VETERINARSKI ARHIV 82 (6), 577-589, 2012

Antibiogram of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, Nigeria

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OJO, O. E., O. G. OGUNYINKA, M. AGBAJE, J. O. OKUBOYE, O. O. KEHINDE, M. A. OYEKUNLE: Antibiogram of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, Nigeria. Vet. arhiv 82, 577-589, 2012.

ABSTRACT

Antimicrobial resistance in bacteria from the family *Enterobacteriaceae* is an important indicator of the emergence of resistant bacterial strains in the community. This study investigated the antimicrobial susceptibility of commensal *Enterobacteriaceae* from free-range chickens to antimicrobial agents using the broth microdilution. In all, 184 isolates (including 104 *Escherichia coli*, 44 *Klebsiella* spp, 20 *Salmonella* spp. and 16 *Enterobacter aerogenes*) were resistant to ampicillin (89.7%), chloramphenicol (73.9%), ciprofloxacin (33.2%), enrofloxacin (60.3%), neomycin (70.7%), norfloxacin (45.7%), streptomycin (78.8%) and tetracycline (73.4%). *Escherichia coli* was resistant to ampicillin (92.3%), chloramphenicol (73.1%), ciprofloxacin (34.6%), enrofloxacin (61.5%), neomycin (76.9%), norfloxacin (46.2%), streptomycin (80.8%) and tetracycline (76.9%). The rate of resistance in *Klebsiella* spp. was ampicillin (90.9%), chloramphenicol (72.7%), ciprofloxacin (54.5%), enrofloxacin (90.9%), neomycin (63.6%), norfloxacin (63.6%), streptomycin (81.8%) and tetracycline (81.8%). *Salmonella* spp. showed resistance to ampicillin (80.0%), chloramphenicol (80.0%), enrofloxacin (20.0%), neomycin (80.0%), norfloxacin (20.0%), streptomycin (80.0%) and tetracycline (35.0%) but were completely susceptible to ciprofloxacin. *Enterobacter aerogenes* was resistant to ampicillin (81.3%), chloramphenicol (75.0%), ciprofloxacin (6.3%), enrofloxacin (18.8%), neomycin (37.5%), norfloxacin (25.0%), streptomycin (56.3%) and tetracycline (75.0%). Overall, 147 (79.9%) out of 184 isolates demonstrated multidrug resistance to at least three unrelated antimicrobial agents. The high rate of antimicrobial resistance in bacterial isolates from free-range birds may have major implications for human and animal health with adverse economic implications.

Key words: multidrug resistance, commensal *Enterobacteriaceae*, free-range chickens

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ISSN 0372-5480
Printed in Croatia

Introduction

Over 80% of the African chicken population is reared under the free-range system (GUEYE, 1998). Free-range poultry keeping indeed constitutes an integral part of many households in rural and peri-urban areas of Nigeria (SONAIYA and SWAN, 2004). It plays an important role in providing additional income and high quality protein with negligible production input from the farmer (SONAIYA and SWAN, 2004). Free-range chickens reared under an extensive management system scavenge for food and receive little or no veterinary care (OBI et al., 2009). They are highly exposed to a myriad of pathogens that limit production capacity. Enteric bacteria in the family *Enterobacteriaceae,* including *Escherichia coli, Salmonella* spp. and *Klebsiella* spp. are major pathogens or secondary complicating invaders hampering the realization of the full potential of free-range poultry production (KILONZO-NTHENGE et al., 2008; OBI et al., 2009). Scavenging free-range chickens may also be asymptomatic carriers and shedders of pathogenic bacteria in their feces, thereby contaminating the environment. These pathogens can thus be transmitted to other animal hosts and humans where they produce disease.

The emergence and wide-spread dissemination of antimicrobial resistant bacteria is becoming commonly encountered worldwide. This is an alarming reality that has attracted the attention of concerned stakeholders, including veterinarians, physicians, microbiologists, livestock producers, public health workers and relevant government agencies in many nations. The emergence of antimicrobial resistant bacterial strains has been linked with the use of antimicrobial agents in animals (ANONYMOUS, 2001). The continuous use of antimicrobials as therapeutic, prophylactic and growth promoter agents creates selective pressure that ultimately leads to the emergence of resistant bacterial strains (ANONYMOUS, 2001; SMITH et al., 2002; BRAOUDAKI and HILTON, 2004).

