Nigerian Veterinary Journal
Vol 34 (1) 720-725

Antibiotic Sensitivity Profile of *Escherichia coli* Isolated from Poultry Farms in Ibadan, Oyo State, Nigeria.

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SUMMARY

A cross sectional study involving 300 cloaca swabs from apparently healthy birds from 8 small-medium scale poultry farms in Ibadan Oyo State was carried out. A total of 201 (67%) *Escherichia coli* isolates were recovered from the birds and they were subjected to in-vitro antibiotic sensitivity test by agar gel diffusion method. Some of the isolates were grown aerobically in antibiotic breakpoints of 8µg/ml of ciprofloxacin, and 32µg/ml of each of chloramphenicol, tetracycline and streptomycin.

High level of drug resistance was observed in virtually all the tested antibiotics, and a 100% resistance level were observed from agar gel diffusion method for; augumentin, nitrofurantoin and amoxicillin. Ciprofloxacin, pefloxacin and ofloxacin were the most sensitive antibiotics despite the high level of resistance of 76.1%, 72.6% and 69.2% respectively observed in them for the bacteria isolates. The two methods used produced similar antibiotic sensitivity. However, going by the high level of drug resistance observed for most of the antibiotics tested from both methods, it is imperative to put necessary measures in place to ascertain achievement of food safety through curtailments of abuse of antibiotics among the small-medium scale poultry farmers, and thus prevent the possible spread of drug resistant pathogens from poultry and poultry products to human.

KEYWORDS: Antibiotic- sensitivity, small-medium poultry farm.

INTRODUCTION

Agricultural sector remains one of the great avenues for economic growth in Nigeria. It employs about two-third of Nigeria's total work force and contributed 42.2% of gross domestic products (GDP) and provides 88% of non-oil earnings in 2007 (Ugwu, 2009). Poultry production was reported to account for 80% of the livestock production (Omotosho and Ladele, 1988; Ojo 2003). Disease outbreaks like bacterial infections are capable of constituting a stumbling block to profitable poultry production, with resultant economic losses to farmers and unavailability of high quality protein to feed the fast-growing human population. Commensal *Escherichia coli* strains may serve as a reservoir for transmissible antimicrobial resistance to other *E. coli* strains that cause extra-intestinal infections or other virulent species (Gennis and Stewart, 1996). Since the introduction of antibiotics into clinical practice over 20 decades ago, it was demonstrated that antibiotics could inhibit bacterial growth *in vitro* in specific, minimal concentrations (MICs); since then, this value has been used to denote susceptibility *in vivo* and to guide clinical practice. The acquisition of resistant mechanisms either by mutations or through interbacterial communication has rendered bacteria more tolerant to antibiotics and more difficult to treat. As a result, susceptibility breakpoints keep changing over time

(Rodriguez-Bano *et al.*, 2012; Falagas *et al.*, 2012).

The current work however focuses on health and public health related issue in terms of the status of antibiotic resistance among bacteria organism using *E coli* as reference pathogen from some small to medium scale farms in Ibadan, Oyo State, Nigeria.

MATERIALS AND METHODS

Sample Collection: In this cross-sectional study, a total of 300 cloaca swabs were obtained from apparently healthy broilers and pullets aged 8weeks to 56 weeks from eight small- medium scales commercial poultry farms in Ibadan, Oyo State, Nigeria. All the farms had history of usage and sometimes abuse/ misuse of antibiotics, without prescription by veterinarian, like tylosin, oxyteteracycline, amoxicillin, docycycline, gentamicin and enrofloxacin. The samples were collected between February and May, 2012.

TABLE I: Interpretive standards for the interpretation of the diameter of zone of inhibition for some antibiotics

Antimicrobial Agent(µg)	Zone Diameter (nearest whole mm for each category)		
	Resistant	Intermediate	Sensitive
QUINOLONES			
Cirofloxacin (10µg)	≤15	16 – 20	≥21
Ofloxacin(5µg)	≤12		
Pefloxacin(5µg)	≤12		
TETRACYCLINES			
Tetracycline (30µg)	≤14	15 – 18	≥19
PENICILLINS			
Amoxicillin (25µg)	≤13		
Augmentin (30µg)	≤19		
AMINOGLYCOSIDES			
Streptomycin (10µg)	≤11	12 – 14	≥15
Gentamicin (10µg)	≤12	13 – 17	≥18
CEPHALOSPORINS	-	-	-
Ceftriazone (30µg)			
PLEUROMUTILIN – DERIVATIVES			
Chloramphenicol (30µg)	≤12	13 – 17	≥18
MACROLIDES			
Erythromycin (30µg)	≤13	14 – 17	≥18
Cotrimazole (10µg)	≤10		

