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# A Three-Year Study on the Prevalence and Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Cloacal Swabs of Wild and Domestic Birds in Ebonyi State, Nigeria

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## Abstract

Antibiotic resistance is a natural biological phenomenon that is vital to microbial colonization and survival in the environment. But this process is of clinical significance because of the ability of antibiotic resistant bacteria to render inefficacious some available antimicrobial agents. This study evaluated the prevalence and antibiotic susceptibility pattern of *Escherichia coli* isolates from cloacal swabs of wild and domestic birds in Ebonyi state, Nigeria over a three (3) year period. Four different bird species including bats, ducks, parrots and pigeons were used for this study. A total of fifty (50) cloacae swabs of each wild and domestic bird species were collected using sterile swab sticks for each year. The samples were analyzed using standard microbiology techniques and the isolates were biochemically confirmed using API kits. Antibiotic susceptibility test on the isolates was conducted using modified Kirby-Bauer disc diffusion method. The results obtained from this study showed that *E. coli* were present at varying levels in the 4 bird species investigated. *E. coli* species were most prevalent in pigeons across the 3 years with the range of 30.43% - 65.63%. This was followed by ducks which recorded between 17.95% - 34.38% across the 3 years. Parrots and bats respectively had 28.26% and 19.57% in 2012; 17.95% and 15.38% in 2014. The result of the antimicrobial susceptibility testing revealed that more than 50% of the isolated *E. coli* was resistant or intermediately resistant to cefuroxime, ceftazidime and cefotaxime. However, the *E. coli* isolates were found to be most susceptible to imipenem, meropenem and ertapenem. Isolates from parrots and bats were generally more resistant to the antibiotics than the same isolates from pigeon and duck samples. Notably, resistance to imipenem was observed only in *E. coli* isolates recovered from parrots and bats at the rate of 22.22%. The carbapenems, imipenem and meropenem were the most active antibiotics tested in this study. Conclusively, this study has shown that E. coli isolates recovered from birds are antimicrobial resistant in nature. The frequency of antibiotic resistant *E. coli* in birds in the community is of public health importance because of their implications in community-acquired infections.

***Keywords:*** *Escherichia coli; Antibiotics; Resistance; Antibiogram; Birds; Nigeria*

## Introduction

From the time when the first sulfa and penicillin were introduced in the 1930s and 1940s, respectively, bacteria have continued to discover a remarkable ability to increase different types of resistance mechanisms to antimicrobials they were susceptible to [1]. Antibiotic resistant bacteria are exceedingly important to human health, but the wild reservoirs of resistant determinants are poorly understood [2]. Their origins in wild life is important to human health because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogens [2]. Wild animals provide a biological mechanism for the spread of antibiotic resistance genes and the antimicrobial-resistant *Escherichia coli* and *Enterococcus* species that originated from wild life species were reported for the first time from Japanese wild birds [3-5]. *E. coli* was also reported 5 years later in South Africa baboons that feed on human refuse [6,7]. *E. coli* is a facultative anaerobic Gram negative bacterium found in the *Enterobacteriaceae*; and they are found in the intestinal tract of warm blooded animals including mammals, poultry and wild birds [8,9]. Faecal *E. coli* is considered to be a key indicator for the selective pressure exerted by the use of antimicrobials on intestinal populations of bacteria [10]. The transfer of antibiotic resistance in enteric bacteria including *E. coli, Salmonella* spp., *Campylobacter* spp. and *Enterococci* spp. from birds to humans have been previously reported and this can occur through the food chain or by direct contact [11-13]. Therefore, the frequent occurrence of antimicrobial resistance in wildlife has some public health implications including the potential to serve as an environmental reservoir of resistance gene and zoonotic diseases [2]. The mobility and migration of avian species are remarkable biological phenomena. At some points in their yearly migrations, they can disperse microorganisms that pose a threat to the health of both animals and the public in diverse geographical areas [14,15]. Birds may also act as bio-indicators of the level of contamination of bacterial pathogens and genetic determinants of antibiotic resistance in the environment [16]. Because of their ease in picking up human and environmental bacteria and their diverse presence in different ecological niches, wild birds could act as transporters or reservoirs of resistant bacteria. They could probably have an important epidemiological role in the dissemination of resistant pathogenic microorganisms present in humans [2,13,17]. This study therefore evaluated the possible involvement of birds such as pigeons, ducks, parrots and bats in the dissemination of antibiotic resistant *E. coli* in Ebonyi State, Nigeria.

