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EVALUATION OF RATIONAL USE OF ANTIBIOTIC IN POULTRY INDUSTRY USING *ESCHERICHIA COLI* ANTIBIOGRAM AS INDEX OF RESISTANCE IN ZARIA, NIGERIA

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ABSTRACT

To control and prevent poultry diseases, breeders are known to administer subtherapeutic and therapeutic levels of antimicrobial agents to chickens via feed and water, which improves feed efficiency and accelerates weight gain but provides a selective pressure for antibacterial resistance. This study was aimed at evaluating the rational use of antibiotic in poultry industry using *Escherichia coli* antibiogram as index of bacteria resistance in Zaria, Nigeria. A total of 382 samples of fresh poultry droppings were collected between July 2018 and March 2019 from commercial chicken farms and plated on Eosine Methyline Blue agar. *Escherichia coli* was isolated and identified by standard microbiological methods and confirmed using Microgen^{TM} kits. Antibiotic susceptibility testing was carried out using Kirby-Bauer disk diffusion technique with 14 different antibiotic discs. The prevalence of *Escherichia coli* in poultry droppings was 83.76%. Among the strains of *Escherichia coli* isolates obtained, 91.30% were resistant to ampicillin and tetracycline each, while 69.57% were resistant to trimethroprim-sulphamethoxazol but no resistance to cefoxitin (0.00%). Furthermore, all the *Escherichia coli* (100%) had multiple antibiotic resistance indices ≥0.21 in this study. In conclusion, use of antibiotic in poultry could have led to emergence of antibiotic resistant bacteria in Nigeria.

Keywords: Antibiotic Resistance, *Escherichia coli*, Poultry droppings.

INTRODUCTION

Escherichia coli is a Gram-negative, non-spore-forming, rod shaped bacteria which is capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface-active agents with similar growth-inhibiting properties. It usually ferment lactose at 37°C within 48 hours, possess the enzymes β-glucuronidase and β-galactosidase and is oxidase-negative (Anon, 1997). *Escherichia coli* is present in large numbers in the normal intestinal flora of humans and animals, however, in other parts of the body, *E. coli* can cause serious diseases, such as urinary tract infections, bacteraemia and meningitis, while a limited number of *E. coli* serotypes can cause acute diarrhoea (Cheesbrough, 2006).

Based on pathogenic features, *E. coli* strains that cause diseases can be grouped into several different categories (pathotypes) including enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), extra-intestinal pathogenic *E. coli* (ExPEC), uropathogenic *E. coli* (UPEC), and enterohemorrhagic *E. coli* (EHEC) (Kim, 2005; Lim *et al*., 2011). Avian pathogenic *Escherichia coli* (APEC) is associated with intestinal and extraintestinal infections in chickens, turkeys, ducks, and other avian species, causing a variety of diseases collectively known as colibacillosis (Huruma *et al*., 2012).

In livestock production, poultry occupies a prominent position in the provision of animal protein and this account for about 25%-30% of local meat production in Nigeria and in the world (FAO, 2006; Agbaje *et al.*, 2010). To control and prevent poultry diseases, breeders are known to administer subtherapeutic and therapeutic levels of antimicrobial agents to chickens via feed and water, which improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). However, this practice has provided a selective pressure for antimicrobial resistance genes, and as a result, many bacteria associated with poultry meat are now resistant to antibacterial agents (Ifigenia*et al*., 2001). Therefore, this study was aimed at evaluating the rational use of antibiotic in poultry industry using *Escherichia coli* antibiogram as index of bacteria resistance in Zaria, North Western, Nigeria.

MATERIALS AND METHODS

The Study Area

Zaria is a town in Kaduna State, in the North-Western part of Nigeria and is located on latitude 11º04N and longitude 7º43E and about 660M above sea level (National Population Census, 2010; Oladimeji and Ojibo, 2012).

Study Design: The study design was a cross-sectional study. Five sampling sites were selected in Zaria Metropolis for this study. They were Samaru, Wusasa, Tudun wada, Zaria City and Sabon Gari., while the sample size was determined using the formula described by Naing *et al.* (2006).

Collection of Samples

A total of 382 faecal samples were proportionately collected from randomly selected poultry farms in Zaria Metropolis (Samaru, Wusasa, Tudunwada, Zaria City and Sabon Gari) between July 2018 and March 2019.

Processing of Samples

Upon arrival at the laboratory, 1g of each faecal sample was homogenized in 9ml of buffered peptone water (Oxoid, UK).

