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Prevalence of cephalosporin-resistant *Escherichia coli* in wild and domestic birds in Jos South Local Government Area, Nigeria

Anueyiagu KN, Elijah OO, David F, Abubakar S, Martins P and Rottemwa R
Federal College of Animal Health and Production Technology, Vom, Nigeria

Corresponding author: anueyiagunnamdi@yahoo.com

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ABSTRACT

Aim: Main purpose of the study was to determine the prevalence of cephalosporin-resistant *Escherichia coli* in wild and domestic birds.

Method and materials: Total 320 freshly voided faecal samples were collected from four different common species of wild birds and domestic birds in the study area. The samples were subjected to standard bacteriological examination. The antibiotics susceptibility pattern of E. coli isolates recovered from the samples were determined using cefepime and ceftriaxone which belongs to cephalosporin group and 7 other antibiotics.

Results: An overall prevalence of 17.06% of E. coli was isolated and from wild birds recorded 6.67% of E. coli while domestic birds recorded 22.73%. The wild birds showed that out of 8 isolates, 7 were resistant to Ceftriaxone while the domestic birds showed that out 50 isolates, 48 were resistant to Ceftriaxone. Some isolates from the domestic birds showed to be resistant to both cephalosporins. Nalidixic Acid showed the highest resistant (87.5% and 100%) from the isolates from both birds while Streptomycin recorded the least (12.5% and 6%).

Conclusion: In conclusion, the presence of resistant *E. coli* in wild birds is indicative that birds have been feeding on contaminated animal wastes or carcasses and drinking of contaminated water. These organisms could also be transmited to domestic birds in other environments because they are migratory birds.

Keywords: AMR, Broilers, *Escherichia coli,* Layers, Noilers, Wild birds.

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Introduction

The forest remains a significant habitat for birds; providing shelter, protection and other resources to about 75% of all tropical birds while 45% are believed to have adapted to habitats already modified through human activities (Bird Life International, 2008**).** Wild birds (such as cattle egrets, pigeon, oxpecker, indigo bird, bush petronia) are not directly exposed to antibiotics but rather indirectly, through environmental contamination. When they feed on contaminated water, animal carcasses or waste, these wild birds could harbour or be infected by resistant bacteria. For example, in Nigeria, egrets are common in South-Western regions, especially during dry season and they are often found among herds of cattle and they feed on cattle ticks and scavenge their faeces. They are also scavengers in cities, particularly in abattoirs and slaughter slabs.

Contaminated habitats where antibiotic-resistant bacteria have been frequently isolated include intensively managed livestock farms, landfills and waste-water treatment facilities (Lemus *et al.,* 2007). Gulls have been found to harbour the same strains of *E. coli* as could be isolated from dumpsites and wastewater treatment centers, and as a result could lead to possible transmission between sewage and birds (Nelson *et al*., 2008). Wild birds have been frequently investigated in the study of the occurrence of AMR bacteria in the environment because many species are relatively ubiquitous, and disperse over relatively long distances (Guenther *et al.,* 2011).

The first antibiotic-resistant strains of *Escherichia coli* bacteria noted in wildlife were isolated in pigeons around 1975 (Sato *et al.,* 1978). Most antibiotic compounds are naturally occurring in the environment (Allen *et al*., 2010) and antimicrobial resistance in soil-dwelling bacteria predates the use of antibiotics (Costa *et al.,* 2011). However, there is a large body of evidence that widespread use of antibiotics by humans and their use in livestock production has increased the prevalence of antimicrobial resistance in bacterial communities of humans, animals, and environment (Knapp *et al.,* 2010). Clinically relevant sequence and extended spectrum beta-lactamases (ESBL) types have been reported among wild birds (Hernandez *et al*., 2010). ESBLs are plasmid-mediated beta-lactamases that hydrolyze and confer resistance to third-generation cephalosporins such ascefotaxime andceftazidime; and organisms producing ESBLs are also resistant to some non-beta lactam antibiotics (i.e., fluoroquinolones). ESBLs are chiefly produced by bacteria in family *Enterobacteriaceae* (for example, *E. coli* and *K. pneumoniae*) but they are also expressed by non-enteric organisms such as the Pseudomonads. *Escherichia coli* strains from domestic animals and poultry have been known to carry same CTX-M enzyme strain which are locally dominant in human isolates (Blanc *et al*., 2006). The use of antimicrobials to improve the welfare, health and productivity of animals becoming very common in the livestock industry. Antimicrobials are used on livestock and poultry farms for as therapeutics, metaphylactics, prophylactics and growth promotion.