## Materials and Methods

**Sample collection:** A total of 500 cloacal samples from four different wild and domestic birds' species (duck, pigeon, parrots, bat) were used for this study for a period of 3 years (2012 - 2014). Each bird from which each sample was collected was labeled appropriately to avoid repetition in sample collection.

**Bacteriological analysis:** Each sample collected was used to inoculate sterile nutrient broth in test tubes (Oxoid, UK) and incubated for 18 - 24h at 37°C. A loopful from each tube was inoculated onto MacConkey (MAC) and Eosin Methylene Blue (EMB) agar (Oxoid, UK), and incubated for 18 - 24h at 37°C. Suspected colonies of *E. coli* were subcultured onto freshly prepared MAC and EMB agar and incubated for 18-24 hrs at 37°C to obtain pure cultures. *E. coli* isolates were identified using API kit [18].

**Antimicrobial susceptibility testing:** All *E. coli* isolates were screened for their susceptibility to different antibiotics using the Kirby-Bauer disc diffusion technique as per the guidelines of Clinical Laboratory Standards Institute (CLSI). A 0.5 McFarland equivalent standard of each of the test organism was inoculated on the surface of Mueller-Hinton (MH) agar plates using a sterile swab stick for each test organism. Antibiotic disks of cefuroxime, CXM (30 μg); ceftazidime, CAZ (30 μg); cefotaxime, CTX (30 μg); ceftriaxone, CRO (30 μg); cefoxitin, FOX (30 μg) amoxicillin/clavulanic acid, AMC (20/10 μg); aztreonam, ATM (30 μg); imipenem, IMI (30 μg); meropenem, MEP (30 μg) and ertapenem, ETP (30 μg) [Oxoid, UK] were aseptically placed on the surface of the inoculated MH agar plates at a distance of 25 mm. The susceptibility plates were incubated for 18 - 24 hrs at 37°C. The diameters of the observed clear zones of inhibition were measured and recorded in line with the CLSI criteria, and as was previously described [18,19].

## Results

The results obtained from this study showed that a total of 117 isolates of *E. coli* were obtained across the three years of study. Of the 117 isolates, 46 (39.32%), 39 (33.33%) and 32 (27.35%) were obtained in 2012, 2013 and 2014, respectively (Table 1).

| Year | Sample source (Birds) | Cloacal swab sample | Percentage occurrence of *E. coli* |
|---|---|---|---|
| 2012 | Ducks | 50 | 10 (21.74) |
| | Pigeons | 50 | 14 (30.43) |
| | Weaver birds | 50 | 13 (28.26) |
| | Bats | 50 | 9 (19.57) |
| | Total | 200 | 46 |
| 2013 | Ducks | 50 | 7 (17.95) |
| | Pigeons | 50 | 19 (48.72) |
| | Weaver birds | 50 | 7 (17.95) |
| | Bats | 50 | 6 (15.38) |
| | Total | 200 | 39 |
| 2014 | Ducks | 50 | 11 (34.38) |
| | Pigeons | 50 | 21 (65.63) |
| | Weaver birds | - | - |
| | Bats | - | - |
| | Total | 100 | 32 |

***Table 1:*** *Prevalence of E. coli obtained from four wild and domestic birds species in 2012, 2013 and 2014.*

In 2012, the 10 E. coli species screened, were 100% susceptible to IMI and ETP; followed by 8 (80.0%) each to FOX and MEP; 7 (70.0%) to ATM; 6 (60.0%) each to CTX and CRO; 3 (30.0%) and 1 (10.0%) to CXM and CAZ, respectively (Figure 1). The highest resistance recorded was 90.0% and to CAZ; followed by 70.0% to CXM, 40.0% each to CXT and CRO; 30.0% to ATM; 20.0% each to FOX and MEP. None of the isolates was resistant to IMI and ETP (Figure 1).

