academicJournals

Vol. 6(3), pp. 91-94, March, 2014
DOI: 10.5897/JVMAH2013.0267

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Cloacal feacal carriage and occurrence of antibiotic resistant *Escherichia coli* in chicken grown with and without antibiotic supplemented feed

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Accepted 31 January, 2014

Drug resistant *Escherichia coli* persist in the intestinal flora of poultry birds, and these serve as route via which they can be transmitted directly to humans, thus contributing to the already growing crisis of antibiotic resistance. The purpose of this study was to determine the cloacal feacal carriage and occurrence of antibiotic resistant *E. coli* isolates from chicken fed with and without antibiotic supplemented feeds. Cloacal feacal swabs (n = 200) were aseptically obtained from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria, and these were inoculated on MacConkey and cystine-lactose-electrolyte-deficient (CLED) media and incubated at 37°C for 18 to 24 h. Suspected colonies of *E. coli* growing on the agar media were subcultured, purified and further characterized using standard microbiology techniques. Antibiogram was investigated using the Kirby-Bauer disk diffusion method as per the clinical laboratory standards institute (CLSI) criteria. A total of 45 *E. coli* was isolated from the 200 cloacal feacal swab samples used for this study. Overall, 28% of *E. coli* were isolated from chicken fed with feed supplemented with antibiotics while only 17% of *E. coli* was isolated from chicken that received feed without antibiotics supplements. All the *E.* coli isolates showed varying rates of resistance and susceptibility to the tested antibiotics. Our results strongly reveal the occurrence of antibiotic resistant *E. coli* from chicken fed with and without antibiotic supplemented feeds. It is very critical that the continuous use of antibiotics in poultry production be strictly monitored, controlled and discouraged in order to contain the emergence and spread of antibiotic resistant bacteria through poultry production.

Key words: Resistance, *Escherichia coli*, poultry, veterinary, Nigeria.

INTRODUCTION

The usage of antimicrobial agents including antibiotics for either clinical or non-clinical reasons is amongst the singular purpose there is for the growing global increase in the emergence and spread of antibiotic resistance genes in pathogenic bacteria. Antibiotics have been continuously used for different veterinary and agricultural purposes including animal husbandry and poultry production where the feeds of poultry birds are constituted with antibiotics (Witte, 1998; Chah and Nweze, 2001). Apart from fighting infection and controlling the population of bacteria, the

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controlling the population of bacteria, the antibiotics are also used as growth promoters in the birds. This scenario allows for the selection of resistance strains of pathogenic and non-pathogenic bacteria including *Escherichia coli* and other bacteria exposed to the antibiotics in the intestinal flora of the birds, and such practices has the potential to increase the frequency of resistant bacteria in the poultry birds (Piddock, 1996; Al Ghamdi et al., 1999; Bisht et al., 2009). Though a natural phenomenon of bacterial genetics and evolution, antibiotic resistance builds up following every usage of antibiotics (whether rational or irrational) including the acquisition of resistance genes from other bacteria in the environment.

The discovery of antibiotics by Alexander Fleming in the early 1920s was one of the most remarkable breakthroughs in the field of medicine; owing to the fact that humanity was saved and is still being saved by these agents from the untoward and killing prowess of pathogenic bacteria (Fernandes, 2006; Jayaramah, 2009; Sundsfjord et al., 2004). But this very significant discovery (that is, the discovery of antibiotics referred to as "magic bullets") however, was inundated by the emergence of resistant strains of bacteria that can thrive even in the face of an antimicrobial onslaught. Antimicrobial resistance limits the life span of a drug, thus making it difficult and even more expensive to treat an infection. Antibiotics have been used and are currently used in the compounding of the feeds of birds and other poultry activities in many parts of the world including Nigeria (Chah and Nweze, 2001; Oyinloye and Ezekiel, 2011; Miranda et al., 2008). Such practices portend danger for public health (human and animal health inclusive) because of the likelihood of the development and transmission of resistant strains of bacteria from poultry birds to humans either directly or through consumption of poultry products.

In this study, the cloacal feacal carriage and frequency of antibiotic resistant *E. coli* from chicken fed with and without antibiotic supplemented feeds was investigated to ascertain the feacal carriage of these pathogens in chicken from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria.