A disinfected blender (using 70% ethanol for disinfection) was used to obtain the homogenate. The homogenate was pre-enriched in peptone water by incubating for 24 hours at 37°C. A loopful of pre-enriched specimen was cultured by streaking on Eosin Methylene Blue (EMB) agar, the plates were inverted and incubated aerobically at 37°C for 24 hours after which the plates were examined for growth. Isolates were characterized and identified using standard conventional biochemical tests and confirmed using Microgen™ kits. (Cheesbrough, 2006).

Antibiotic Susceptibility Testing of *Escherichia coli*

The antibiotic susceptibility testing of *Escherichia coli* isolates were carried out using the Kirby-Bauer disk diffusion technique according to the methods recommended by the Clinical Laboratory and Standards Institute (CLSI, 2018) to determine susceptibility or resistance profiles of the isolates, discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared pre-dried Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed (20mm away from each other) on the inoculated Mueller-Hinton agar plates. The antibiotic discs used were Ampicillin (10µg), Cefotaxim (30µg), Ceftazidime (30µg), Amoxicillin-Clavulanic acid (20/10µg), Cefoxitin (30µg), Etarpenem (10µg), Azithromycin (15µg), Aztreonam (30µg), Tetracycline (30µg), Trimethroprim-Sulphamethoxazol (1.25/23.75µg), Chloramphenicol (30µg), Gentamicin (10µg), Ciprofloxacin (5µg) and Nitrofurantoin (300µg) (Oxoid, UK). The reference standard strain used as control in this study was *Escherichia coli* ATCC 25922. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimetre (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistance were made according to CLSI. (2018) manual. The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotic to which the isolate was resistant to, by the total number of antibiotic tested (Olayinka *et al.,* 2004).

Determination of Multiple-Antibiotic Resistance Index

The multiple-antibiotic resistance (MAR) index was determined for each isolate by dividing the number of antibiotic to which the isolate was resistant to, by the total number of antibiotic tested (Olayinka *et al.,* 2004).

$$\text{MAR index} = \frac{\text{Number of antibiotic the isolate was resistant to}}{\text{Total number of antibiotic tested.}}$$

Data Analysis. The data were coded and entered into Microsoft Excel®2007, and then exported to Statistical Package for Social Sciences (SPSS) windows version 20.0 (IBM, USA) for appropriate statistical analysis. The prevalence of *E. coli* in chicken droppings were expressed using descriptive statistics and Chi square ($\chi 2$) to measure the association between prevalence across the study locations. Association were reported as statistically significant if *P* value was less than 5% ($P< 0.05$).

RESULTS

Prevalence of *Escherichia coli* in Poultry Droppings from Zaria Metropolis

Table 1 showed the prevalence of *Escherichia coli* in poultry droppings sampled from Zaria Metropolis. The highest detection rate was recorded in sampling locations Tudun Wada 41(100%), Zaria City 136(100%) and Sabon Gari 55(100%). The least detection rate was observed in samples obtained from sampling location Samaru 41(50%). Besides, across locations, there was a statistical significant difference ($p < 0.05$) in the prevalence of *E coli* in poultry droppings where sampling location Zaria City (35.60%) had the highest point prevalence while Samaru (10.73%) and Tudun Wada (10.73%) locations had the least point prevalence of *E. coli.*

Antibiogram of *Escherichia coli* Isolated from Poultry Droppings in Zaria Metropolis.

Table 2, showed the results of antibiotic resistance profile of 23 randomly selected out of 320 *E. coli* isolated from poultry faecal samples in Zaria. Out of the 23 *Escherichia coli* isolates tested, 21(91.3%) were resistant to ampicillin and tetracycline each, while 16(69.57%), 12(52.17%) and 11(47.83*%)* expressed resistance to Sulfamethoxazol-Trimethoprim, Cefotaxim and Ceftazidime respectively. However, the least resistance by the isolates were to Etarpenem 1(4.35%), while non-expressed resistance to Cefoxitin (0%). Table 2 further showed the antibiogram of *Escherichia coli* isolated from poultry droppings in Zaria Metropolis. The results showed that *22(95.65%)* of the *Escherichia coli* isolates were susceptible to Etarpenem while 9(39.13%), were intermediately resistant to Amoxicillin-Clavulanic acid. Table 3 showed the multiple antibiotic resistance pattern and indices of *Escherichia coli* isolated from poultry droppings in Zaria. The results showed that all the *Escherichia coli* isolates 23(100%) were resistant to three or more antibiotics; 1(4.35%) was resistant to three antibiotics, 6(26.09%) to four antibiotics, 4(17.39%) to six and seven antibiotics while 2(8.70%) and 1(4.35%) were resistant to nine and ten antibiotics respectively. It was also observed that all the 23(100%) of the *Escherichia coli* isolates from poultry droppings had multiple antibiotic resistance indices ≥0.21 as shown in Table 3.