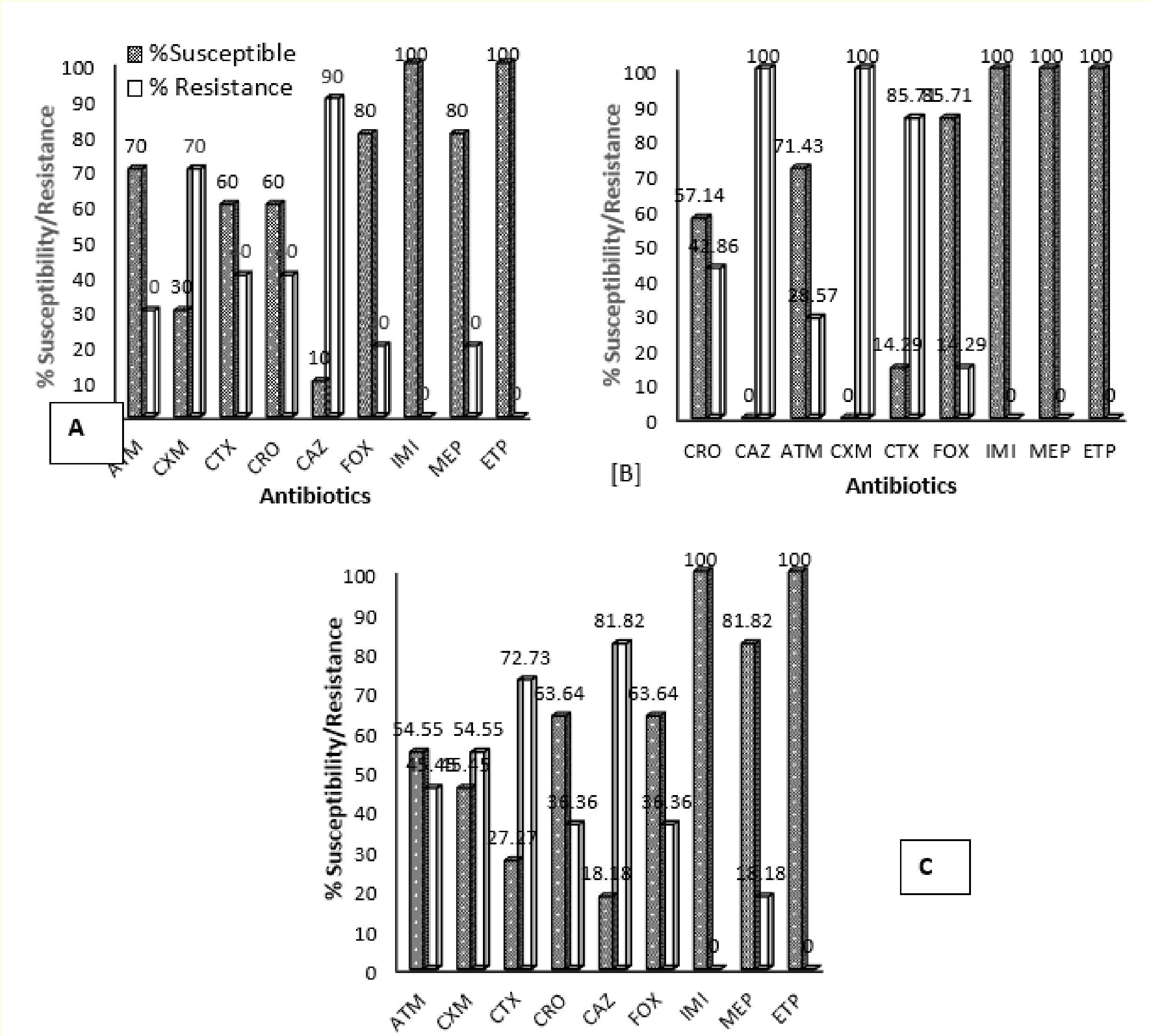


***Figure 1:*** *Antibiotic Susceptibility and Resistance Patterns of E. coli Isolated from Duck Cloacal Swabs in 2012 [A], 2013 [B] and 2014 [C].*