Resident intestinal flora constitutes a common reservoir of antimicrobial resistance genes (LEE et al., 2006; SRINIVASAN et al., 2008). The occurrence of antimicrobial resistance in enteric bacteria, especially *Enterobacteriaceae,* is an indication of the emergence of resistant bacterial strains in the community (KIJIMA-TANAKA et al., 2003). Regular surveillance and monitoring of antimicrobial resistance in commensal *Enterobacteriaceae* of animal origin is required as part of the strategy for early detection of antimicrobial resistance in the community (GOODYEAR, 2002; KIJIMA-TANAKA et al., 2003). This is necessary for policy formulation in the prevention and control of widespread dissemination of antimicrobial resistant bacteria strains in the environment and possible transmission of zoonotic resistant bacteria to humans (ANONYMOUS, 2001). It also provides valuable information for clinicians in empirical antibiotic prescription for treatment of infection, pending the outcome of laboratory investigations (OKEKE et al., 1999).

The present study therefore examines the susceptibility of commensal *Enterobacteriaceae* isolated from the feces of free-range chickens to antimicrobial agents (including quinolones) commonly used in treatment of bacterial infections in humans and animals.

Materials and methods

Sample collection. From October 2008 to June 2010, a total of 153 fecal samples were collected by cloacal swabs from apparently healthy free-range chickens in households and a major chicken market in Abeokuta, Nigeria. The samples were preserved in ice-packs and immediately transferred to the laboratory for microbiological analysis.

Isolation and identification of bacteria. Each sample was inoculated onto MacConkey agar (CM 0115 Oxoid® Basingstoke, UK) and incubated at 37 °C for 18 to 24 hours. After incubation, the MacConkey agar plates were examined for bacterial growth. Discrete colonies of lactose fermenting (pink) and non-lactose fermenting (cream) bacteria were identified and selected. Selected colonies were purified on MacConkey 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 accompanying computer software package (Oxoid Microbact® 2000 version 2.03).

Antimicrobial susceptibility testing. The identified isolates were tested for susceptibility to ampicillin (Amp), chloramphenicol (Chl), ciprofloxacin (Cip), enrofloxacin (Enr), neomycin (Neo), norfloxacin (Nor), streptomycin (Str) and tetracycline (Tet), by determining the minimum inhibitory concentration (MIC) using the broth micro-dilution method with an antimicrobial concentration ranging from 0.25-512 µg/µL, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (ANONYMOUS, 2008). The antimicrobial MIC breakpoint concentrations for bacterial isolates were: 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 (ANONYMOUS, 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.

Data analysis. Data were expressed in absolute values and in percentages. The geometric mean, median and mode of MIC values were determined using the Microsoft Office Excel 2007 software package. Rates of antimicrobial resistance were compared among bacterial genera by the Chi-square test at a $P<0.05$ probability level using the Statistical Software Package for Social Sciences (ANONYMOUS, 2007).

Results

In this study, a total of 184 bacterial isolates belonging to the family *Enterobacteriaceae* were obtained from the feces of free-range chickens. These included *E. coli* (104), *Klebsiella pneumoniae* (33), *Klebsiella oxytoca* (11), *Salmonella* spp. (20), and *Enterobacter aerogenes* (16). Overall, the isolates were resistant to ampicillin (89.7%), chloramphenicol (73.9%), ciprofloxacin (33.2%), enrofloxacin (60.3%), neomycin (70.7%), norfloxacin (45.7%), streptomycin (78.8%) and tetracycline (73.4%) (Table 1). *Escherichia coli* showed resistance to ampicillin (92.3%), chloramphenicol (73.1%), ciprofloxacin (34.6%), enrofloxacin (61.5%), neomycin (76.9%), norfloxacin (46.2%), streptomycin (80.8%) and tetracycline (76.9%) (Table 2). *Klebsiella* spp. were resistant to ampicillin (90.9%), chloramphenicol (72.7%), ciprofloxacin (54.5%), enrofloxacin (90.9%), neomycin (63.6%), norfloxacin (63.6%), streptomycin (81.8%) and tetracycline (81.8%) (Table 3). The rate of *Salmonella* spp. resistance to the tested antimicrobial agents is as follows: ampicillin (80.0%), chloramphenicol (80.0%), ciprofloxacin (0.0%), enrofloxacin (20.0%), neomycin (80.0%), norfloxacin (20.0%), streptomycin (80.0%) and tetracycline (35.0%) (Table 4). Isolates of *Enterobacter aerogenes* were resistant to ampicillin (81.2%), chloramphenicol (75.0%), ciprofloxacin (6.3%), enrofloxacin (18.8%), neomycin (37.5%), norfloxacin (25.0%), streptomycin (56.3%) and tetracycline (75.0%) (Table 5).

