neomycin (Neo), norfloxacin (Nor), streptomycin (Str) and tetracycline (Tet) were determined by the broth micro-dilution technique to determine the minimum inhibitory concentration (MIC) using antimicrobial concentrations ranging from 0.25-512 µg/µL according to the standard guidelines by Clinical and Laboratory Standards Institute (2008). The antimicrobial MIC were determined with reference to the respective antimicrobial breakpoint concentrations for bacterial isolates (ampicillin, 32 µg/µL; chloramphenicol, 32 µg/µL; ciprofloxacin, 4 µg/µL ; enrofloxacin, 4 µg/µL; neomycin, 16 µg/µL; norfloxacin, 4 µg/µL; streptomycin, 64 µg/µL and tetracycline, 16 µg/µL) (CLSI, 2008). Isolates with minimum inhibitory concentrations (MIC) higher than the breakpoint for the respective antimicrobial agents were regarded as resistant while those with MIC equal to or lower than the breakpoint were regarded as susceptible.

Statistical Analysis:

Data were expressed in absolute values and in percentages. The geometric mean of MIC values were determine using Microsoft Office Excel 2007 software package. Rates of antimicrobial resistance were compared between isolated from apparently healthy and diseased birds by Chi-square test at $p<0.05$ probability level using Statistical Software Package for Social Sciences (SPSS, version 16, 2007).

RESULTS

A total of 504 bacterial isolates belonging to three genera in the family *Enteroobateriaceae* were obtained in this study. The isolates comprised of *E. coli* (471), *Klebsiella spp* (28) and *Salmonella enterica* (5) (Table 1). Overall, the isolates showed resistance to ampicillin (88.5%), chloramphenicol (62.3%), ciprofloxacin (74.8%), enrofloxacin (81.0%), neomycin (83.9%), norfloxacin (78.8%), streptomycin (91.3%) and tetracycline (83.3%) (Table 1). Rates of resistance to chloramphenicol, ciprofloxacin, enrofloxacin, neomycin and norfloxacin were significantly higher ($p<0.05$) in bacterial isolates from diseased chickens than in those from apparently healthy chickens. However, there was no significant difference ($p>0.05$) in the rates of antimicrobial resistance in isolates from diseased chickens and turkeys.

Antimicrobial resistance in bacterial isolates from apparently healthy commercial chickens

The rates of antimicrobial resistance in *E. coli* isolates from commercial chickens is ampicillin 74.4%, chloramphenicol 37.8%, ciprofloxacin 36.1%, enrofloxacin 48.8%, neomycin 57.6%, norfloxacin 46.5%, streptomycin 76.7% and tetracycline 57.6%. The geometric mean MIC was highest (121.5 µg/µL) for streptomycin and lowest (3.0 µg/µL) for norfloxacin (Table 2).

Klebsiella isolates from apparently healthy commercial chickens showed resistance to ampicillin (75.0%), chloramphenicol (25.0%), ciprofloxacin (12.5%), enrofloxacin (37.5%), neomycin (50.0%), norfloxacin (12.5%), streptomycin (62.5%) and tetracycline (62.5%). The geometric mean MIC was highest (152.2 µg/µL) for ampicillin and lowest (0.5 µg/µL) for ciprofloxacin (Table 3).

All the five *Salmonella* isolates from apparently healthy commercial chicken were resistant to ampicillin, four (80.0%) were resistant to streptomycin, two (40.0%) showed resistance to each of chloramphenicol, enrofloxacin and neomycin while only one (20.0%) was resistant to each of ciprofloxacin, norfloxacin and tetracycline. The geometric mean MIC was highest (168.9 µg/µL) for ampicillin and lowest (0.5 µg/µL) for ciprofloxacin (Table 4).