SOURCE: Clinical and Laboratory Standards Institute (CLSI), 2008

Bacteriological Analysis: cloaca swab samples were streaked on MacConkey agar and incubated aerobically at 37°C for 24 to 48hours. All the isolates that fermented lactose were sub cultured onto Eosin Methylene Blue agar and incubated at 37°C for 24 to 48hours. Colonies that produced typical metallic sheen on EMB were further subjected to morphological and biochemical analysis by standard methods (Barrow and Felthams, 1993; Garcia and Isenberg, 2007). In vitro antibiotic sensitivity test on the isolates were first of all carried out by the agar disc diffusion method (Matsen and Barry, 1974) using 13 antibiotic. The antibiotics used were: Amoxicillin (AMX), 25µg; Ofloxacin (OFL),5µg; Streptomycin (STR), 10µg, Chloramphenicol (CHL),30µg, Ceftriazone (CRO), 30µg, Gentamicin (GEN),10µg, Pefloxacin (PEF), 5µg, Cotrimoxazole (COT),25µg, Ciprofloxacin (CPX),10µg , Erythromycin (ERY),5µg , Tetracycline (TET), 30µg , Nitrofurantoin (NIT), 200µg, Augumentin (AUG)30µg, using oxoid(R) Iso-sesitest agar and Oxoid(R) antibiotic disc. Interpretive standard guideline (CLSI, 2008) shown in Table 1 served as the basis of categorizing the resistance patterns of the isolates to, sensitive, intermediate resistance and resistant strains. Subsequently, 199 of the isolates earlier tested with agar disc diffusion method were grown aerobically to determine and compare the antibiotic resistant pattern in breakpoint concentration of 8µg/ml for ciprofloxacin, and 32µg/ml for each of: chloramphenicol, tetracycline and streptomycin with the results from the agar disc diffusion method (all obtained from SIGMA-ALDRICH) according to standard method (CLSI, 2009). Resistance was ascribed if flocculent growth was observed after 16hours of anaerobic growth at 37°C.

RESULTS

TABLE II: Resistance patterns of *E coli* to commonly used antibiotics by agar gel diffusion methods

Antibiotic(µg)	% Sensitive	% with intermediate resistance	% Resistant	Total isolates tested
CPX(10µg)	37(18.4%)	11(5.5%)	153(76.1%)	201
TET(30µg)	-	-	58(100%)	58
PFX (5µg)	42(20.9%)	13(6.5%)	146(72.6%)	201
AUG(30µg)	-	-	58(100%)	58
CRO(30µg)	4(2.0%)	17(8.5%)	180(89.6%)	201
NIT (200µg)	-	-	58(100%)	58
GEN(10µg)	8(3.9%)	21(10.4%)	172(85.6%)	201
COT(25µg)	29(14.4%)	11(5.5%)	161(80.1%)	201
OFL(5µg)	47(23.4%)	15(7.5%)	139(69.2%)	201
AMX(25µg)	-	-	201(100%)	201
CHL(30µg)	7(4.9%)	10(7.0%)	126(88%)	143
STR(10µg)	4(2.8%)	3(2.1%)	136(95.1%)	143
ERY(5µg)	1(0.6%)	1(0.6%)	141(98.6%)	143

Key: AMX=Amoxycillin – 25µg, OFL= Ofloxacin – 5µg, STR=Streptomycin – 10µg, CHL=Chloramphenicol -30µg, CRO=Ceftriazone – 30µg, GEN=Gentamicin – 10µg, PEF=Pefloxacin 5µg , COT=Cotrimoxazole – 25µg,CPX= Ciprofloxacin – 10µg , ERY= Erythromycin – 5µg , Tetracycline – 30µg , NIT=Nitrofurantoin – 200µg, AUG=Augumentin 30µg

Escherichia coli were isolated from 201/300(67%) of the cloaca swab processed. Table 2 shows the respective numbers and percentages of the *Escherichia coli* that were either sensitive, or of intermediate resistance or completely resistant to each of the 13 antibiotics tested through agar disc diffusion methods. A100% level of resistance was observed in the number of isolates tested for Augumentin, nitrofurantoin, amoxicillin. High level resistances of 98.6% (erythromycin), 95.1% (streptomycin), 89.69% (ceftriaxone), 88% (chloramphenicol), 85.6% (gentamicin), 80.1% (cotrimazole), 76.1% (ciprofloxacin), 72.6% (pefloxacin), and 69.2% (ofloxacin) were observed in the bacteria isolates.

Also from table 2, the antibiotics with highest level of sensitivity in the bacteria isolates include; ofloxacin (23.4%), pefloxacin (20.9%), and ciprofloxacin (18.4%) followed closely by cotrimazole (14.4%). The isolates displayed lower levels of sensitivities for gentamicin, chloramphenicol, streptomycin, ceftriaxone and erythromycin. Table 3 shows the comparative resistance pattern obtained from antibiotic breakpoint method for the four selected antibiotics compared with the results from the disc diffusion method in the bacteria isolates.