MATERIALS AND METHODS

Study area and collection of cloacal feacal swab samples

This research was carried out in the Microbiology Department of Ebonyi state university, Abakaliki, Nigeria in line with ethical consideration of the 2004 Declaration of the World Medical Association (WMA) in Helsinki regarding principles guiding experiments that involves human and non-human subjects (WMA, 2004). A total of two hundred (200) birds from two poultry farms were included in this study. One of the poultry farm used antibiotic supplemented feed in the growth of their birds while the other used non-antibiotics supplemented feed. However, the type and name of antibiotic used for compounding the feed was not made known by the manufacturer of the feed. In each, cloacal feacal samples were randomly taken from 20 days old and 40 days old chicks in two different batches designated flock A (chicken fed with antibiotic supplemented feed) and flock B (chicken fed with feed without antibiotic supplements), and these were transported to the laboratory in normal saline tubes and stored at 4°C until use.

Isolation and identification

Cloacal feacal swabs were inoculated on MacConkey and cystein lactose electrolyte deficient (CLED) agar plates (Oxoid UK) and incubated at 37°C for 18 to 24 h. *E. coli* grows on MacConkey and CLED medium as smooth pink colonies and yellow colonies, respectively. Only these colonies were counted and further analyzed after 18 to 24 h incubation at 37°C. Suspected colonies of *E. coli* was grown on Mueller-Hinton (MH) agar (Oxoid UK) plates after series of subculturing on MacConkey and CLED agar plates. The isolates were cultured on nutrient agar plates (Oxoid UK) and characterized by Gram staining, triple sugar iron agar (TSIA), indole test and citrate test (Cheesbrough, 2000).

Antibiogram

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method as per the CLSI criteria, formerly National Committee for Clinical Laboratory Standards (NCCLS) (CLSI, 2012) on Mueller-Hinton agar plates using single antibiotic disks of chloramphenicol (10 µg), tetracycline (30 µg), sulphamethoxazole/ trimethoprim (15 µg), ciprofloxacin (5µg), gentamicin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), amoxicillin/clavulanic acid (30 µg), and ampicillin (10 µg). These antibiotics were selected and used for this study because they (including some related antibiotics) have been used regularly in poultry production and for other agricultural purposes either on the prescription of a veterinary doctor or on self-medication. The plates were incubated at 37°C for 18 to 24 h, and the inhibition zone diameters (IZDs) measured with a meter rule and recorded as recommended by the CLSI (CLSI, 2012).

RESULTS

Overall, 200 cloacal feacal samples (100 samples from each poultry farm) were examined microbiologically for the cloacal feacal carriage of *E. coli*. A total of 45 *E. coli* isolates was isolated from the 200 cloacal feacal swab samples employed in this study. Cloacal feacal samples from chicken fed with feeds supplemented with antibiotics (flock A) showed a 14% carriage of *E. coli* in their feaces while chicken fed with feeds without any antibiotics supplements (flock B) showed a total of 8.5% *E. coli* carriage in their feacal samples (Table 1). The antibiotics used to supplement the feed that was given to flock A chicken was not documented on the bag of the feed but it was carefully recorded by the manufacturer that the feed was compounded with antibiotics that could serve as growth promoters as well as control bacterial population in the poultry birds. However, the restriction of the use of antibiotics in the production of poultry birds is not yet restricted by the Nigerian government, though the practice has been greatly discouraged by concerned authorities. The results of antimicrobial susceptibility of the *E. coli* isolates to some selected antibiotics are shown in Tables 2 and 3. Of the two batches of chicken populations included

Table 1. Percentage frequency of *Escherichia coli* isolation from the chicken**.**

Age of chicken (day old)	% Chicken given feed supplemented with antibiotics (Flock A: n=100)	% Chicken given feed without antibiotics supplement (Flock B: n=100)
20	9	13
40	19	4
Total	28	17

Table 2. Results of susceptibility of *Escherichia coli* isolated from chicken grown with antibiotics supplemented feed.

Susceptibility pattern	CHL	TET	SXT	CIP	GEN	CTX	CAZ	CFO	AMC	AMP
Susceptible (n)	6	10	14	13	6	14	4	12	16	6
Resistant (n)	20	14	13	14	18	13	14	16	10	19
Intermediate (n)	2	4	1	1	4	1	10	0	2	3

n = number of isolates, CHL=chloramphenicol, AMP=ampicillin, TET= tetracycline, SXT=sulphamethoxazole/trimethoprim, CIP=ciprofloxacin, GEN=gentamicin, CTX=cefotaxime, CAZ=ceftazidime, CFO=cefoxitin, AMC=amoxicillin/clavulanic acid.