Table 1: Antimicrobial resistance in bacterial isolates from apparently healthy and diseased chickens and turkeys in Abeokuta, Nigeria

Bacterial isolates by source (number tested)	Number (%) of resistant isolates							
	Ampicillin	Chloram-phenicol	Ciproflox-acin	Enrofloxacin	Neomycin	Norfloxacin	Streptomycin	Tetracycline
Escherichia coli								
Apparently healthy chickens (172)	128.0 (74.4)	65.0 (37.8)	62.0 (36.1)	84.0 (48.8)	99.0 (57.6)	80.0 (46.5)	132.0 (76.7)	99.0 (57.6)
Diseased chickens (194)	194.0 (100.0)	146.0 (75.3)	194.0 (100.0)	194.0 (100.0)	194.0 (100.0)	194.0 (100.0)	194.0 (100.0)	194.0 (100.0)
Diseased Turkeys (105)	94.0 (89.5)	83.0 (79.1)	101.0 (96.2)	105.0 (100.0)	105.0 (100.0)	101.0 (96.2)	105.0 (100.0)	101.0 (96.2)
Subtotal (471)	416 (88.3)	294 (62.4)	357 (75.8)	383 (81.3)	398 (84.5)	375 (79.6)	431 (91.5)	394 (83.7)
Klebsiella species								
Apparently healthy chickens (8)	6.0 (75.0)	2.0 (25.0)	1.0 (12.5)	3.0 (37.5)	4.0 (50.0)	1.0 (12.5)	5.0 (62.5)	5.0 (62.5)
Diseased chickens (15)	14.0 (93.3)	12.0 (80.0)	14.0 (93.3)	15.0 (100.0)	14.0 (93.3)	15.0 (100.0)	15.0 (100.0)	15.0 (100.0)
Diseased Turkeys (5)	5.0 (100.0)	4.0 (80.0)	4.0 (80.0)	5.0 (100.0)	5.0 (100.0)	5.0 (100.0)	5.0 (100.0)	5.0 (100.0)
Subtotal (28)	25 (89.3)	18 (64.3)	19 (67.9)	23 (82.1)	23 (82.1)	21 (75.0)	25 (89.3)	25 (89.3)
Salmonella serotypes								
Apparently healthy chickens (5)	5.0 (100.0)	2.0 (40.0)	1.0 (20.0)	2.0 (40.0)	2.0 (40.0)	1.0 (20.0)	4.0 (80.0)	1.0 (20.0)
Diseased chickens	-	-	-	-	-	-	-	-
Diseased Turkeys	-	-	-	-	-	-	-	-
Subtotal (5)	5.0 (100.0)	2.0 (40.0)	1.0 (20.0)	2.0 (40.0)	2.0 (40.0)	1.0 (20.0)	4.0 (80.0)	1.0 (20.0)
Overall total (504)	446 (88.5)	314 (62.3)	377 (74.8)	408 (81.0)	423 (83.9)	397 (78.8)	460 (91.3)	420 (83.3)

Table 2: Minimum inhibitory concentration of antimicrobial agents for *Escherichia coli* isolated from apparently healthy

Antimicrobial agents	Number of iso-late tested	Range of tested antimicrobial concentration (µg/ µL)	Lowest MIC (µg/ µL)	Highest MIC (µg/ µL)	Geometric mean MIC (µg/ µL)	MIC50 (µg/ µL)	MIC90 (µg/ µL)	Number (%) sensitive	Number (%) resistant
Ampicillin	172	0.25-512	0.5	>512.0	73.1	128.0	512.0	44.0 (25.6)	128.0 (74.4)
Chloram-phenicol	172	0.25-512	≤0.25	>512.0	22.3	16.0	256.0	107.0 (62.2)	65.0 (37.8)
Ciprofloxacin	172	0.25-512	≤0.25	>512.0	4.3	2.0	64.0	110.0 (63.9)	62.0 (36.1)
Enrofloxacin	172	0.25-512	≤0.25	>512.0	5.4	2.0	64.0	88.0 (51.2)	84.0 (48.8)
Neomycin	172	0.25-512	≤0.25	>512.0	17.7	32.0	256.0	73.0 (42.4)	99.0 (57.6)
Norfloxacin	172	0.25-512	≤0.25	>512.0	3.0	2.0	32.0	92.0 (53.5)	80.0 (46.5)
Streptomycin	172	0.25-512	1.0	>512.0	121.5	256.0	512.0	40.0 (23.3)	132.0 (76.7)
Tetracycline	172	0.25-512	≤0.25	>512.0	16.1	16.0.	512.0	73.0 (42.4)	99.0 (57.6)