Table 1: Prevalence of *Escherichia coli* in Poultry Droppings Sampled from Poultry Farms in Zaria Metropolis

Locations of Poultry Farms Sampled	Number of Samples Collected	*E. coli* Positive Samples (%)	Prevalence (%)	$\chi 2$	df	P-value
Samaru (A)	82	41(50)	10.73	124.465	4	0.000
Tudun Wada (B)	41	41(100)	10.73			
Wusasa (C)	68	47(69)	12.30			
Zaria City (D)	136	136(100)	35.60			
Sabon Gari (E)	55	55(100)	14.40			
Total	382	320(83.76)	83.76			

$\chi 2$ = Chi square, df = degree of freedom.

Table 2: Antibiogram of *Escherichia coli* Isolated from Poultry Droppings in Zaria Metropolis

Antibiotic (Disc Content µg)	Symbol	Antibiotic Class	Susceptibility (n=23) R(%)	I(%)	S(%)
Ampicillin (10)	AMP	Penicillins	21(91.30)	1(4.35)	1(4.35)
Cefotaxim (30)	CTX	Cephalosporins*	12(52.17)	0(0)	11(47.83)
Ceftazidime (30)	CAZ	Cephalosporins*	11(47.83)	0(0)	12(52.17)
Amoxicillin-Clavulanic acid (20/10)	AMC	β-Lactam Combination agents*	2(8.70)	9(39.13)	12(52.17)
Cefoxitin (30)	FOX	Cephalosporins**	0(0)	2(8.70)	21(91.30)
Etarpenem (10)	ETP	Penems	1(4.35)	0(0)	22(95.65)
Azithromycin (15)	AZM	Macrolides	9(39.13)	0(0)	14(60.87)
Aztreonam (30)	ATM	Monobactams	9(39.13)	0(0)	14(60.87)
Tetracycline (30)	TE	Tetracyclins	21(91.30)	0(0)	2(8.70)
Trimethroprim-Sulphamethoxazol (1.25/23.75)	SXT	Folate Pathways Antagonists	16(69.57)	0(0)	7(30.43)
Chloramphenicol (30)	C	Phenicols	6(26.09)	0(0)	17(73.91)

Gentamicin (10)	CN	Aminoglycosides	3(13.04)	1(4.35)	19(82.61)
Ciprofloxacin (5)	CIP	Quinolones and Flouroquinolones	4(17.39)	0(0)	19(82.61)
Nitrofurantoin (300)	F	Nitrofurans	3(13.04)	4(17.39)	16(69.57)

R= Resistance, I= Intermediate, S= Susceptible, n= number, *=ESBL Indicator, **=ESBL AmpC Indicator.

Table 3: Multiple Antibiotic Resistance Pattern and indices of *Escherichia coli* Isolated from Poultry Droppings in Zaria Metropolis

Number of Antibiotic Combination	Resistance Phenotype	Droppings n=23 (%)	MARI
3	AMP, SXT, TE,	1(4.35)	0.21
4	AMP, AMC**, TE, SXT	2(8.7)	0.29
	AMP, AZM, TE, SXT	1(4.35)	
	AMP, FOX*, TE, SXT	1(4.35)	
	AMP, CAZ, CTX, TE	1(4.35)	
	AMP, ATM, CAZ, CTX	1(4.35)	
5	AMC, AMP, AZM, SXT, TE.	1(4.35)	0.36
	AMP, CAZ, CTX,TE, SXT	1(4.35)	
	AMP, CAZ, CTX, C, TE	1(4.35)	
6	AMP, ATM, TE, SXT, CIP, F	1(4.35)	0.43
	AMP, CTX, ATM, AZM, TE, F*	1(4.35)	
	AMP, AMC*, CAZ, CTX, TE, SXT	2(8.7)	
	AMP, AMC*, CAZ, CTX, ATM, TE	1(4.35)	
7	AZM, ATM, TE, SXT, C, CN*, F*	1(4.35)	0.5
	AMP, AMC*, AZM, TE, SXT, CN, F*	1(4.35)	
	AMP, AMC*, AZM, CAZ, CTX, TE, SXT	1(4.35)	
	AMP, AMC*, AZM, CAZ, CTX, ATM, C	1(4.35)	
8	AMP, AZM, ATM, TE, SXT, CN, CIP, F	1(4.35)	0.57
	AMP, FOX*, TE, SXT, C, CN, CIP, F*	1(4.35)	
9	AMP, AMC*, ATM, CAZ, CTX, ETP, TE, C, F	1(4.35)	0.64
10	AMP, AMC, AZM, ATM, CAZ, CTX, TE, SXT, C, CIP	1(4.35)	0.71