In 2013, all the *E. coli* species screened were observed to be susceptible to IMI, MEP and ETP, 7 (100%). The isolates were totally resistant to CAZ and CXM, 7 (100%) each; 6 (85.71%) of the isolates (Figure 1). In 2014, the 11 *E. coli* species screened were absolutely (100%) susceptible to IMI and ETP. In 2012, it was observed that all the 14 *E. coli* species screened were susceptible to IMI and ETP (100%). In 2013, all 19 *E. coli* species screened were susceptible to IMI and ETP (Figure 2). All the isolates were found to be resistant to CTX, 18 (94.74%). In 2014, the result showed all the isolates of *E. coli* screened to be susceptible only to IMI, 21 (100%); 20 of the 21 isolates (95.23%) and 18 (85.71%) were respectively susceptible to ETP and MEP.

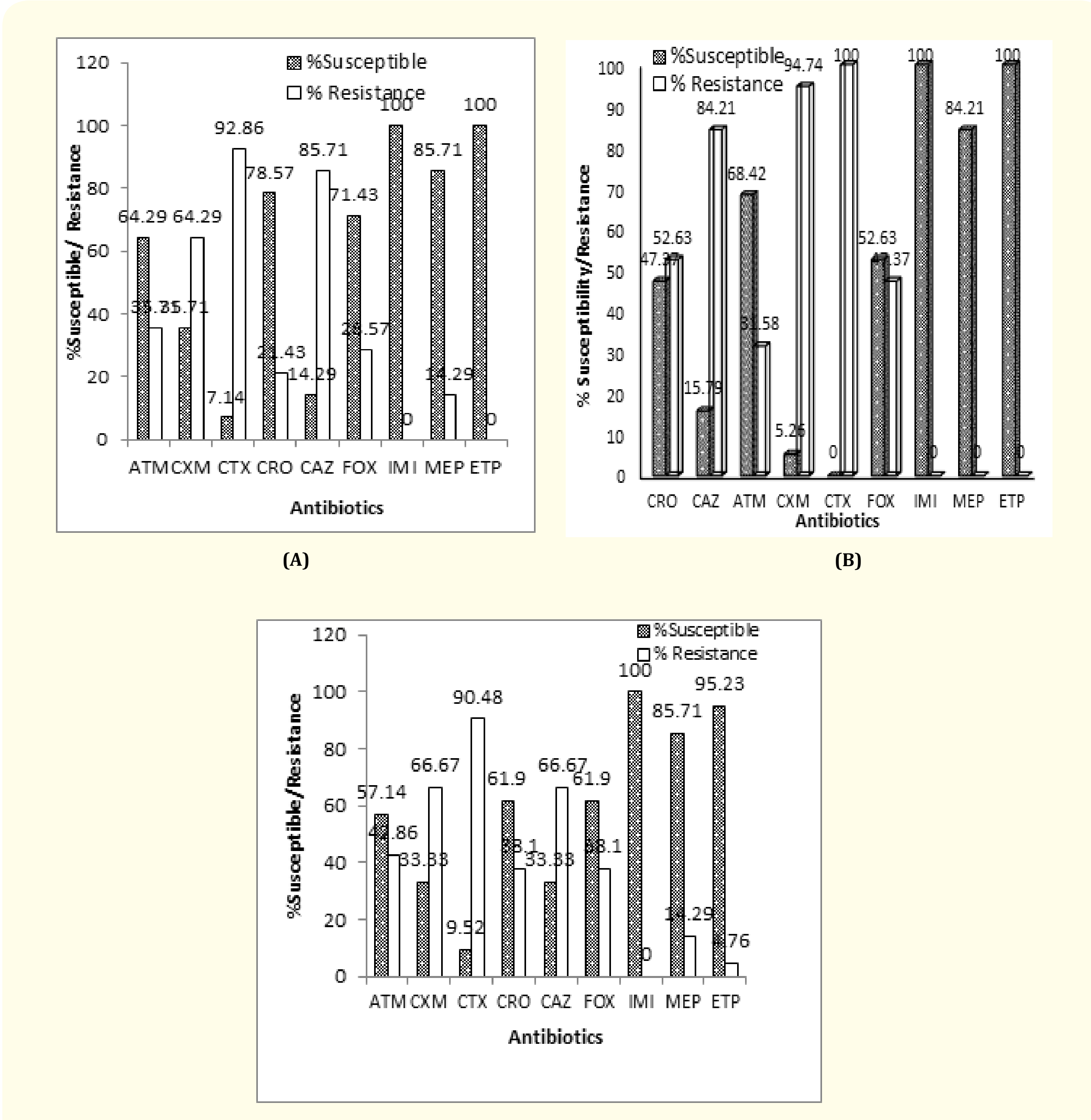