Table 3: Minimum inhibitory concentration of antimicrobial agents for *Klebsiella spp* isolated from apparently healthy intensively-reared chickens in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (µg/ µL)	Highest MIC (µg/ µL)	Geometric mean MIC (µg/ µL)	MIC50 (µg/ µL)	MIC90 (µg/ µL)	Number (%) sensitive	Number (%) resistant
Ampicillin	8	0.25-512	512.0	512.0	152.2	512.0	512.0	2.0 (25.0)	6.0 (75.0)
Chloramphenicol	8	0.25-512	16.0	256.0	26.9	16.0	256.0	6.0 (75.0)	2.0 (25.0)
Ciprofloxacin	8	0.25-512	0.25	4.0	0.5	0.25	4.0	7.0 (87.5)	1.0 (12.5)
Enrofloxacin	8	0.25-512	2.0	8.0	2.0	2.0	8.0	5.0 (62.5)	3.0 (37.5)
Neomycin	8	0.25-512	16.0	32.0	10.4	16.0	32.0	4.0 (50.0)	4.0 (50.0)
Norfloxacin	8	0.25-512	1.0	4.0	1.2	1.0	4.0	7.0 (87.5)	1.0 (12.5)
Streptomycin	8	0.25-512	128.0	128.0	69.8	128.0	128.0	3.0 (37.5)	5.0 (62.5)
Tetracycline	8	0.25-512	128.0	128.0	26.9	128.0	128.0	3.0 (37.5)	5.0 (62.5)

Table 4: Minimum inhibitory concentration of antimicrobial agents for *Salmonella* serotypes isolated from apparently healthy intensively-reared chickens in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (μg/ μL)	Highest MIC (μg/ μL)	Geometric mean MIC (μg/ μL)	MIC50 (μg/ μL)	MIC90 (μg/ μL)	Number (%) sensitive	Number (%) resistant
Ampicillin	5	0.25-512	64.0	>512.0	168.9	128.0	512.0	0.0 (0.0)	5.0 (100.0)
Chloramphenicol	5	0.25-512	0.5	128.0	13.9	8.0	128.0	3.0 (60.0)	2.0 (40.0)
Ciprofloxacin	5	0.25-512	≤0.25	8.0	0.5	0.25	8.0	4.0 (80.0)	1.0 (20.0)
Enrofloxacin	5	0.25-512	0.5	256.0	5.3	1.0	256.0	3.0 (60.0)	2.0 (40.0)
Neomycin	5	0.25-512	4.0	>512.0	16.0	4.0	512.0	3.0 (60.0)	2.0 (40.0)
Norfloxacin	5	0.25-512	≤0.25	16.0	0.9	0.5	16.0	4.0 (80.0)	1.0 (20.0)
Streptomycin	5	0.25-512	16.0	>512.0	147.0	256.0	512.0	1.0 (20.0)	4.0 (80.0)
Tetracycline	5	0.25-512	0.5	128.0	6.1	4.0	128.0	4.0 (80.0)	1.0 (20.0)

Antimicrobial resistance in bacterial isolates from diseased chickens:

All 194 *E. coli* isolates from diseased chickens were all resistant to tested antimicrobials except chloramphenicol to which 146 (75.3%) of the isolates were resistant. The geometric mean MIC was highest (156.4 µg/µL) in ampicillin and least (38.1 µg/µL) in ciprofloxacin (Table 5).

Klebsiella isolates from diseased chickens showed 100% resistance to enrofloxacin, norfloxacin, streptomycin and tetracycline; 93.3% resistance to ciprofloxacin, ampicillin and neomycin and 80.0% resistance to chloramphenicol. The geometric mean MIC was highest (406.4 µg/µL) in ampicillin and least (46.3 µg/µL) in ciprofloxacin (Table 6).

Antimicrobial resistance in bacterial isolates from diseased turkeys:

Escherichia coli isolates from diseased turkeys were all resistant to enrofloxacin, neomycin and streptomycin. The rate of resistance to ciprofloxacin, norfloxacin and tetracycline was 96.2% each while resistance was 89.5% and 79.1% for ampicillin and chloramphenicol respectively. The geometric mean MIC was highest (146.1 µg/µL) in streptomycin and least (95.7 µg/µL) in norfloxacin (Table 7).