DISCUSSION

The antibiotic resistant profile obtained from the screening of eight small- medium scale commercial poultry farms sampled in this study is worrisome. The results shows a very high level of resistance in virtually all the groups of commonly used and even in drug of last resort for treatment of life threatening disease conditions such as cephalosporin and even quinolones/ fluoroquinollone based drugs. For instance, the results from the disc diffusion method showed that 100% of the respective number of *E coli* isolates tested exhibited resistance to tetracycline, penincillin derivative drugs like augumentin and amoxicillin as well as in the wide spectrum nitrofuran (nitrofurantoin).

TABLE III: Comparative percentage resistance patterns of *E coli* to four antibiotics for breakpoint and agar gel diffusion methods

Antibiotics	% resistance using breakpoint method	% resistance for agar gel diffusion method
Ciprofloxacin	195/199 (98%) at 8µg/ml	153/201 (76%) at 10µg/ml
Chloramphenicol	165/199 (83%) at 32 µg/ml	126/143 (88%) at 30µg/ml
Tetracycline	169/199 (85%) at 32 µg/ml	143/143 (100%) at 30µg/ml
Streptomycin	186/199 (93%) at 32 µg/ml	136/143 (95%) at 10µg/ml

Out of the two macrolides tested, Cotrimazole was more sensitive 29% than erythromycin with 0.6% sensitivity. This observation may be due to the fact that the latter is more commonly used and possibly abused by farmer than the former. Of the aminoglycosides, the isolates exhibited a higher percentage of resistance 95.1% in streptomycin than for Gentamicin (85.6%). From this study, the isolates displayed, 88% level of resistance for the pleuromutilin derivative; Chloramphenicol

which used to be the drug of last resort for life threatening infection like typhoid fever. The high level of resistance 89.6% displayed by the isolates for ceftriazone (a third generation cephalosporin) is more worrisome. Cephalosporin group of drugs are together with the quinolone/ flouroquinolone group of drugs that are still the current drugs of last resort in life- threatening infections in most parts of the world, Nigeria inclusive.

From this study, although the level of resistance observed for the quinolone/ fluoroquinolone group of drugs in the isolates tested is still relatively high, that is 76.1%, 72.6% and 69.2% respectively for ciprofloxacin, pefloxacin and ofloxacin. They are all the same the group most sensitive compared with the other groups of antibiotics. There were 18.4%, 20.9% and 23.9% level of sensitivity for ciprofloxacin, pefloxacin and ofloxacin respectively in the isolates.

From table 3 the results obtained from breakpoint method at 8µg/ml for ciprofloxacin, and 32 µg/ml for each of chloramphenicol, tetracycline and streptomycin compared with that from disc diffusion method showed some apparent differences for ciprofloxacin and tetracycline. For example, 98% resistance was observed for breakpoint method and 76% for disc diffusion for ciprofloxacin. For tetracycline, 85% in breakpoint method compared with 100% for disc diffusion method. The results obtained in both methods for chloramphenicols and streptomycin is quite close; that is 83% compared with 88% for chloramphenicol and 93% compared with 95% for streptomycin. The acquisition of resistant mechanisms is known to render bacteria more tolerant to antibiotics and more difficult to treat. As a result, susceptibility breakpoints keep changing over time (Rodriguez-Bano *et al.*, 2012; Falagas *et al.*, 2012). From the result obtained in this study, it may be difficult to conclude on the most sensitive/ most reliable method between the breakpoint and disc diffusion method for determination of resistance of the tested isolates, there is however the need for studies to determine the respective antibiotic breakpoints for pathogen of interest in Nigeria.

Also in Nigeria, while the increase in the number of small scale poultry farming is desirable for our economy and the supply of the much needed, animal protein, the observation from this study brings to focus a very important factor relating to the need for judicious use of antibiotics by small- medium scale poultry farmers. The history of antibiotic usage gathered from the sampled farms was suggestive of misuse/abuse of antibiotics such as tylosin, oxyteteracycline, amoxicillin, docycycline, gentamicin and enrofloxacin at various times. These antibiotics abuse may be responsible for the high level of drug resistance observed from this study. There is also the public health implication of the observed high level of antibiotic drug resistance because of possible spread of these multidrug resistant pathogens to humans. The observation from this study underscores the importance of investigating various factors responsible for the misuse or abuse of antibiotics among small-medium scale poultry farmers in Nigeria, with the view to curtailing drug resistance due to antibiotic selective pressure. It is therefore important that necessary attention should be given to food safety and prevent the possible public health risk of transferring drug resistant pathogen to the public from poultry and poultry products emanating from this very important sector of our economy.

Conclusion: It is of utmost importance to pay close attention to food safety in terms of prevention of the spread of antibiotic resistance to the public through poultry and poultry products. This should be one of the major factors while the stakeholders in Agricultural sectors are considering necessary measures for the growth and improvement of livestock sectors in Nigeria.

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