

Antibiotic Resistant *Escherichia coli* Isolates from Barn Swallow Droppings in Ishaka Town, Uganda

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Abstract Considerable concern has been raised over the problem of antibiotic resistance in bacteria from humans and farm animals. However, in spite of this concern, the spread of resistance into wider ecosystems has not received the desired attention. The transfer of antibiotic resistance to wildlife is an important risk for environmental health. Detection of resistance to antibiotics in populations of wild animals usually entails examination of isolates of the common intestinal bacterium *Escherichia coli*. Bird populations sympatric to areas with human settlements and areas that have high density of livestock are colonised with antibiotic resistant *E. coli* strains. Data regarding occurrence of antibiotic-resistant bacteria in African domestic and wild mammals is very limited. There is therefore a need to carry out studies in other parts of the African continent to find out if sympatric animals represent a possible reservoir for antibiotic resistant microbes. This study was therefore carried out to analyze faecal samples from barn swallow (*Hirundo rustica*) droppings in Ishaka Town, Uganda, for the presence of antibiotic resistant *E. coli* strains. Results of the study showed that, out of the one hundred and sixteen (116) fecal samples of Barn Swallow droppings collected, twenty seven isolates of *Escherichia coli* were obtained. All the 27 isolates (100%) were resistant to one or more of the tested antibiotics. Out of the 27 isolates, 20 (74.1%) were multi-drug resistant isolates (resistant to three or more classes of antimicrobials). Such birds can act as reservoirs for antibiotic resistant microbes if enough attention is not given to the problem. Further research is recommended to determine the prevalence of antibiotic resistant *Escherichia coli* from barn swallows residing in other parts of the country and even on the continent and the whole world at large.

Keywords: drug resistance, wild birds, *Escherichia coli*, barn swallow, reservoirs

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1. Introduction

Bacteria have a very unique ability to adapt to changes in their environment, and to develop mechanisms to protect themselves against toxic compounds. Their ability to develop resistance mechanisms to antimicrobial drugs has reached catastrophic proportions, rendering increasingly more infections difficult or impossible to treat [1]. Majority of reports indicate that the main force behind emergence of drug resistance is the use and misuse of antimicrobial drugs during the past few decades, but there is also evidence for the epidemic spread of drug-resistant bacteria as a contributing factor [2]. There is evidence that resistance genes can be found even in a region where no selection pressure for resistance development exists, thus implying that dissemination of drug-resistant bacteria is worldwide [3]. Although wild bird populations are not influenced directly by antibiotic practice, it has been

demonstrated that antibiotic resistant strains of *E. coli* are found in faeces of wild birds. This includes strains with various antibiotic resistance genes [4]. The problem of antibiotic resistance in bacteria from humans and farm animals has raised considerable concern. In spite of this, the spread of resistance into wider ecosystems has not received the desired attention [5]. Detection of antibiotic resistance in populations of wild animals usually entails examination of isolates of the common intestinal bacterium *Escherichia coli* [6]. The transmission of antibiotic resistance from livestock and humans remains a poorly understood mechanism, with wild animals acting as reservoirs of the resistance that may be eventually amplified and spread to the environment. It should be considered that the transfer of antibiotic resistance to wildlife is an important risk for environmental health [7]. In a study conducted in the inland Hamilton Harbour on Lake Ontario, Canada, it was found out that faeces of wild geese, ducks and gulls could have been a more prominent contributor of antibiotic resistant *E. coli* to a beach near