All five (100%) *Klebsiella* isolates from diseased turkeys were resistant to ampicillin, enrofloxacin, neomycin, norfloxacin and tetracycline while four (80.0%) were resistant to chloramphenicol and ciprofloxacin. The geometric mean MIC was highest (512.0 µg/µL) in ampicillin and least (55.7 µg/µL) in ciprofloxacin (Table 8).

Table 5: Minimum inhibitory concentration of antimicrobial agents for *Escherichia coli* isolated from diseased intensively-reared chickens in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (μg/ μL)	Highest MIC (μg/ μL)	Geometric mean MIC (μg/ μL)	MIC50 (μg/ μL)	MIC90 (μg/ μL)	Number (%) sensitive	Number (%) resistant
Ampicillin	194	0.25-512	128.0	>512.0	156.4	128.0	128.0	0.0 (0.00)	194.0 (100.0)
Chloramphenicol	194	0.25-512	8.0	>512.0	67.3	8.0	128.0	48.0 (24.7)	146.0 (75.3)
Ciprofloxacin	194	0.25-512	16.0	64.0	38.1	16.0	64.0	0.0 (0.00)	194.0 (100.0)
Enrofloxacin	194	0.25-512	64.0	>512.0	111.4	64.0	128.0	0.0 (0.00)	194.0 (100.0)
Neomycin	194	0.25-512	16.0	256.0	66.3	16.0	128.0	0.0 (0.00)	194.0 (100.0)
Norfloxacin	194	0.25-512	16.0	>512.0	83.4	16.0	128.0	0.0 (0.00)	194.0 (100.0)
Streptomycin	194	0.25-512	128.0	>512.0	139.5	128.0	128.0	0.0 (0.00)	194.0 (100.0)
Tetracycline	194	0.25-512	64.0	>512.0	112.6	64.0	128.0	0.0 (0.00)	194.0 (100.0)

Table 6: Minimum inhibitory concentration of antimicrobial agents for *Klebsiella spp* isolated from diseased intensively-reared chickens in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (μg/ μL)	Highest MIC (μg/ μL)	Geometric mean MIC (μg/ μL)	MIC50 (μg/ μL)	MIC90 (μg/ μL)	Number (%) sensitive	Number (%) resistant
Ampicillin	15	0.25-512	16.0	>512.0	406.4	512.0	512.0	1.0 (6.7)	14.0 (93.3)
Chloramphenicol	15	0.25-512	0.5	128.0	67.0	128.0	128.0	3.0 (20.0)	12.0 (80.0)
Ciprofloxacin	15	0.25-512	2.0	64.0	46.3	64.0	64.0	1.0 (6.7)	14.0 (93.3)
Enrofloxacin	15	0.25-512	32.0	128.0	67.0	64.0	128.0	0.0 (0.0)	15.0 (100.0)
Neomycin	15	0.25-512	8.0	128.0	55.7	64.0	128.0	1.0 (6.7)	14.0 (93.3)
Norfloxacin	15	0.25-512	16.0	>512.0	70.2	64.0	512.0	0.0 (0.0)	15.0 (100.0)
Streptomycin	15	0.25-512	64.0	>512.0	222.9	256.0	512.0	0.0 (0.0)	15.0 (100.0)
Tetracycline	15	0.25-512	16.0	>512.0	185.3	256.0	512.0	0.0 (0.0)	15.0 (100.0)