Key: *= Intermediate-Resistance,AMP =Ampicillin, CTX =Cefotaxim, CAZ =Ceftazidime, AMC=Amoxicillin-Clavulanic acid, FOX =Cefoxitin, ETP =Etarpenem, AZM =Azithromycin, ATM =Aztreonam, TE =Tetracyclin, SXT =Sulphamethoxazol-Trimethroprim, C =Chloramphenicol, CN =Gentamicin, CIP =Ciprofloxacin, F =Nitrofurantoin.

DISCUSSION

Prevalence of *Escherichia coli* in Poultry Droppings from Zaria Metropolis

The prevalence of *Escherichia coli* in poultry droppings sampled from Zaria Metropolis were determined and it was observed that the highest detection rate was recorded in sampling locations Tudun Wada 41(100%), Zaria City 136(100%) and Sabon Gari 55(100%) which all had 100% incidence of *E. coli* in poultry droppings sampled from each location. This could affirm the claim that *E. coli* are part of the enteric flora of the chickens and therefore could reliably serve as index of faecal contamination. The least detection rate was observed in samples obtained from sampling location Samaru 41(50%). This might be due to low discriminatory

power of conventional culture based technique in the detection of *E. coli* and not necessarily the absence of *E. coli* in (50%) of the poultry droppings from such location. It could also be due to improvement in hygiene and farm management practices observed in Samaru location. Besides, across locations, there was a statistical significant difference ($p < 0.05$) in the prevalence of *E. coli* in poultry droppings where sampling location Zaria City (35.60%) had the highest point prevalence while Samaru (10.73%) and Tudun Wada (10.73%) locations had the least point prevalence of *E. coli* in poultry droppings. The highest point prevalence observed in sampling location Zaria City (35.60%) could be due to poor hygiene practices observed in this location and use of well water as the source of poultry drinking water by majority of the poultry farms visited in Zaria City. Also, it could be due to the fact that Zaria City location had the highest number of samples analyzed.

Antibiogram of *Escherichia coli* as Index of Resistance Associated with Poultry Droppings in Zaria Metropolis.

According to the guidelines by Clinical and Laboratory Standards Institute, (CLSI, 2018), Fourteen commonly used antibiotics in the study area were used for the susceptibility testing. The results of the antibiotic resistance profile of 23 out of 320 *E. coli* isolated from poultry dropping samples in Zaria Metropolis, showed that out of the 23 *Escherichia coli* isolates tested, 21(91.3%) were resistant to ampicillin and tetracycline while 16(69.57%), 12(52.17%) and 11(47.83%) were resistant to trimethoprim-sulfamethoxazol, cefotaxim and ceftazidime respectively. This may be due to previous exposure of these *E. coli* strains to antibiotic use which may have led to the development of antibiotic resistance through mutation or acquisition of resistance genes. The occurrence of antibiotic resistance *E. coli* suggest that frequent use of antibiotics in poultry industry for disease prevention or as growth promoters may bring about an increase in resistance to antibiotics not only in pathogenic bacterial strains but also in strains forming part of the endogenous floral of chickens such as *E. coli* as seen in this study. The implication of this is that multidrug resistant bacterial of poultry origin may spread into the human population by direct contacts or through the food chain Kolář *et al.* (2002). This findings is in agreement with the work of Omoya and Ajayi. (2016), who reported that more than 90% of Gram negative pathogens isolated from poultry droppings in Akure, Nigeria, were resistant to Amoxicillin and Ceftriaxone, while 80.15% were resistant to tetracycline. However, in this study, the least resistance by the *E. coli* isolates from poultry droppings were to etarpenem 1(4.35%), while none was recorded in cefoxitin (0%), which were therefore, the most active antibiotic while ampicillin, tetracycline and trimethoprim-sulfamethoxazol were the least active.

The results also showed that 22(95.65%) of the *Escherichia coli* isolates were susceptible to etarpenem, this may be to its high cost and as such may not be frequently used by poultry farmers. The multiple antibiotic resistance pattern and indices of *Escherichia coli* isolated from poultry droppings in Zaria, showed that all (23, 100%) the *Escherichia coli* isolates were resistant to three or more antibiotics; 1(4.35%) was resistant to three antibiotics, 6(26.09%) to four antibiotics, 4(17.39%) to six and seven antibiotics while 2(8.70%) and 1(4.35%) were resistant to nine and ten antibiotics respectively. It was also observed that all the *Escherichia coli* isolates 23(100%) from poultry droppings had multiple antibiotic resistance indices (MARI) ≥ 0.21. This according to Olayinka *et al.* (2004), MAR index greater than 0.2 implies that the strains of such bacteria originated from an environment where several antibiotics were irrationally used or abused.