Hamilton than municipal wastewater sources [8]. Various strains of *E. coli* colonise wild bird populations, including the *E. coli*O157 strain that is pathogenic to humans [6]. Faecal sample isolate strains of *E. coli* resistant to antibiotics have been found at varying prevalence in wild bird populations. Most notably, bird populations sympatric to areas with human settlements and areas that have high density of livestock are colonised with antibiotic resistant *E. coli* strains [9,10,11,12]. This poses a potential risk to public health. House sparrows living with cattle and coming into contact with cattle waste on farms in the Czech Republic were found to be infected by resistant *E. coli* strains with different characteristics from those found in cattle [13]. There is reported occurrence of *E. coli* strains that are pathogenic to humans and animals in the eggs and nestlings of house sparrows [14]. Corvids and gulls that feed on garbage dumps and in areas with urbanization are frequently colonised with resistant strains of *E. coli* and *salmonella spp*. Such birds are considered to form important reservoirs and vectors of these resistant strains in the environment [15,16]. Seagulls of diverse species colonised with resistant *E. coli* strains producing extended-spectrum beta-lactamase have been reported in Portugal [17]. Resistant strains carrying class 1 and 2 integrons have been isolated in Canada geese (*Branta canadensis*) in the United States and black-headed gulls in the Czech Republic [18,19]. Strains that are resistant to fluoroquinolones have also been reported in gulls living in Greenland [3]. Resistance to penicillin G, ampicillin, cephalothin, and sulfathiazole has been reported in a high frequency of *E. coli* isolates from migratory Canada geese sampled on the eastern shore of Maryland in the United States [20]. Bacteria displaying commensalism in humans and animals constitute hidden reservoirs of antimicrobial drug resistance [21]. There are reported cases of antimicrobial drug-resistant bacteria in wild animals with little or no contact with human settings [22,23]. There is limited data regarding occurrence of antibiotic-resistant bacteria in African domestic and wild mammals [24]. Studies conducted in Senegal and Kenya showed that rats represent a possibly important reservoir for antibiotic-resistant Enterobacteriaceae including extended-spectrum beta-lactamase producing strains [24,25]. Therefore, a great impact on human health can be brought about by antibiotic resistance in animals. This can occur especially in an environment where humans and animals share the same ecosystem [25]. In order to determine the extent of the spread of extended-spectrum beta-lactamases, it is necessary to characterize antibiotic-resistant bacteria in each geographical area and environments including wildlife, especially in areas where resources are limited and antibiotics are unregulated [26]. In view of this, there is therefore a need to carry out studies in other parts of the African continent to find out if sympatric animals represent a possible reservoir for antibiotic resistant microbes. In this study, we were particularly interested in analysing faecal samples from barn swallow (*Hirundo rustica*) droppings in Ishaka Town, Uganda, for the presence of antibiotic resistant *E. coli* strains. This bird's habit of nesting in buildings associated with human habitation has led it to be one of the most familiar bird species in the world [27]. Because this species of birds come into close contact with human habitats [28,29], one recent study(the first of its kind) has implicated them as a

potential source of *Clostridium difficile* in animals and humans [30]. Ishaka Town area of Uganda is fairly densely populated with this species of birds.

2. Materials and Methods

2.1. Bird Faecal Samples

The faecal samples were collected from twenty residential areas and ten educational institutions housing nests of Barn swallows in Ishaka town, Uganda. Individual faecal samples were transported to the Microbiology laboratory of Kampala International University-Western Campus, Ishaka. They were then placed overnight in buffered peptone water at 37°C.

2.2. Culturing and Isolation of Antibiotic Resistant *E. coli* Strains

The samples were cultivated for *E. coli* on selective chromogenic media for *E. coli* and coliform bacteria (CM0956; Oxoid, United Kingdom). A single colony of each plate was tested for susceptibilities to antimicrobial agents by the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (31). Susceptibilities to the following antibacterial substances were tested: ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), streptomycin (10 µg), Sulfamethoxazole (25 µg) and Cotrimoxazole (25µg).

2.3. Isolation of Extended-Spectrum Beta-lactamase and Quinolone-resistant Strains

This was done according to the method described by Literak *et al.* [32]. By this method, samples from peptone buffered water were enriched in MacConkey broth and subcultivated on MacConkey agar (MCA) containing ceftazidime (2 mg liter⁻¹) to detect the presence of *E. coli* strains with extended-spectrum beta-lactamase. These were then subcultivated on MacConkey agar containing ciprofloxacin (0.05 mg liter⁻¹) to isolate *E. coli* strains resistant to fluoroquinolone. To detect *E. coli* strains resistant to nalidixic acid and other quinolones, samples from the buffered peptone water were also enriched in MacConkey broth and subcultivated on MacConkey agar containing nalidixic acid (20 mg liter⁻¹).

2.4. Data Interpretation

Diameter of zones of inhibition was measured to the nearest millimeter using a transparent ruler. This was indicative of the susceptibility of *E. coli* to the different antibiotic discs used. It was then compared with the standard zone of inhibition of *E. coli* by the respective antibiotic disc.