Table 3. Results of susceptibility of *Escherichia coli* isolated from chicken grown with feed without antibiotics supplement.

Susceptibility pattern	CHL	TET	SXT	CIP	GEN	CTX	CAZ	CFO	AMC	AMP
Susceptible (n)	15	16	14	11	13	12	13	15	17	17
Resistant (n)	1	1	1	5	2	3	1	1	0	0
Intermediate (n)	1	0	2	1	2	2	3	1	0	0

n=number of isolates, CHL=chloramphenicol, AMP=ampicillin, TET= tetracycline, SXT=sulphamethoxazole/trimethoprim, CIP=ciprofloxacin, GEN=gentamicin, CTX=cefotaxime, CAZ=ceftazidime, CFO=cefoxitin, AMC=amoxicillin/clavulanic acid

in this study (that is, flock A and flock B), the highest degree of resistance of the *E. coli* isolates to the tested antibiotics was detected in chicken fed with feeds supplemented with antibiotics (flock A) (Table 2). Similar tendency of resistance was also detected in chicken fed with feeds without any antibiotic supplements (flock B); however, the trend and frequency of resistance in *E. coli* isolates from flock B chicken is lower than the number and degree of resistance observed in *E. coli* isolates from flock A chicken (Table 3).

DISCUSSION

The resistance of pathogenic bacteria to antibiotics is not a new phenomenon in both the practice of human and veterinary medicine but it is a problem that is becoming more dangerous, and must be contained in order to protect and extend the efficacy and shelf life of available antibiotics. This is very important due to the slow pace in research and development of novel antibiotics and other antimicrobials that can effectively assuage the resistance problem that pathogenic bacteria express *in vivo* against antibiotics. Antibiotics apart from being used for human medicine are also used for other veterinary purposes both for prescription reasons to control bacteria invasion and as growth promoters to increase poultry bird's production.

In this study, the feacal carriage and occurrence of antibiotic resistant *E. coli* was investigated amongst chicken from two poultry farms in Abakaliki metropolis, Ebonyi state of Nigeria. From our study, we discovered that there was a higher percentage of the isolation of *E. coli* (28 %) from flock A chicken than from flock B chicken (17%). A possible reason for the low occurrence of *E. coli* in flock B chickens compared to flock A chickens could be attributed to the retention of resistant bacterial strains in the alimentary canal of the poultry birds and the non-exposure of the birds to initial antibiotic challenge which is one of the prerequisite that allows pathogenic bacteria to develop resistance towards a particular drug via selective pressure.

E. coli strains are routinely found in the gut as part of the indigenous microbiota. However, some strains of *E. coli* have been implicated in a number of resistant infections in humans, and this includes *E. coli* strains that harbour multidrug resistance genes such as extended spectrum beta lactamase (ESBL) enzymes amongst others (Rupp and Paul, 2003). The frequency of feacal carriage of *E. coli* in chicken given feed supplemented with antibiotics in this study is in line with available data that reveal the impact and effect of antibiotics when they are used for non-human purposes such as in the production of livestocks. A recent work carried out in Owerri, Nigeria also reported over 40% increase in the isolation of *E. coli* from poultry birds in Owerri metropolis,

Imo state of Nigeria (Duru et al., 2013).

In Saudi Arabia, antibiotic resistant *E. coli* has been isolated from chicken including patients and poultry workers (Al Ghamdi et al., 1999). Antimicrobial resistance is a serious global health problem that knows no bother, and that strikes at the core of infectious disease control. It has the potential to halt, and possibly even to roll back some of the many progresses achieved in the medical sciences as is related to antimicrobial chemotherapy. Overcrowding, poor poultry sanitation, and over usage of antibiotics in the production of poultry birds are some of the factors contributing to the upward trend of antimicrobial resistance development and spread in bacteria emanating from poultry farms. Antibiotics are infrequently used as a prophylactic measure as well as a growth promoting agent in the raring of poultry birds. This gives room for high antibiotic selection and resistance development amongst bacterial population.