CONCLUSION

The study established an isolation rate of *Escherichia coli* (83.76%) in poultry droppings and also revealed that *Escherichia coli* isolates obtained from poultry droppings had high resistance to Ampicillin (91.30%), Tetracycline (91.30%) and Trimethoprim-Sulfamethoxazol (69.57%), but no resistance to Cefoxitin (0.00%), Therefore, the best antibiotic that could be used for the treatment of infections associated with *Escherichia coli* in poultry farms located in Zaria Metropolis, Kaduna State, Nigeria, is Cefoxitin, however, indiscriminate use of Cefoxitin should be discourage to avoid the development of resistance to this antibiotic in the future. Also, all the *Escherichia coli* isolates 23(100%) from poultry droppings had multiple antibiotic resistance indices (MARI) ≥ 0.21. This according to Olayinka *et al.* (2004), MAR index greater than 0.2 implies that the strains of such bacteria originated from an environment where several antibiotics were abused. This, therefore, indicate an irrational use of antibiotic in poultry farms in Zaria, Nigeria as observed in this study.

REFERENCES

Agbaje, M., Davies, R., Oyekunle, M.A., Ojo, O.E., Fasina, F.O. and 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 Microbiological Research*, 4:796-800.

Anon, A. (1997). *The Microbiology of water*. *Weekly Epidemiological Record (WHO, Geneva)*, **72**(14):97-100.

Bower, C.K. and Daeschel, M.A. (1999). Resistance responses of microorganisms in food environments. *International Journal of Food Microbiology*. 50:33–44.

Cheesbrough, M. (2006). District laboratory practice in tropical countries (2nd ed.). Cambridge: Cambridge University Press, pp 178–179.

Clinical and Laboratory Standards Institute (CLSI) (2018). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. 950 West Valley Road, Suite 2500 Wayne, PA 19087 USA.

FAO, (2006). World agriculture: towards 2030/2050 interim report. Rome.

Huruma, N.T., Sarah, L., Paul, S.G. and Bruno, M.G. (2012). Identification of Avian Pathogenic *Escherichia coli* Genes that are Induced in Vivo during Infection in Chickens. *Journal of Applied and Environmental Microbiology* 78(9):3343–3351.

Ifigenia, G., John, W.H. and Alexander, V.H. (2001). Genotypic Analysis of *Escherichia coli* Strains from Poultry Carcasses and Their Susceptibilities to Antimicrobial Agents. *Applied and Environmental Microbiology.* 67(4):1940-1944.

Kim, J.P. (2005). A case of hemolytic uremic syndrome with hemorrhagic colitis due to *Escherichia coli* O111 infection. *Korean Journal of Gastroenterology*. 45:365-368.

Kolář, M., Pantůček, R., Bardoň, J., Vágnerová, I., Typovská, H., Válka, I. and Doškař, J. (2002). Occurrence of antibiotic-resistant bacterial strains isolated in poultry. *Journal of Veterinary Medicine Czech*, 47(2–3): 52–59.

Lim, J., Lee, K.M., Kim, S.H., Kim, Y., Park, W. and Park, S., (2011). YkgM and ZinT proteins are required for maintaining intracellular zinc concentration and producing curli in enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 under zinc deficient conditions. *International Journal of Food Microbioliology*, 149:159-170.

National Population Census. (2010). Population distribution, Nigeria demographic and health survey. 2006, Federal Republic of Nigeria gazette volume **3**, p 18.

Oladimeji, J. and Ojibo, S.D. (2012). Effects of effluents from sewage treatment plant on the aquatic organisms. *Journal of Water, Air and Soil Pollution,* **22**:309-316.

Olayinka, B., Olonitola, O., Olayinka, A. and Agada, E. (2004). Antibiotic susceptibility pattern and multiple antibiotic resistance index of *Pseudomonas aeruginosa* urine isolates from a university teaching hospital. *African Journal Clinical and Experimental Microbiology*, ***5***(2):198-200. doi:10.4314/ajcem.v5i2.7377.

Omoya, F.O. and Ajayi, K.O. (2016). Antibiotic Resistance Pattern of Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Nigeria. *Federal University of Technology Akure Journal of Research in Sciences*, 12(2), 219 -227.

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