Table 7: Minimum inhibitory concentration of antimicrobial agents for *Escherichia coli* isolated from diseased intensively-reared chickens in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (μg/ μL)	Highest MIC (μg/ μL)	Geometric mean MIC (μg/ μL)	MIC50 (μg/ μL)	MIC90 (μg/ μL)	Number (%) sensitive	Number (%) resistant
Ampicillin	105	0.25-512	16.0	>512.0	107.1	16.0	128.0	11.0 (10.5)	94.0 (89.5)
Chloram-phenicol	105	0.25-512	16.0	>512.0	99.6	16.0	128.0	22.0 (20.9)	83.0 (79.1)
Ciprofloxacin	105	0.25-512	2.0	128.0	97.0	64.0	128.0	4.0 (3.8)	101.0 (96.2)
Enrofloxacin	105	0.25-512	32.0	256.0	104.3	64.0	128.0	0.0 (0.0)	105.0 (100.0)
Neomycin	105	0.25-512	16.0	>512.0	117.5	128.0	128.0	0.0 (0.0)	105.0 (100.0)
Norfloxacin	105	0.25-512	2.0	256.0	95.7	64.0	128.0	4.0 (3.8)	101.0 (96.2)
Streptomycin	105	0.25-512	128.0	>512.0	146.1	128.0	128.0	0.0 (0.0)	105.0 (100.0)
Tetracycline	105	0.25-512	8.0	>512.0	124.7	128.0	128.0	4.0 (3.8)	101.0 (96.2)

Table 8: Minimum inhibitory concentration of antimicrobial agents for *Klebsiella spp* isolated from disease intensively-reared turkeys in Abeokuta, Nigeria

Antimicrobial agents	Number of isolate tested	Range of tested antimicrobial concentration	Lowest MIC (μg/ μL)	Highest MIC (μg/ μL)	Geometric mean MIC (μg/ μL)	MIC50 (μg/ μL)	MIC90 (μg/ μL)	Number (%) sensitive	Number (%) resistant
Ampicillin	5	0.25-512	512.0	>512.0	512.0	512.0	512.0	5.0 (100.0)	5.0 (100.0)
Chloramphenicol	5	0.25-512	8.0	256.0	128.0	256.0	256.0	4.0 (80.0)	4.0 (80.0)
Ciprofloxacin	5	0.25-512	2.0	128.0	55.7	128.0	128.0	4.0 (80.0)	4.0 (80.0)
Enrofloxacin	5	0.25-512	4.0	128.0	64.0	128.0	128.0	5.0 (100.0)	5.0 (100.0)
Neomycin	5	0.25-512	32.0	256.0	168.9	256.0	256.0	5.0 (100.0)	5.0 (100.0)
Norfloxacin	5	0.25-512	64.0	128.0	111.4	128.0	128.0	5.0 (100.0)	5.0 (100.0)
Streptomycin	5	0.25-512	256.0	>512.0	445.7	512.0	512.0	5.0 (100.0)	5.0 (100.0)
Tetracycline	5	0.25-512	128.0	256.0	147.0	128.0	256.0	5.0 (100.0)	5.0 (100.0)

DISCUSSION

In the present study, *E. coli* was the predominant isolate from apparently healthy and diseased poultry birds. This agrees with earlier report by Kilonzo-Nthenge *et al.* (2008) that *E. coli* is a major cause of morbidity and mortality in poultry worldwide (Kilonzo-Nthenge *et al.,* 2008). Apart from being a primary cause of diseases, *E. coli* is also implicated as an opportunistic pathogen capable of complicating infections caused by other pathogens. Other bacteria identified in the present study (*Salmonella enterica* isolates and *Klebsiella spp.*) are also known to induce clinical diseases in poultry (Kilonzo-Nthenge *et al.,* 2008). *Salmonella* species was the least encountered and was detected only in apparently healthy chickens. Although a major avian pathogen of high economic importance, the presence of *Salmonella* in apparently healthy birds showed that birds may harbour *Salmonella* without clinical manifestations (Agbaje *et al.,* 2010). Apparently healthy carriers may thus serve as sources of persistent *Salmonella* infection in the flock.