***Figure 2:*** *Antibiotic Susceptibility and Resistance Pattern of E. coli Isolated from Pigeon Cloacal Swabs in Abakaliki in 2012 [A], 2013 [B] and 2014 [C].*

In 2012, the result showed all the *E. coli* species screened to be totally susceptible to IMI and ETP, 13 (100%); 11 (84.62%) were susceptible to MEP; 8 (61.54%) each to CRO and ATM; 7 (53.85%), 3 (23.08%), 2 (15.38%) and 1 (7.69%) to FOX, CAZ, CXM and CTX, respectively (Figure 3). In 2013, all the isolates (7) screened were susceptible to IMI, MEP, FOX and ETP (100%); 5 (71.43%) and 4 (57.14%) were respectively susceptible to ATM and CRO.

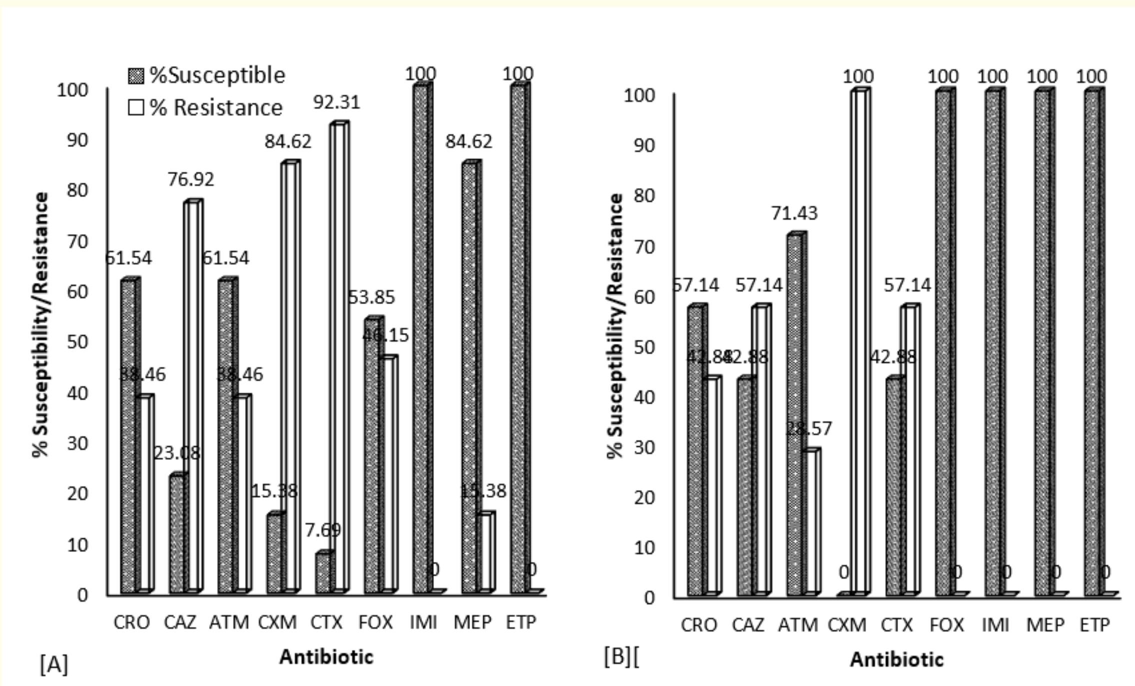


***Figure 3:*** *Antibiotic Susceptibility and Resistance Pattern of E. coli Isolated from Weaver bird Cloacal Swabs in Abakaliki in 2012 [A] and 2013 [B].*