The observed differences in the rates of resistance to ampicillin, chloramphenicol, neomycin and streptomycin among the bacterial species were not statistically significant ($P>0.05$). However, the rates of resistance to the fluoroquinolones were significantly higher ($P<0.05$) in *Klebsiella* spp. (ciprofloxacin 54.5%, enrofloxacin 90.9% and norfloxacin 63.6%) than in *E. coli* (ciprofloxacin 34.6%, enrofloxacin 18.8% and norfloxacin 25.0%), *Salmonella* spp. (ciprofloxacin 0%, enrofloxacin 20.0% and norfloxacin 20.0%) and *Enterobacter aerogenes* (ciprofloxacin 6.3%, enrofloxacin 18.8% and norfloxacin 25.0%). Resistance to tetracycline was significantly lower ($P<0.05$) in *Salmonella* (35.0%) than in *Klebsiella* spp. (81.8%), *E. coli* (76.9%) and *Enterobacter aerogenes* (75.0%).

In all the bacterial species except *Salmonella,* ampicillin had the highest geometric mean MIC values (Tables 1-5). The highest geometric MIC value for *Salmonella* was obtained for streptomycin (Table 4). The lowest geometric mean MIC values for all the bacterial species was for ciprofloxacin (Tables 1-5).

There was a high rate of multidrug resistance (resistance to three or more unrelated antimicrobials) among the isolates. In all, 147 (79.9%) of the 184 isolates showed resistance to at least three unrelated antimicrobial agents. Thirty-nine (21.2%) of the 184 isolates were resistant to all eight tested antimicrobials, while only 12 (6.5%) of the 184 isolates were susceptible to all antimicrobials. A total of 26 antimicrobial resistance patterns were observed among the isolates (Table 6).

Table 1. The minimum inhibitory concentration (MIC) of antimicrobial agents on *Enterobacteriaceae* isolates from free-range chickens in Abeokuta, Nigeria

Antimicrobial agents	No. of isolates tested	Range of antimicrobial concentration (μg/μL)	MIC_{50}	MIC_{90}	Geometric mean MIC (μg/μL)	Median MIC (μg/μL)	Mode MIC (μg/μL)	Lowest MIC (μg/μL)	Highest MIC (μg/μL)	Number (%) resistant	Number (%) sensitive
Ampicillin	184	0.25-512	512.0	512.0	161.8	512.0	512.0	≤0.25	>512.0	165.0 (89.7)	19.0 (10.3)
Chloramphenicol	184	0.25-512	64.0	512.0	54.5	64.0	64.0	≤0.25	>512.0	136.0 (73.9)	48.0 (26.1)
Ciprofloxacin	184	0.25-512	2.0	64.0	2.4	2.0	0.25	≤0.25	>512.0	61.0 (33.2)	123.0 (66.8)
Enrofloxacin	184	0.25-512	8.0	64.0	6.7	8.0	16.0	≤0.25	>512.0	111.0 (60.3)	73.0 (39.7)
Neomycin	184	0.25-512	64.0	512.0	41.5	64.0	512.0	≤0.25	>512.0	130.0 (70.7)	54.0 (29.3)
Norfloxacin	184	0.25-512	4.0	256.0	5.6	4.0	0.25	≤0.25	>512.0	84.0 (45.7)	100.0 (54.3)
Streptomycin	184	0.25-512	512.0	512.0	116.1	384.0	512.0	≤0.25	>512.0	145.0 (78.8)	39.0 (21.2)
Tetracycline	184	0.25-512	128.0	512.0	50.9	128.0	512.0	≤0.25	>512.0	135.0 (73.4)	49.0 (26.6)

Table 2. The minimum inhibitory concentration (MIC) of antimicrobial agents on *Escherichia coli* isolates from free-range chickens in Abeokuta, Nigeria