The present study showed varying degrees of antimicrobial resistance in bacterial species isolated from apparently healthy chickens in the study area. In all the bacteria species, the highest rates of antimicrobial resistance and geometric mean MIC were recorded in ampicillin and streptomycin. Ampicillin and streptomycin are first generation antimicrobials which are commonly used antimicrobials in the livestock industry. This may account for the higher rates of resistance to these drugs. Generally, there was moderate level of neomycin resistance which did not exceed 50.0% except in *E. coli* (57.6%). Among the antimicrobials from different classes represented in this study, resistance to the fluoroquinolones was relatively low. However, it is evident that bacteria are developing resistance to the fluoroquinolone which are considered the drug of choice for the treatment of gastroenteritis in humans (Guerrant *et al.,* 2001). Among fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin) resistance rate and geometric mean MIC were observed to be highest for enrofloxacin and lowest for ciprofloxacin. Enrofloxacin resistance rate was as high as 48.8% in *E. coli* and ciprofloxacin resistance as low as 12.5% in *Klebsiella* spp. A previous study in Nigeria showed that enrofloxacin is the most commonly administered fluoroquinolones in poultry production in the study area (Ogunleye *et al.,* 2008). This may be responsible for the higher rates of resistance to enrofloxacin than to other fluoroquinolones. Fluoroquinolone resistant avian *E. coli* has been reported in other regions of the world (White *et al.,* 2000; Thorsteinsdottir *et al.,* 2010; Chen *et al.,* 2011) and may be transmitted to humans through the food chain (Warren *et al.,* 2008). The continued efficacy of fluoroquinolone therapy in the treatment of human diseases can be achieved by regulating the use of these drugs in humans and disallowing their use in food animals (Cheng *et al.,* 2012).

The presence of drug resistant bacteria in apparently healthy chicken as observed in the present study has implications for poultry production and public health. Non-pathogenic resistant bacteria resident in apparently healthy birds may share their resistant trait and confer resistance on virulent pathogens or acquire virulent traits from pathogenic bacteria (Yaron *et al.,* 2000; Osterloh**,** 2004). Exchange of resistance and virulence genes is common among enteric bacteria especially the enterobacteriaceae (Balis *et al.,* 1996; Yaron *et al.,* 2000). Close contact between humans and birds, consumption of contaminated poultry products and environmental contamination may increase the possible transmission of resistant bacteria more so it has been reported that transmission of resistant clones and plasmids from poultry to humans is a common occurrence (Van dan Boggard *et al*., 2001).

The overall high rates of antimicrobial resistance observed among the bacterial isolates in this study were due largely to high resistance observed in isolates from diseased birds. When considered separately, resistance rates were significantly lower ($p<0.05$) in isolates from apparently healthy birds than in isolates from diseased birds. The present study suggests that antimicrobial resistant bacteria may predominate in disease outbreaks. Antimicrobial resistant *Salmonella* spp. have been reported to be more invasive than susceptible strains thereby producing more severe and fatal infections (Helms *et al.,* 2004). The present study investigated outbreaks of diarrhoea refractory to antimicrobial therapy accompanied by high mortality of over 60%. *Escherichia coli* was isolated from all the clinical samples submitted for bacteriological examination. Few isolates of *Klebsiella* spp were also obtained from the samples. High levels of antimicrobial resistance of between 80.0% and 100% (100% in most cases) were observed among the bacterial isolates. Involvement of multi-drug resistant bacteria in disease outbreak as observed in the present study could undermine the efficacy of therapeutic intervention in the control of bacterial infections. The direct effect of orally administered drug on enteric bacteria may alter the integrity of gastrointestinal microflora leading to the eradication of susceptible strains and proliferation of resistant ones (Zhoa *et al*., 2001). This may lead to an increase in the prevalence of antimicrobial resistance in isolates recovered from animals while on antimicrobial therapy. In the present study, antimicrobial agents had been administered to the sick birds before sample collection and as such may account for the high level of antimicrobial resistance in the bacterial isolates (Boothe and Debavalya 2011). Antimicrobial usage during an outbreak may therefore eliminate competing susceptible bacteria co-habiting the gut with resistant pathogens. This will aid the proliferation of the resistant pathogen and increase the damage done to the host. The high rates of antimicrobial resistance in bacteria may also contribute to the persistence of pathogens in poultry flock because of the ineffectiveness of chemoprophylactic eradication approach.

CONCLUSION

The present study showed high level of antimicrobial resistance in clinical and non-clinical bacterial isolates from intensively reared birds in the study area. The major factors selecting for antimicrobial resistance in bacteria are antimicrobial use, overcrowding and poor sanitation. These factors are typical of many intensive poultry farming and may explain the high prevalence of antimicrobial resistance in bacteria as encountered in this study. Antimicrobial resistance in avian bacterial pathogens is a threat to profitable poultry production, protein availability and public health.