Antimicrobial usage is an important driver of antimicrobial resistance (Chantziaras *et al*., 2014). This factor plays a role in prevalence of antimicrobial resistance considering the high and indiscriminate usage of antimicrobials in veterinary and human medicine and animal production in Nigeria as in many African countries (Kimera *et al*., 2020). This issue was made worse by lack of good antimicrobial stewardship, easy access to antimicrobials, substandard antimicrobials and employment of untrained staff who administer antimicrobials to animals (Hasan *et al.,* 2012). Antimicrobial resistance (AMR) is a becoming a majorpublic health threat to both human and animal health, with an increasing number of infections no longer responding to once-standard treatments (WHO, 2014). Cephalosporin-resistant bacteria, including those producing extended spectrum β-lactamases (ESBLs), are particularly of great concern to public health, due to their resistance to commonly prescribed beta-lactam antibiotics, common co-resistance to other antimicrobial agents, and their increasing global prevalence (McDanel *et al*., 2017).

World Bank reports provide the most recent estimates on the current and projected global economic impact of AMR from a societal perspective (Taylor *et al.,* 2014, Jonas et al., 2017). Estimates on the indirect economic impact of AMR ranged from 0.06% to 6.08% reduced global Gross Domestic Product (GDP) by 2050 (Taylor *et al.,* 2014). Similar to The AMR Review, the World Bank reports that the economic burden of AMR is not distributed equally at different levels of economic status, and that the negative impacts are more pronounced in low-income countries than in high-income countries. The World Bank report also noted that AMR in animals would result from declining livestock production due to greater prevalence of untreated disease, which would also be more pronounced in low-income countries. World Bank estimates on the global direct economic impact of AMR ranged from an average of $3.5 billion/year among Organization for Economic Co-operation and Development countries to $1.2 trillion USD/year worldwide by 2050, due to healthcare related costs of AMR (Jonas et al., 2017).

Escherichia coli is a Gram-negative bacterium that has been known for ages to easily and frequently exchange genetic information through horizontal gene transfer with other related bacteria. Hence, it may exhibit characteristics based on the source of isolation. *E. coli* is a commensal organism living in the intestines of both humans and animals. However, some strains have been reported to cause gastrointestinal illnesses (Tenaillon *et al*., 2010). Tetracycline which is commonly used in poultry has been reported to be one of the drugs bacteria are most resistant to. There is a reported tetracycline resistance in poultry even without the administration ofthis antibiotic (Bogaard & Stobberingh, 2000)

Wild bird represents one of the environmental sources by which bacteria is transferred across different livestock farms. Thus, understanding the risk to animal health requires active surveillance for Antimicrobial (Cephalosporin) resistant bacteria in wild birds. This would help to enlighten farmers on AMR bacterial transmission pathway and to provide information for further study on the extent of cross-transmission among wild birds and livestock animals. The objective of this study is to determine the prevalence of cephalosporin-resistant *Escherichia coli* in wild and domestic birds.

Materials and Methods

Study Area

Jos South is a Local Government Area in Plateau State located between Latitude: 9° 47' 59.99" N and Longitude: 8° 51' 59.99" E. It has an area of 5,104km2 and a population of 306,716 at the 2006 census (Plateau State Government, 2022).

Sample collection

Freshly voided fecal samples were collected from chickens and wild birds. 320 samples were aseptically collected using spoon and stool container from apparently healthy chickens in poultry farms and 120 samples from wild birds found in the locality of the poultry farms. All samples collected were transported in cold boxes to the Central Diagnostic Laboratory of National Veterinary Research Institute, Vom and processed within 3 hours of sample collection for the presence of *Escherichia coli.*

Bacterial Examination of the Feacal sample

The samples collected were inoculated into 9ml of sterile peptone water and incubated overnight at 37^{o}C for pre-enrichment. A loop full of peptone water broth was streaked on Blood Agar and incubated overnight37^{o}C. Purified presumptive isolates were inoculated onto Eosin Methylene Blue agar (EMB) using quadrant streak method (Cheesebrough, 2000). The plate was incubated aerobically at 37^{o}C for 24hours. The plates were examined for gross colony morphology, pigmentation such as greenish metallic sheen between 24 and 48 hours. Presumptive colonies of *E. coli* were selected and purified on Nutrient agar, and purified isolates were subjected to biochemical tests and antibiotic susceptibility tests.

Detection of Cephalosporin Resistant E coli

A standardized Kirby Bauer disk diffusion method utilizing the Muller–Hinton Agar (MHA) plate technique was performed as per the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI, 2008). McFarland standard of the test organism was inoculated on the surface of the already prepared dry agar plate. The plate was allowed to stand for 30 minutes to allow effective diffusion and then standard cephalosporin antimicrobial (cefepime 10µg and ceftriaxone 30µg), disks were placed on dry seeded Muller-Hinton Agar and incubated at 37^{o}C between 18-24 hours. Zones of growth inhibition were then measured to the nearest millimeter and recorded.