Antimicrobial agents	No. of isolates tested	Range of antimicrobial concentration (μg/μL)	MIC_{50}	MIC_{90}	Geometric mean MIC (μg/μL)	Median MIC (μg/μL)	Mode MIC (μg/μL)	Lowest MIC (μg/μL)	Highest MIC (μg/μL)	Number (%) resistant	Number (%) sensitive
Ampicillin	104	0.25-512	512.0	512.0	228.6	512.0	512.0	≤0.25	>512.0	96.0 (92.3)	8.0 (7.7)
Chloramphenicol	104	0.25-512	64.0	512.0	46.1	64.0	64.0	≤0.25	>512.0	76.0 (73.1)	28.0 (26.9)
Ciprofloxacin	104	0.25-512	2.0	32.0	2.3	2.0	0.25	≤0.25	>512.0	36.0 (34.6)	68.0 (65.4)
Enrofloxacin	104	0.25-512	8.0	64.0	6.7	8.0	8.0	≤0.25	>512.0	64.0 (61.5)	40.0 (38.5)
Neomycin	104	0.25-512	64.0	512.0	45.6	64.0	32.0	≤0.25	>512.0	80.0 (76.9)	24.0 (23.1)
Norfloxacin	104	0.25-512	4.0	512.0	5.9	4.0	0.25	≤0.25	>512.0	40.0 (46.2)	56.0 (53.8)
Streptomycin	104	0.25-512	512.0	512.0	149.2	512.0	512.0	≤0.25	>512.0	84.0 (80.8)	20.0 (19.2)
Tetracycline	104	0.25-512	128.0	512.0	57.9	128.0	512.0	≤0.25	>512.0	80.0 (76.9)	24.0 (23.1)

Table 3. The minimum inhibitory concentration (MIC) of antimicrobial agents on *Klebsiella* species from free-range chickens in Abeokuta, Nigeria

Antimicrobial agents	No. of isolates tested	Range of antimicrobial concentration (µg/µL)	MIC_{50}	MIC_{90}	Geometric mean MIC (µg/µL)	Median MIC (µg/µL)	Mode MIC (µg/µL)	Lowest MIC (µg/µL)	Highest MIC (µg/µL)	Number (%) resistant	Number (%) sensitive
Ampicillin	44	0.25-512	512.0	512.0	281.4	512.0	512.0	16.0.	>512.0	40.0 (90.9)	4.0 (9.1)
Chloramphenicol	44	0.25-512	256.0	512.0	94.9	256.0	256.0	≤0.25	>512.0	32.0 (72.7)	12.0 (27.3)
Ciprofloxacin	44	0.25-512	8.0	256.0	7.9	8.0	2.0	≤0.25	>512.0	24.0 (54.5)	20.0 (45.5)
Enrofloxacin	44	0.25-512	16.0	128.0	22.6	16.0	16.0	≤0.25	>512.0	40.0 (90.9)	4.0 (9.1)
Neomycin	44	0.25-512	128.0	512.0	43.2	96.0	256.0	≤0.25	>512.0	28.0 (63.6)	16.0 (36.4)
Norfloxacin	44	0.25-512	16.0	256.0	11.0	16.0	16.0	≤0.25	>512.0	28.0 (63.6)	16.0 (36.4)
Streptomycin	44	0.25-512	256.0	512.0	183.9	256.0	512.0	4.0	>512.0	36.0 (81.8)	8.0 (18.2)
Tetracycline	44	0.25-512	64.0	512.0	60.1	64.0	512.0	≤0.25	>512.0	36.0 (81.8)	8.0 (18.2)

Table 4. The minimum inhibitory concentration (MIC) of antimicrobial agents on *Salmonella* from free-range chickens in Abeokuta, Nigeria

Antimicrobial agents	No. of isolates tested	Range of antimicrobial concentration (µg/µL)	MIC_{50}	MIC_{90}	Geometric mean MIC (µg/µL)	Median MIC (µg/µL)	Mode MIC (µg/µL)	Lowest MIC (µg/µL)	Highest MIC (µg/µL)	Number (%) resistant	Number (%) sensitive
Ampicillin	20	0.25-512	128.0	512.0	119.4	128.0	512.0	4.0	>512.0	16.0 (80.0)	4.0 (20.0)
Chloramphenicol	20	0.25-512	64.0	512.0	52.0	64.0	64.0	0.25	>512.0	16.0 (80.0)	4.0 (20.0)
Ciprofloxacin	20	0.25-512	0.25	4.0	0.6	0.25	0.25	0.25	4.0	0.0 (0.0)	20.0 (100.0)
Enrofloxacin	20	0.25-512	2.0	8.0	1.3	2.0	0.25	0.25	32.0	4.0 (20.0)	16.0 (80.0)
Neomycin	20	0.25-512	64.0	512.0	42.2	64.0	64.0	0.25	>512.0	16.0 (80.0)	4.0 (20.0)
Norfloxacin	20	0.25-512	2.0	16.0	1.7	1.5	0.25	0.25	64.0	4.0 (20.0)	16.0 (80.0)
Streptomycin	20	0.25-512	256.0	512.0	181.0	256.0	512.0	16.0	>512.0	16.0 (80.0)	4.0 (20.0)
Tetracycline	20	0.25-512	16.0	512.0	22.6	16.0	512.0	0.25	>512.0	7.0 (35.0)	13.0 (65.0)