In this study, the antimicrobial susceptibility of the *E. coli* isolates from cloacal feacal sample swabs of chicken fed feeds with and without antibiotic supplements was investigated against a battery of 10 selected antibiotics. Higher degree of antibiotic resistance was detected in *E. coli* isolates from cloacal feacal swab samples of chicken fed with feed supplemented with antibiotics (flock A). However, a lesser degree and number of *E. coli* isolates from cloacal feacal swab samples of chicken fed with feeds without antibiotic supplements (flock B) was resistant to the tested antibiotics. Generally, a higher occurrence of resistance was found in cloacal feacal samples of flock a chicken compared with those from flock B chicken. Resistance of *E. coli* isolates from poultry origin to some conventional antibiotics has been reported both within and outside Nigeria (Zhang et al., 2010; Duru et al., 2013; Gray et al., 2004). Antibiotic resistant *E. coli* may persist in the intestinal tract of these poultry birds for a long period of time with or without the use of antibiotics, and these can serve as route via which they can be transmitted to human population directly or through consumption. The continuous usage of antibiotics outside the health system, especially in veterinary and livestock purposes still continues in Nigeria and other parts of the emerging economies. For us not to go back to the pre-antibiotic era when they were barely no conventional antibiotics as we now have to treat many bacterial related diseases, it is very important that urgent and consolidated efforts are put in place and sustained in order to abate the problem of antibiotic resistance.

Conclusion

Conclusively, our results confirm the feacal carriage of antibiotic resistant *E. coli* in poultry birds reared in Abakaliki metropolis, Ebonyi state of Nigeria, making it the first presumptive study to be conducted on the matter in this part of Nigeria. Co-operation between human, animal health and scientists in the agriculture profession is very important in containing antibiotic resistance in poultry farms since the use of antibiotics in food animal production also contributes immensely to the increased drug resistance that we now face in the world today.

REFERENCES

Al Ghamdi MS, El Morsy F, Al Mustafa ZH, Al Ramadhan M, Hanif M (1999). Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Trop. Med. Inter. Health 4:278-283.

Bisht R, Katiyar A, Singh R, Mittal P (2009). Antibiotic Resistance – A Global Issue of Concern. Asian J. Pharm. Clin. Res. 2 (2):34-39.

Chah KF, Nweze NE (2001). Antibiotic use in poultry production in Nsukka, Southeast Nigeria. Proc. Nig. Soc. Anim. Prod, 26:69-72.

Cheesbrough M (2002). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press. pp. 54-59.

Clinical and Laboratory Standards Institute (Formerly National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Susceptibility Testing; 15thInformational Supplement 2012; (M100-S15).

Duru C, Nwanegbo E, Adikwu M, Ejikeugwu C, Esimone C (2013). Extended-Spectrum Beta-Lactamase–Producing *Escherichia Coli* Strains of Poultry Origin in Owerri, Nigeria. World J. Med. Sci.8 (4):349-354.

Fernandes P (2006). Antibacterial discovery – the failure of success? Nature Biotechnol. 24(12):1.

Gray JT, Hungerford LL, Fedorka-Cray PJ (2004). Extended spectrum-cephalosporin resistance in *Salmonella* enteric isolates of animal origin. Antimicrob. Agents Chemother. 48:3179–81.

Jayaramah R (2009). Antibiotic Resistance: an overview of mechanisms and a paradigm shift. Curr. Sci., 96(11):1475-1484.

Lapara TM, Firl SJ, Onan LJ, Ghosh S, Yan T, Sadowsky MJ (2006). Municipal Waste – Water Treatment: A Novel Opportunity to show the Proliferation of Antibiotic – Resistant Bacteria? Fall 18-23.

Miranda JM, Franco CM, Vazquez BI, Fente CA, Barros-Velazquez J, Cepeda A (2008). Evolution of resistance of poultry intestinal *Escherichia coli* during three commonly used antimicrobial therapeutic treatments in poultry. Poult. Sci. 87(8):164-1648.

Oyinloye JMA, Ezekiel CN (2011). Extended-Spectrum Beta-Lactamase (ESBL) – Producing Multidrug Resistant *Enterobacteriaceae* from Commercial Poultry Feeds in Nigeria. Annals Biol. Res. 2(2):250-254.

Piddock LJV (1996). Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? J. Antimicrob. Chemother. 38:1–3.

Rupp ME, Paul D (2003). Extended Spectrum β-Lactamase (ESBL) Producing *Enterobacteriaceae*. Drugs, 63(4)353-356.

Sundsfjord A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmevoll S,
Witte W (1998). Medical consequences of antibiotic use in agriculture. Sci.279:996-997.

World Medical Association (WMA) Declaration of Helsinki. Ethical Principles for Medical Research involving Human Subjects. Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004. 9.10.2004.

Zhang CH, Liu YL, Wang JH (2010). Detection of ESBLs and Antimicrobial susceptibility of *Escherichia coli* isolated in Henan, China. J. Anim. Vet. Adv. 9(15):2030-2034.