REFERENCES

Agbaje, M., Davies, R., Oyekunle, M.A., Ojo, O.E., Fasina, F.O., Akinduti, P.A. 2010. Observation on the occurrence and transmission pattern of *Salmonella gallinarum* in commercial poultry farms in Ogun State, South Western Nigeria. *African Journal of Microbiology Research,* 4 (9): 796–800.

Balis, E., Vatopoulos, A.C., Kanelopoulou, M., Mainas, E., Hatzoudis, G., Kontogianni, V., Malamou-Lada, H., Kitsou-Kiriakopoulou, S., Kalapothaki, V., 1996. Indications of in vivo transfer of an epidemic R plasmid from *Salmonella enteritidis* to *Escherichia coli* of the normal human gut flora. *Journal of Clinical Microbiology*, 34: 977–979.

Barbosa, T.M., Levy, S.B. 2000. The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates*, 3: 303–311.

Bergeron, C.R., Prussing, C., Boerlin, P., Daignault, D., Dutil, L., Reid-Smith, R.J., Zhanel, G.G., Manges, A.R. 2012. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. *Emerging Infectious Diseases*, 18 (3): 415-521.

Boothe, D.M., Debavalya, N. 2011. Impact of routine antimicrobial therapy on canine fecal *Escherichia coli* antimicrobial resistance: A pilot study. *International Journal of Applied Research in Veterinary Medicine*, 9 (4): 396-406.

Carrique-Mas J.J. and Davies R.H. 2008. Sampling and bacteriological detection of *Salmonella* in poultry and poultry premises: a review. *Revue Scientifique et Technique de Office International des Epizooties* 27 (3): 665 – 677.

Chen, X., Pan, W., Zhang, W., Pan, Z., Gao, S., Jiao, X. 2011. Quinolone resistance in *Escherichia coli* and *Salmonella* spp. isolates from diseased chickens during 1993 -2008 in China. *African Journal of Microbiology Research,* 5 (19): 3078-3083.

Cheng, A.C., Turnidge, J., Collignon, P., Looke, D., Barton, M., Gottlieb, T. 2012. Control of Fluoroquinolone Resistance through Successful Regulation, Australia. *Emerging Infectious Diseases,* 18 (9): 1453-1460.

CLSI, 2008. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard-Third edition Vol 28 (8), CLSI document M31-A3, 1 – 99. Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne Pennsylvania, USA.

DANMAP 2011. The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032. Available from http://www.danmap.org. Accessed 19th September, 2012.

Guerrant, R.L., Van Gilder, T., Steiner, T.S., Thielman, N.M., Slutsker, L., Tauxe, R.V., Hennessy, T., Griffin, P.M., DuPont, H., Sack. R.S., Tarr, P., Neill, M., Nachamkin, I., Reller, L.B., Osterholm, M.T., Bennish, M.L., Pickering, L.K. 2001. Practice guidelines for the management of infectious diarrhea. *Clinical Infectious Diseases*, 32: 331–351.

Harbarth, S., Samore, M.H. 2005. Antimicrobial Resistance Determinants and Future Control. *Emerging Infectious Diseases*, 11 (6): 794-801.

Helms, M., Simonsen, J., Molbak, K. 2004. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *Journal of Infectious Diseases*, 190: 1652–1654.

Kabir, S.M.L. 2010. Avian colibacillosis and Salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health,* 7: 89–114.

Kijima-Tanaka, M., Ishihara, K., Morioka, A., Kojima, A., Ohzono, T., Ogikubo, K., Takahashi, T., Tamura, Y. 2003. A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan. *Journal of Antimicrobial Chemotherapy,* 51: 447–451.

Kilonzo-Nthenge, A., Nahashon, S.N., Chen, F., Adefope, N. 2008. Prevalence and Antimicrobial Resistance of Pathogenic Bacteria in Chicken and Guinea Fowl. *Poultry Science* 87: 1841-1848.

MacPherson, D.W., Gushulak, B.D., Baine, W.B., Bala, S., Gubbins, P.O., Holtom, P., Segarra-Newnham, M. 2009. Population mobility, globalization, and antimicrobial drug resistance. *Emerging Infectious Diseases*, 15 (11): 1727–1732.

Momtaz, H., Rahimi, E., Moshkelani, S. 2012. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinarni Medicina*, 57 (4): 193–197.