Table 5. The minimum inhibitory concentration (MIC) of antimicrobial agents on *Enterobacter aerogenes* from free-range chickens in Abeokuta, Nigeria

Antimicrobial agents	No. of isolates tested	Range of antimicrobial concentration (µg/µL)	MIC_{50}	MIC_{90}	Geometric mean MIC (µg/µL)	Median MIC (µg/µL)	Mode MIC (µg/µL)	Lowest MIC (µg/µL)	Highest MIC (µg/µL)	Number (%) resistant	Number (%) sensitive
Ampicillin	16	0.25-512	256.0	512.0	139.6	192.0	512.0	16.0	512.0	13.0 (81.2)	3.0 (18.8)
Chloramphenicol	16	0.25-512	256.0	512.0	98.7	192.0	512.0	4.0	512.0	12.0 (75.0)	4.0 (25.0)
Ciprofloxacin	16	0.25-512	0.25	4.0	0.9	0.25	0.25	0.25	16.0	1.0 (6.3)	15.0 (93.7)
Enrofloxacin	16	0.25-512	2.0	8.0	1.8	1.5	0.25	0.25	512.0	3.0 (18.8)	13.0 (81.2)
Neomycin	16	0.25-512	16.0	512.0	19.9	16.0	16.0	0.25	512.0	6.0 (37.5)	10.0 (62.5)
Norfloxacin	16	0.25-512	4.0	32.0	2.7	3.0	4.0	0.25	64.0	4.0 (25.0)	12.0 (75.0)
Streptomycin	16	0.25-512	128.0	512.0	94.5	128.0	512	4.0	512.0	9.0 (56.3)	7.0 (43.7)
Tetracycline	16	0.25-512	256.0	512.0	66.8	256	512	0.25	512.0	12.0 (75.0)	4.0 (25.0)

Table 6. Antimicrobial susceptibility patterns of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, Nigeria

Phenotypic resistance groups	Antimicrobial resistance patterns	Number of resistant isolates				
		Escherichia coli	*Klebsiella* species	*Salmonella* species	*Enterobacter* species	Total
R1	AmpChlCipEnrNeoNorStrTet	20	18	0	1	39
R2	AmpChlCipEnrNeoNorTet	4	0	0	0	4
R3	AmpChlCipEnrNeoStrTet	0	6	0	0	6
R4	AmpChlEnrNeoNorStrTet	8	1	4	2	15
R5	AmpCipEnrNeoNorStrTet	4	0	0	0	4
R6	AmpChlCipEnrStrTet	4	0	0	0	4
R7	AmpChlNeoNorStrTet	8	0	0	1	9
R8	AmpChlEnrNorStrTet	4	1	0	0	5
R9	AmpChlEnrNeoStrTet	16	3	0	0	19
R10	AmpChlEnrStrTet	0	3	0	0	3
R11	AmpChlNeoStrTet	4	0	2	2	8
R12	AmpEnrNorStrTet	0	2	0	0	2
R13	AmpChlNeoStr	4	0	8	0	12
R14	AmpChlStrTet	0	0	0	3	3
R15	AmpNeoStrTet	8	0	0	0	8
R16	AmpChlNeo	0	0	1	0	1
R17	AmpChlStr	0	0	1	0	1
R18	AmpChlTet	0	0	0	3	3
R19	AmpEnrNor	0	6	0	0	6
R20	CipEnrNeo	4	0	0	0	4
R21	NeoStrTet	0	0	1	0	1
R22	AmpChl	4	0	0	0	4
R23	AmpStr	4	0	0	0	4
R24	StrTet	0	2	0	0	2
R25	Amp	4	0	0	1	5
R26	Susceptible to all	4	2	3	3	12