3. Results

Out of the one hundred and sixteen (116) fecal samples of Barn Swallow droppings collected, twenty seven (23.3%) isolates of *E. coli* were obtained. The 27 *E. coli*

isolates were therefore subjected to further analysis to identify isolates that are resistant to the antibiotics used in the study. All the 27 isolates (100%) were resistant to one or more of the tested antibiotics. Out of the 27 isolates, 20 (74.1%) were multi-drug resistant (resistant to three or more classes of antimicrobials) isolates. As shown in **Table 1**, susceptibility to Ciprofloxacin was noted to be highest, while resistance to Ampicillin was highest. None of the isolates was resistant to ciprofloxacin, whereas two isolates (7.4%) were intermediately susceptible and twenty five isolates (92.6%) were highly susceptible to the antibiotic. This meant that the microbes had not developed resistance against the antibiotic. All the 27 isolates (100%) were resistant to Ampicillin as evidenced by no zone of inhibition. Resistance of more than 50% was observed towards two other antibiotics, namely ceftazidime and streptomycin at 55.6% and 51.9% respectively. The 15 isolates that were resistant to ceftazidime were hence regarded as being extended-spectrum beta-lactamase (ESBL) producing strains. Nine (9) of the isolates were

resistant to the quinolone nalidixic acid, which represented 33.3% of all the isolates. Out of these nine quinolone resistant isolates, three of them (33.3%) were highly resistant, as evidenced by completely no zone of inhibition. **Figure 1** below shows the numbers of susceptible and resistant isolates.

Table 1. Resistance pattern of *E. coli* isolated from Barn Swallow droppings

Antibiotic	No. of resistant isolates (n=27)	Percentage
Ciprofloxacin	00	0%
Cotrimoxazole	12	44.4%
Chloramphenicol	11	40.7%
Streptomycin	14	51.9%
Nalidixic acid	09	33.3%
Ceftazidime	15	55.6%
Sulfamethoxazole	13	48.2%
Ampicillin	27	100%