Antimicrobial susceptibility testing

Cephalosporin-resistant *E. coli* were then subsequently tested against multiple antibiotics such as gentamicin (10 µg), erythromycin (15 µg), streptomycin (30 µg), ampicillin (30 µg), Augmentin (30 µg), Nalidixic acid (30 µg), Tarivid (10 µg), and ciprofloxacin (10 µg). The plates were then incubated at 37°C for 24 hours in Mueller–Hinton agar (MHA). An inhibition zone diameter of each antimicrobial was measured and interpreted as resistant (R), intermediate (I), and sensitive (S) zones according to CLSI guidelines (CLSI, 2008).

Data Analysis

Data obtained was analyzed using R 4.2.1 for windows. The result obtained was analyzed calculated using the Chi-square analysis to determine the statistical association. P-value less than 0.05 was considered statistically significant.

Results and Discussion

Out of a total of 340 fecal samples collected aseptically from wild and domestic birds, an overall prevalence of 17.06% of *E. coli* was isolated. Isolates from wild birds recorded 6.67% of *E. coli* while domestic birds recorded 22.73% (Table 1). Among the wild birds, Pied Crow recorded the highest *E. coli* prevalence of 33.33% while Yellow Weavers had the least. Among domestic birds, Layers had the highest *E. coli* prevalence of 38.18% while Noilers had the least of 11.43%. The Chi-square test showed that there was no significant statistical difference between the *E. coli* isolates among the wild birds with p-value greater than 0.05 while among the domestic birds, there was significant statistical difference between the *E. coli* isolates with p-value less than 0.05.

The prevalence of Cephalosporins resistant *E. coli* in wild birds where the *E. coli* isolates were tested against two Cephalosporin antibiotics namely, Cefepime and Ceftriaxone was observed (Table 2). There was more resistance of the *E. Coli* isolates to Ceftriaxone than Cefepime. The wild birds showed that out of 8 isolates, 7 were resistant to Ceftriaxone while the domestic birds showed that out 50 isolates, 48 were resistant to Ceftriaxone. Some isolates from the domestic birds showed to be resistant to both cephalosporins. The Chi-square test showed that there was no significant statistical difference between the *E. coli* isolates between the two cephalosporins with p-value greater than 0.05.

Resistance of *E. coli* isolates in wild and domestic birds to different antibiotics was recorded (Tables 3 and 4).

Table 1: Prevalence of *E. coli* in wild birds

Types of birds	No of samples	No positive	Percentage (%)	χ^2	Df	p- value
Wild birds						
Cattle egrets	86	4	4.65	5.31	3	0.150
Wild pigeon	14	2	14.29			
Yellow weavers	17	1	5.88			
Pied crow	3	1	33.33			
Total	**120**	**8**	**6.67**			
Domestic						
Broilers	80	15	13.33	11.76	2	0.003**
Layers	80	28	38.18			
Noilers	60	7	11.43			
Total	**220**	**50**	**22.73**			
Grand Total	**340**	**58**	**17.06**			

Table 2: Prevalence of Cephalosporins resistant *E. coli* in birds

Birds	**No of positive isolate**	**No of resistant (%)**	
		Cefepime	**Ceftriaxone**
Wild	**8**	**1 (12.5)**	**7 (87.5)**
Cattle egret	4	0	3
Wild pigeon	2	0	2
Yellow weaver	1	0	1
Pied crow	1	1	1
Domestic	**50**	**12 (24)**	**48 (96)**
Broilers	7	1	6
Layers	36	10	36
Noilers	7	1	6
χ^2		0.5244	1.0159
Df		1	1
p- value		0.468	0.314

Table 3: Resistance of *E. coli* isolates in wild birds to different antibiotics

Antibiotics	Sensitive No(%)	Intermediate No(%)	Resistant No(%)	χ^2	Df	p- value
Tarivid	4(50)	1(12.5)	3(37.5)	28.06	12	0.005**
Augmentin	0(0)	4(50)	4(50)			
Gentamycin	4(50)	2(25)	2(25)			
Streptomycin	4(50)	3(37.5)	1(12.5)			
Nalidixic Acid	1(12.5)	0(0)	7(87.5)			
Ampicillin	0(0)	2(25)	6(75)			
Ciprofloxacin	0(0)	5(62.5)	3(37.5)			

Table 4: Resistance of *E. coli* isolates in domestic birdsto different antibiotics