Discussion

Findings from the present study show that free-range birds harbor antimicrobial resistant bacteria. Over 70% of the isolates from the present study showed resistance to ampicillin, chloramphenicol, neomycin and streptomycin. The isolates were also resistant to the fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin) but at a lower rate. Resistance to tetracycline was lower in *Salmonella* spp. (35.0%) than in the other genera, in which it was above 70%. *Escherichia coli* and *Klebsiella* spp. also showed higher rates of resistance to the fluoroquinolones than did *Salmonella* and *Enterobacter aerogenes*. The observed higher resistance to ampicillin, chloramphenicol, neomycin, streptomycin and tetracycline could be because these drugs are older antimicrobial agents that were in use for a long period of time before the introduction of the fluoroquinolones. The high incidence of bacterial resistance to the older first line antimicrobial agents has resulted in an increase in the prescription and use of the newer generation fluoroquinolones. The consequence of this behavioral change over the years is the gradual emergence of resistant bacteria strains refractory to fluoroquinolone treatment (HOGE et al., 1998; KURUTEPE et al., 2005).

Escherichia coli is commonly used as the indicator bacterium for the surveillance and monitoring of the emergence of antimicrobial resistance (KIJIMA-TANAKA et al., 2003). The rate of *E. coli* resistance to ampicillin (92.7%), tetracycline (73.1%), streptomycin (80.8%) and neomycin (76.9%) observed in the present study is higher than that observed in *E. coli* isolates from healthy commercial and free-range chickens in Australia (ampicillin 26.7%, neomycin 6.0%, streptomycin 10.8%, and tetracycline 40.6%) (OBENG et al., 2012). Moreover, no resistance was observed to the fluoroquinolones, contrary to the observations in the present study. In Australia, the use of antimicrobials in animal production is regulated and fluoroquinolones are prohibited for use in all food-producing animals, including poultry (OBENG et al., 2012). However, in Nigeria, there is no policy guiding the use of antimicrobials in animals. Consequently, there is a high level of indiscriminate use of antimicrobials in animals (ALO and OJO, 2007). In another study conducted in Maiduguri, located in an Arid region of Nigeria, *E. coli* isolates from the tissues of apparently healthy and sick chickens showed resistance to ampicillin (66.7%), chloramphenicol (66.7%), ciprofloxacin (16.7%) and tetracycline (63.3%) (MAMZA, et al., 2010). These are lower than the rates obtained in the present study conducted in Abeokuta, located in the tropical rainforest region of Nigeria. Factors such as levels of dependence on antimicrobial usage in animals, degrees of environmental pollution and prevailing climatic conditions from region to region may contribute to antimicrobial selection pressure and the emergence of resistant bacteria, the persistence and distribution of resistant bacteria, as well as the exposure of hosts to resistant bacteria. All these will influence the overall prevalence of antimicrobial resistant bacteria within an ecosystem.

The high incidence of multi-drug resistant *E. coli* in free-range chickens, as observed in the present study, may be due to the continuous exposure of these birds to resistant bacteria in the environment. Although free-range chickens hardly receive any modern veterinary attention, they are exposed to potentially resistant bacteria harbored by other hosts (with previous exposure to antimicrobials) living in the same environment. Free range chickens may acquire resistant bacteria by contact with carriers or by ingestion of food and water that have been contaminated by fecal materials from other scavenging animals, which are more likely to receive veterinary care and treatment with antimicrobials. Poor sanitation and environmental pollution with human excreta due to inadequate toilet facilities, as observed in many rural communities in Nigeria, may expose free-range chickens to resistant bacteria of human origin. Poor management of effluent generated by abattoirs and commercial farms also contributes to environmental pollution and, hence, possible exposure of free-range chickens to resistant bacteria.

Furthermore, in developing countries, including Nigeria, there is easy access to antimicrobials and owners of free-range chickens may administer antimicrobial preparations to sick animals without recourse to professional advice. Free-range chickens may also be directly exposed to antimicrobials through improper disposal of the containers of used antimicrobial agents.