Nataro. P., Kaper, J.B., 1998. Diarrhoeagenic *Escherichia coli*. *Clinical Microbiology Reviews,* 11(1): 142-201.

Ogunleye, A.O., Oyekunle, M.A., Sonibare, A.O. 2008. Multidrug resistant *Escherichia coli* isolates of poultry origin in Abeokuta, South Western Nigeria. *Veterinarski Arhiv* 78 (6): 501–509.

Osterloh, M. 2004. Transfer of antibiotic resistant genes between *Escherichia coli* and *Salmonella typhimurum*. *Cantaurus*, 12: 13-14.

Schwarz, S., Chaslus-Dancla, E. 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Veterinary Research* 32: 201–225.

Silbergeld, E.K., Graham, J., Price, L.B., 2008. Industrial Food Animal Production, Antimicrobial Resistance, and Human Health. *Annual Review of Public Health* 29: 151-169.

Soulsby, L. 2007. Antimicrobials and animal health: a fascinating nexus. *Journal of Antimicrobial Chemotherapy* 60, Suppl. 1: i77–i78.

Statistical Package for Social Sciences (SPSS) version 16, 2007. SPSS Inc. 233 South Wacker Drive, 11th floor Chicago, Illinois 60606. http://www.spss.com

Stordeur, P., Marlier, D., Blanco, J., Oswald, E., Biet, F., Dho-Moulin, M., Mainil, J., 2002. Examination of *Escherichia coli* from poultry for selected adhesion genes important in disease caused by mammalian pathogenic *E. coli*. *Veterinary Microbiology*, 84: 231-241.

Sunde, M., Norström, M. 2006. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. *Journal of Antimicrobial Chemotherapy,* 58 (4): 741-747.

Thorsteinsdottir, T.R., Haraldsson, G., Fridriksdottir, V., Kristinsson, K.G., Gunnarsson, E. 2010. Broiler Chickens as Source of Human Fluoroquinolone-Resistant

Escherichia coli, Iceland. *Emerging Infectious Diseases*, 16 (1): 133-135.

Van den Bograad, A.E., London, N., Driessen, C., Stobberingh, E.E., 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, 47: 763-771.

Varga C., Rajić A., McFall M.E., Avery B.P., Reid-Smith R.J., Deckert A., Checkley S.L., McEwen S.A. 2008. Antimicrobial resistance in generic *Escherichia coli* isolated from swine faecal samples in 90 Alberta finishing farms. *The Canadian Journal of Veterinary Research* 72:175–180.

Warren, R.E., Ensor, V.M., O'Neill, P., Butler, V., Taylor, J., Nye, K., Harvey, M., Livermore, D.M., Woodford, N., Hawkey, P.M. 2008. Imported chicken meat as a potential source of quinolone-resistant Escherichia coli producing extended-spectrum β-lactamases in the UK. *Journal of Antimicrobial Chemotherapy* 61: 504–508.

White, D.G., Piddock, L.J.V., Maurer, J.J., Zhao, S., Ricci, V., Thayer, S.G. 2000. Characterization of Fluoroquinolone Resistance among Veterinary Isolates of Avian. *Antimicrobial agents and Chemotherapy*, 44 (10): 2897–2899.

WHO (World Health Organisation), 2001. WHO global strategy for containment of antimicrobial resistance. WHO/CDS/CSR/DRS/2001.2. http://www.who.int/csr/resources/publications/drugresist/en/EGlobal_Strat.pdf. Accessed 19th September, 2012.

WHO (World Health Organisation), 2007. Critically important antimicrobials for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use : report of the second WHO Expert Meeting, Copenhagen, 29 -31 May 2007. http://www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf. Accessed 19th September, 2012.

Yaron, S., Kolling, G.L., Simon, L., Matthews, K.R. 2000. Vesicle-mediated transfer of virulence genes from *Escherichia coli* O157:H7 to other enteric bacteria. *Applied and Environmental Microbiology*, 66 (10): 4414–4420.

Zhao S., White D.G., Ge B. Ayers S., Friedman S., English L., Wagner D., Gaines S. and Meng J. 2001. Identification and characterization of integron-mediated

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