Antibiotics	Sensitive No (%)	Intermediate No (%)	Resistant No (%)	χ^2	Df	p- value
Tarivid	21(42)	5(10)	24(48)	279.46	12	0.000***
Augmentin	43(86)	0(0)	7(14)			
Gentamycin	3(6)	4(8)	43(86)			
Streptomycin	21(42)	26(52)	3(6)			
Nalidixic Acid	0(0)	0(0)	50(100)			
Ampicillin	2(4)	0(0)	48(96)			
Ciprofloxacin	14(28)	24(48)	12(24)			

Nalidixic Acid showed the highest resistant (87.5% and 100%) from the isolates from both birds while Streptomycin recorded the least (12.5% and 6%).The Chi-square test showed that there was significant statistical difference between the Sensitive, Intermediate and resistant antibiotics reaction on the *E. coli* isolates among both types of birds with p-value less than 0.05.

The overall prevalence rate (6.7%) of *Escherichia coli* in wild birds recorded in this study was less than the figures reported by Ejikeugwu *et al.,* 2015 and Hasan *et al.,* 2012; 52.12% in Ebonyi state and 73.3% in Bangladesh respectively while Fashae *et al.,* 2021, recorded 26/28 (92.9%) and 2/24(8.3%), cattle egrets and whistling duck faecal samples, respectively.However, Brittingham et al., 1988 recorded 1% prevalence in U.S.A. which was lower than what was observed in this study, therefore variability in the prevalence of E. coli in wild bird may be attributed to location, season, method of sampling, sample size, species of wild bird, health status of birds sampled etc.

The overall prevalence of *E. coli* among the domestic birds in this study was 22.73% which was similar to the report of Aworh et al., (2020) which had 26.8%. Layers had the highest prevalence of 38.18% followed by broiler of 13.33%. This is contrary to the conducted Kitii et al (2021) which reported 49.5 from layer and 50.5% from broilers chickens. The disparity may be due to the type of sample collected in this work which was freshly voided feces while Kitii et al (2020) was cloacae samples.

Wild birds in this study recorded a range of 25% to 87.5% prevalence of *E. coli* multidrug resistance and12.5% to 87.5% prevalence of cephalosporins resistance *E. coli*. Ong *et al*., 2020 recorded similar results for wild birds with 3.8% to 73.1% prevalence of resistant *E. coli* to different antibiotics classes. The high multidrug resistance shown by the sample isolates from some of the wild birds such as cattle as egret, wild pigeon and pied crow is explainable because they are birds of prey and scavengers. They obtain their diet from a wider range from ground, tree, including injured animals, small mammals, young birds, eggs, any scraps of human food, fruit and around slaughter houses.They are not directly exposed to antibiotics but rather indirectly, through environmental contamination.Domestic birds in this study recorded a range of 14% to 100% prevalence of *E. coli* multidrug resistance and 24% to 96% prevalence of cephalosporins resistance *E. coli*. Kiitiet al 2021 observed a prevalence of 86.76%*E. coli* in both broilers and layers were multidrug resistant. This prevalence of multidrug resistant *E. coli* isolates is also higher than the one (63.4%) obtained from local and imported retail chicken carcasses in Qatar (Eltai *et al*, 2020) and also higher than 68% multidrug resistant E. coli isolates obtained from healthy chicken farms in the region of Dakar, Senegal (Vounba et al, 2019). Rahman et al. (2020) also reported a slightly lower prevalence of multidrug-resistant E. coli isolates obtained from broiler and layer chickens, which was 49.23% for broilers and 51.09% for layer chicken in Sylhet Division, Bangladesh.Most of the isolates were resistant to at least more than one antibiotic agent, thereby satisfying the definition of multidrug resistance proposed by Schwarz *et al.,* 2010 that is, bacteria exhibiting resistance to at least one agent in at least three antimicrobial classes..

Conclusion

It was concluded that E. coli isolated from wild birds, broiler, layers and noilers in Jos Metropolis were resistant to at least one antimicrobial agent and any of the two cephalosporins used in the study. The presence of resistant *E. coli* in wild birds is indicative that these have been feeding on contaminated animal wastes or carcasses and drinking of contaminated water. These could also transmit such organisms to domestic birds in other environments because these are migratory birds. However, high prevalence in domestic birds does not necessarily mean high morbidity or mortality of chickens in farms sampled. This is because most *E. coli* isolates are not pathogenic. However, the high prevalence showed that hygiene level in domestic birds sampled are quite low because presence of *E. coli* in food and water is an indicator of fecal contamination. Antimicrobial surveillance and bio-security measures are therefore recommended for domestic birds.

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