In 2012, 7 of the 9 isolates of E. coli screened (77.78%) were found to be susceptible to each of IMI, ETP and MEP; followed by 6 (66.67%) each to CRO and FOX; 5 (55.56%), 2 (22.22). The isolates were totally (100%) resistant to CTX; 8 (88.89%), 7 (77.78%), 4 (44.44%) were respectively resistant to CXM, CAZ and ATM; whereas only 3 (33.33%) were resistant to each of CRO and FOX and 2 (22.22%) were resistant to each of IMI, MEP and ETP (Figure 4). In 2013, the 6 E. coli species screened were all susceptible only to IMI, 5 of them (83.33%) were susceptible to ETP; 2 (33.33%) to each of CRO, FOX and MEP. All the isolates were 100% resistant to CXM and CTX; 83.33% of them were resistant to each of CAZ and ATM; followed by 66.67% to each of CRO, FOX and MEP; and only 16.67% was resistant to ETP; and none to IMI (Figure 4).

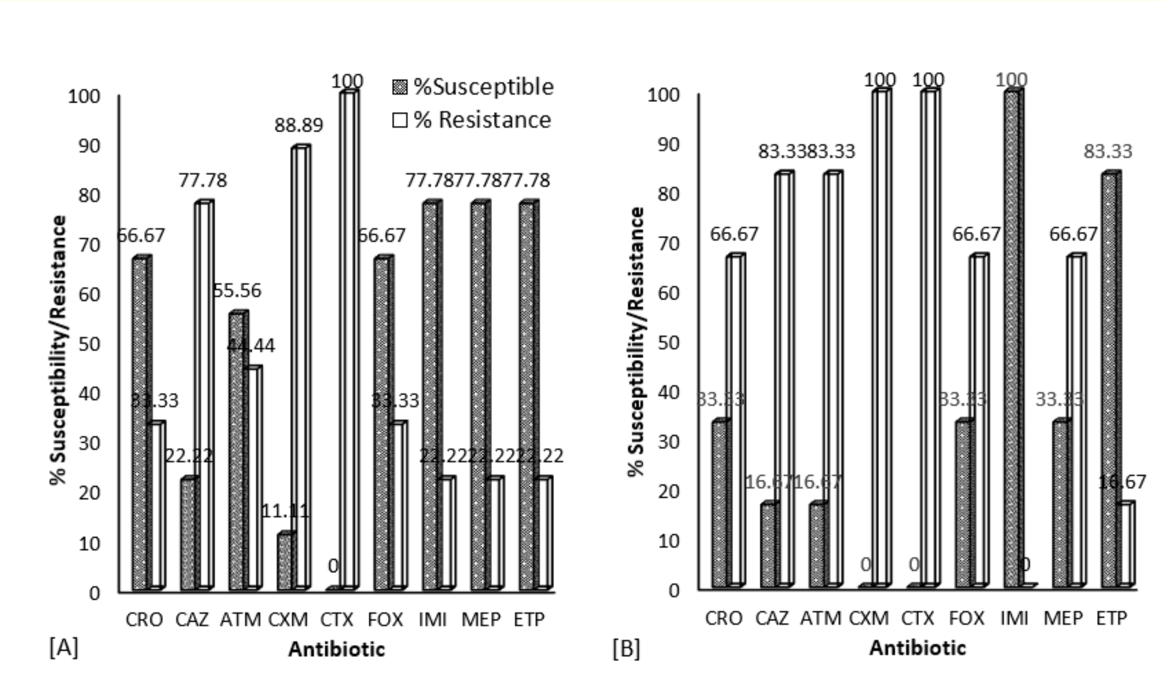


***Figure 4:*** *Antibiotic Susceptibility and Resistance Patterns of E. coli Isolated from Bat Cloacal Swabs in Abakaliki in 2012 [A] and 2013 [B].*