The present study revealed high levels of antimicrobial resistance in bacteria isolated from free-range chickens that rarely receive direct antimicrobial chemotherapy. These resistant bacteria are potential pathogens associated with diseases in avian and mammalian species, including humans (HART and KARIUKI, 1998; NWENEKA et al., 2009; KILONZO-NTHENGE et al., 2008; KABIR, 2010). Free-range birds are reared as scavengers and thus are exposed to a large amount of microorganisms in the environment. They also contribute to contamination of the environment by fecal shedding of bacteria. The findings in the present study therefore suggest a possible high level of antimicrobial resistant bacteria in the environment. Contamination of the environment by resistant bacteria from free-range chicken constitutes a public health hazard because of the possible transmission of these potential pathogens to humans through contact and consumption of contaminated food substances (TAUXE et al., 1989; ANONYMOUS, 2001; AUBRY-DAMON et al., 2004).

The findings in the present study provide valuable data for monitoring the continued emergence, persistence and dissemination of resistant bacteria in the community. In future surveillance studies, the MIC values obtained in the present study will be useful in comparative quantitative assessment of the level of antimicrobial resistance in bacteria within the community. Most developed countries have organized structures for systematic monitoring and reporting of antimicrobial resistance in pathogenic and non-pathogenic bacteria from humans and animals, but this is not the case in developing countries (OKEKE et al., 1999; GOODYEAR, 2002). Surveillance programs are required as the basis for policy formulation in the prevention and control of antimicrobial resistant bacteria in human and animal populations (ANONYMOUS, 2000).

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Received: 9 November 2011
Accepted: 27 June 2012

OJO, O. E., O. G. OGUNYINKA, M. AGBAJE, J. O. OKUBOYE, O. O. KEHINDE, M. A. OYEKUNLE: Antibiogram nekih bakterija porodice *Enterobacteriaceae* izdvojenih iz pilića u slobodnom sustava držanja u Abeokuti u Nigeriji. Vet. arhiv 82, 577-589, 2012.

SAŽETAK

Otpornost bakterija porodice *Enterobacteriaceae* na antimikrobne lijekove važan je pokazatelj pojave otpornih sojeva u populaciji. U ovom je radu mikrodilucijskim postupkom bila istražena osjetljivost na antimikrobne lijekove bakterija porodice *Enterobacteriaceae* izdvojenih iz pilića u slobodnom sustavu držanja. Od 184 izolata (104 izolata bakterije *Escherichia coli*, 44 *Klebsiella* spp., 20 *Salmonella* spp. i 16 *Enterobacter aerogenes*) na ampicilin je bilo otporno 89,7% izolata, na klormafenikol 73,9%, ciprofloksacin 33,2%, enrofloksacin 60,3%, neomicin 70,7%, norfloksacin 45,7%, streptomicin 78,8% i tetraciklin 73,4%. Izolati bakterije *Escherichia coli* bili su otporni na ampicilin (92,3%), kloramfenikol (73,1%), ciprofloksacin (34,6%), enrofloksacin (61,5%), neomicin (76,9%), norfloksacin (46,2%), streptomicin (80,8%) i tetraciklin (76,9%). Stopa otpornosti bakterija roda *Klebsiella* bila je za ampicilin 90,9%, kloramfenikol 72,7%, ciprofloksacin 54,5%, enrofloksacin 90,9%, neomicin 63,6%, norfloksacin 63,6%, streptomicin 81,8% i tetraciklin 81,8%. Izolati *Salmonella* spp. pokazivali su otpornost na ampicilin (80,0%), kloramfenikol (80,0%), enrofloksacin (20,0%), neomicin (80,0%), norfloksacin (20,0%), streptomicin (80,0%) i tetraciklin (35,0%), ali su u potpunosti bili osjetljivi na ciprofloksacin. Izolati *Enterobacter aerogenes* su bili otporni na ampicilin (81,3%), kloramfenikol (75,0%), ciprofloksacin (6,3%), enrofloksacin (18,8%), neomicin (37,5%), norfloksacin (25,0%), streptomicin (56,3%) i tetraciklin (75,0%). Sveukupno je 147 (79,9%) od 184 izolata pokazivalo višestruku otpornost na najmanje tri nesrodna antimikrobna lijeka. Veliki postotak bakterijskih izolata iz slobodno držanih pilića na antimikrobne lijekove može biti od znatne važnosti za ljudsko i životinjsko zdravlje s nepovoljnim gospodarskim učinkom.

Ključne riječi: višestruka otpornost na lijekove, *Enterobacteriaceae,* slobodno držani pilići