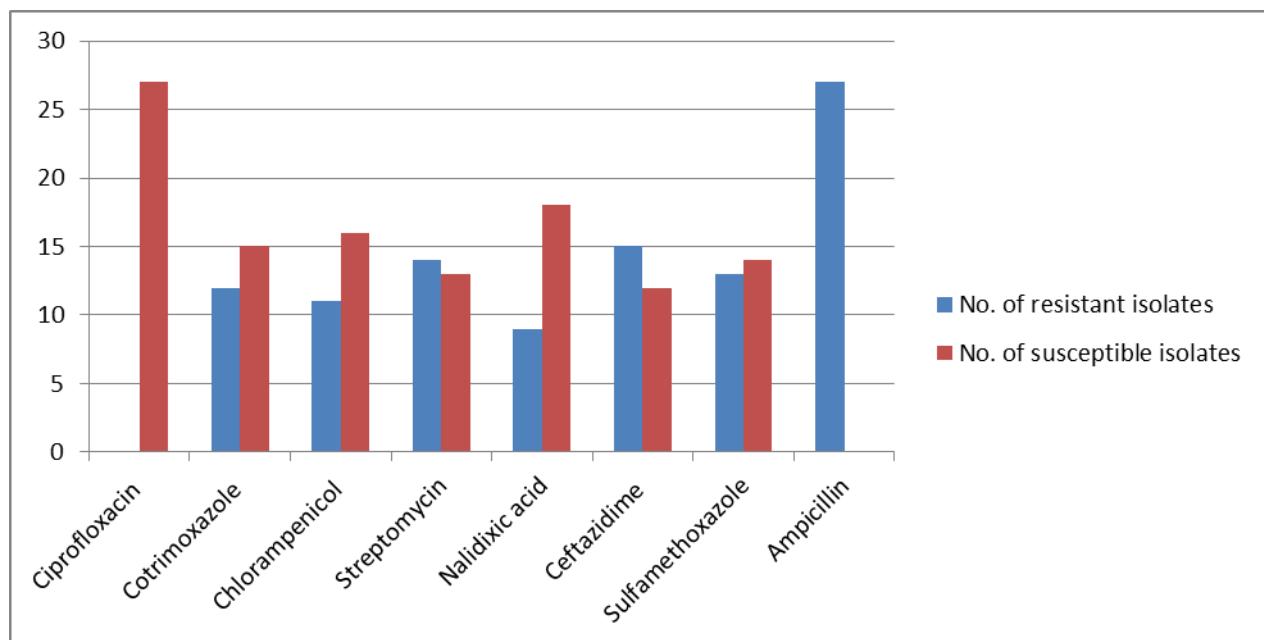


Figure 1. Distribution of resistant and susceptible isolates to the various antibiotics

4. Discussion

The prevalence of *E. coli* in Barn Swallow droppings was low according to the findings of this study. Generally, the carriage of antibiotic resistance was observed to be high. Resistance to Ciprofloxacin was observed to be the least occurring. This suggests that the antibiotic is still effective against the isolated microbes. However, this finding seems to be opposite to the findings of another study which indicated that *E. coli* isolated from the Asian Open Bill Stork (*Anastomus oscitans*) were resistant to the antibiotic [33]. Findings of this study indicated the presence of a high number of multi-drug resistant *E. coli* in the samples obtained. This is in agreement with another recent study done in Taif Province of Saudi Arabia [34] that found multidrug resistance in *E. coli* isolated from both migratory and non-migratory birds (*Hirundo rustica* included). However, it is worth noting that the Saudi

Arabian study only used two birds of *Hirundo rustica* species because the analysis was performed on a heterogeneous sample of birds. In our study, fifteen *E. coli* isolates were found to be ESBL producing strains. Although these ESBL producing strains were first isolated in 2006 from wild birds, more reports of their prevalence have been published in recent years, especially from European countries [35-44]. A recent comparative study on ESBL-producing *E. coli* carriage in raptors from Germany and Mongolia [45] found higher carriage rates in German than Mongolia (13.8% and 10.8% respectively). There are several factors which have been postulated to contribute to the prevalence of antibiotic resistance among wild birds in any given geographic area. Notably, the various characteristics of an area are more likely to contribute to antibiotic resistance than its actual location in the world. Natural preservation state, livestock and human densities, and the remoteness of an area have been put forward as important factors. In this study, the bird faecal samples were obtained from residential areas and

schools which are located around two major hospitals, namely Kampala International University Teaching Hospital and Ishaka Adventist Hospitals. There are several other smaller clinics which serve the population in and around Ishaka. It is probable that since antibiotics are highly prescribed in these health institutions, microbes could have evolved mechanisms of becoming resistance to them. Another important thing to note is that *Hirundo rustica* living in this area are frequently seen flying in and around these hospitals, where they could be easily picking the antibiotic resistant microbes from. There are other studies which have shown that the level of resistance in microbes seem to correlate with the degree of association to human activities [46,47]. Hospital waste water has been shown to be a reservoir of antibiotic resistant microbes in Nigeria [48]. This can significantly be a source from which wild birds pick-up antibiotic resistant *E. coli*. Studies done elsewhere have shown that aquatic associated species are seemingly more prone to pick up antibiotic resistance including ESBL-producing strains [49]. The fact that migrating birds travel long distances in short time frames and the large number of reports from many places concerning carriage of antibiotic resistant bacteria by wild birds leads to the genuine concern that spread of antibiotic resistance can be facilitated by wild birds. It is important to note that the bird species used in this study is a migratory bird. It will therefore not be surprising that the antibiotic resistant microbes isolated from this bird's droppings could be found elsewhere in the continent or even in another continent. In a study conducted in Chile in recent years, the prevalence of ESBL-producing *E. coli* among Franklin's gulls (*Leucophaeus pipixcan*) was found to be more than twice as high as in humans within the same geographical area [44].

5. Conclusion

In conclusion, our study and other studies done elsewhere have shown the carriage of antibiotic-resistant *E. coli* in wild birds. Such birds can act as reservoirs for antibiotic resistant microbes if enough attention is not given to the problem. Further research is recommended to determine the prevalence of antibiotic resistant *E. coli* from barn swallows residing in other parts of the country and even on the continent at large.

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Competing Interests

The authors have no competing interests

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