## Discussion

*Escherichia coli* isolates were more prevalent in pigeons (46.15%), and this was followed by ducks (23.93%), weaverbirds (17.07%) and bats (12.82%). This is in line with previous studies which reported that *E. coli* was the most prevalent Gram negative bacteria recovered from birds [20-23]. Wild and domestic bird species, including the long and short distance migrants occupying diverse ecological niches and adapting to varying feeding patterns, can as well host and contribute to the spread of multidrug resistant bacteria species [24,25]. Our result also showed that the *E. coli* species isolated from duck and pigeon cloacal swabs were generally more susceptible to the antibiotics used than those isolated from weaverbirds and bats. This could be associated with their closeness to humans who also use these antibiotics. Isolates from duck samples were most susceptible to imipenem (IMP), ertapenem (ETP), meropenem (MEP) and cefoxitin (FOX). Their percentage susceptibilities across the 3 years ranged from 54.55 - 100%. More than 50% of the *E. coli* isolates were also resistant to cefuroxime (CXM), cefotaxime(CTX) and ceftazidime (CAZ) over the 3 years under study. The susceptibility and resistance patterns of the E. coli isolated from pigeons were similar to those isolated from duck. Their percentage susceptibilities to IMP, ETP and MEP ranged from 84.21 - 100% while their resistance to CXM, CAZ and CTX ranged from 64.29 - 100%. *E. coli* isolated from parrots and bats were also most susceptible to IMP, ETP and MEP and most resistant to CTX, CXM and CAZ. In parrots, the *E. coli* susceptibility to IMP, MEP, and ETP ranged from 82.62 - 100% while their percentage resistance to CAZ, CXM and CTX ranged from 57.14 - 100%. In bats, susceptibility of *E. coli* to IMP, MEP and ETP ranged from 66.67 - 100% while their percentage resistance to CTX, CXM and CAZ was as high as 77.78 - 100%. The *E. coli* isolates from parrots and bats were generally more resistant to the antibiotics used when compared to the same isolates from pigeons and ducks. From this study, it is a matter of concern that *E. coli* isolated from birds are resistant to some commonly used antibiotics. This is most disturbing because the birds in question do not receive any direct antibiotic treatment as humans. These birds are highly migratory, feeding from diverse environments and could potentially contribute highly to the spread of drug resistant microbes [1]. The high resistance of the isolates from these birds could be linked to their feeding behavior. They could feed at poorly managed hospital waste dumps; come in contact with human and animal excreta and may later visit any close-by water body to drink, making them both potential reservoirs and active spreaders of drug resistant bacteria [1]. Hassan [1] has also reported resistance to some classes of antibiotics by *E. coli* isolated from both commercial and free-range poultry birds that even have limited migration capacity and feed in less diverse environment. Similarly, antibiotic resistant *E. coli* have been previously obtained from ducks and geese [5,16], birds of prey [26,27], gulls [28-30] and feral pigeons [31]. Antibiotic resistance is a global phenomenon that bedevils the health sector and compromises our ability to effectively manage and treat some bacterial related infections. The emergence and spread of drug resistant bacteria which is very notable in the hospital environment is now being experienced in the non-hospital environment. This present study gives impetus to this rising incidence of antibiotic resistant bacteria in the non-hospital environment, and the possible damage that they cause to antimicrobial therapy.

## Conclusion

This study reported the prevalence and antibiogram of *E. coli* isolates recovered from different wild and domestic bird species over a three (3) year period. And the results from this study shows that *E. coli* isolates from birds could be multidrug resistant in nature. Birds inclusive of migratory and domestic birds could serve as reservoir for antibiotic resistant bacteria due to their feeding habits or other environmental factors. It is important to be on the lookout for antibiotic resistant bacteria from birds, and prevent those circumstances that could make these birds disseminator's and reservoirs of antibiotic resistant Gram negative bacteria inclusive of *E. coli*.

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## Declaration of Interest

The authors report no declaration of interest.

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