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Antibiotic Sensitivity Patterns of *E. coli* Isolated from Barn Swallow Droppings in Bushenyi and Sheema Towns, Uganda

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Abstract

Introduction: The objective of this research was to determine the occurrence of antimicrobial resistant *E. coli* from barn swallow droppings. There is evidence suggesting that wild birds can spread resistant bacteria through migration and that resistant bacteria can be transmitted from birds to humans and vice versa. Development of antibiotic resistance among pathogenic bacteria in wild living birds remains a major alarming public health concern. Spreading of antimicrobial resistant organisms may result into exposing antibiotic resistant genes to humans making treatment difficult and expensive. This study was focused on analysis of fecal samples from barn swallow (Hirundo rustica) droppings in Bushenyi Town and Sheema town, Uganda, for the presence of antibiotic resistant *E. coli* strains. ***Materials and methods:*** Bird fecal samples were cultured to isolate *E. coli***.** The isolates were subjected to antibiotic sensitivity testing using standard procedures. The results were interpreted using Clinical and Laboratory Standards Institute guidelines. ***Results*****:** Out of 58 samples tested, 21 samples were positive for E. coli which is equivalent to 36.2%. All isolates were found resistant to Augmentin, amoxicillin and Ampiclox. Resistance to other antibiotics starting from the most resistant bacteria were as follows; erythromycin (85.7%), ceftazidime (71.4%) and chloramphenicol (61.9%). It was noted that *E. coli* is still susceptible to gentamicin (100%), ofloxacine (90.5%) and imipenem (76.2%) in Sheema and Bushenyi. Resistance to ciprofloxacin (57.1%) and Nalidixic acid (23.9%) is still intermediate; All isolates were resistant to one or more of the tested antibiotics. 77.8% and 83.3% of isolates from Sheema and Bushenyi respectively were resistant to at least three or more classes of antibiotics tested (Multi-drug resistance). ***Conclusion***: Detection of antibiotic resistant *E. coli* in Barn swallows not only indicates a potential risk of transferring resistance to humans but also shows the status of antibiotic resistance within the ecosystem in which the Barn swallows interact with humans. This study has shown that isolated *E. coli* from bird droppings are resistant to most antibiotics that were used. This can potentially pose a risk of spread of resistance from wild birds to humans in these areas. Much attention is needed to reduce the risks of transmission of resistance from birds and help us to better understand the dissemination of antibiotic resistance in the environment.

Keywords: *E. coli*; barn swallows; AMR; antibiotic resistance; antimicrobial resistance; Uganda; wild birds; sympatric birds

1. Introduction

Antimicrobial resistance (AMR) is currently a world threat in the field of infectious diseases (Spellberg & Gilbert, 2014). This emergence of AMR has been demonstrated both in humans and

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veterinary practice (Grobbel *et al*., 2006). Although investigators tend to point this occurrence of drug resistant organisms as due to extensive use of antibiotics in medicine and agriculture (Sarmah, Meyer, & Boxall, 2006), current frequent detection of multi-resistant bacteria in birds is also significant as a contributing factor in the transmission of antimicrobial resistance into the environment (Guenther *et al*., 2010). Birds have a strong interaction with humans and livestock. Apart from livestock, detection of Multi Drug Resistance (MDR) is also associated with high human density, distant regions like high mountains (Caprioli *et al*., 1991) and particularly contaminated water which allows mixing of bacteria from different regions with exchange of antibiotic resistant genes (Baquero, Martinez, & Canton, 2008). Birds, through food contacts, wastes, soil and surface water in urban areas predispose them to acquisition of resistant bacteria (Cole *et al*., 2005b) and subsequently become a vehicle for dispersing resistant bacteria over greater geographical areas (Allen & Donato, 2010). Research suggest that birds may be acting as a reservoir for the spreading of resistance (Dolejska, Cizek, & Literak, 2007), with detection of similar resistant genes of both human and livestock origin in birds (Veldman, van Tulden, Kant, Testerink, & Mevius, 2013). It has also been demonstrated that human-associated *E. coli* strains and the resistant *E. coli* strains found in gulls are concordant indicating that the resistant bacteria isolated from birds is as a result of human dissemination (Bonnedahl *et al*., 2010; Simoes *et al*., 2010).

Gulls have been found to carry same strain of *E. coli* as can be isolated from landfills and waste water thus the possibility of transmission between sewage and birds. Red-billed choughs feeding on soil invertebrates can easily pick up resistant bacteria from contaminated manure (Allen & Donato, 2010).

There is a significant high prevalence of pathogenic Escherichia spp in migrating birds than non-migrating birds confirmed by detection of virulence factors in the isolates (Shobrak & Abo-Amer, 2014). E coli is a normal flora of the gastrointestinal tract of birds, animals and humans but it is capable of being pathogenic to both (Levine, 1987).

The number of studies describing presence of antimicrobial resistant E. coli in wildlife has increased significantly (Cole et al., 2005b; Dolejska et al., 2007; Guenther et al., 2010; Okullu et al., 2016). Development of antibiotic resistance among pathogenic bacteria in wild and sympatric birds remains a major alarming public health concern. Barn swallow (Hirundo rustica) live in areas with high human density leading to cross contamination between their fecal matter and human food and water sources which poses a possible risk of acquiring resistant strains of E. coli. It is therefore necessary to determine the prevalence of antibiotic resistant *E. coli* from Barn swallows residing in major towns of Uganda in order to determine the extent of geographical spread of antimicrobial resistant bacteria (Okullu et al., 2016).

2. Materials and Methods

2.1. Bird Fecal Sample Collection

58 Barn swallow fecal samples were collected from homesteads in Sheema and Bushenyi towns (25 in Sheema and 33 in Bushenyi town respectively)

The samples were collected from 20 residential areas, 2 health facilities and 3 educational institutions housing nests of Barn swallows in Bushenyi and Sheema towns.

Samples were collected on the ground under the nest using a sterile sampling spoon into a receptacle sterile container. The containers were then labeled and placed in the ice pack containing vessel.

The containers were transported to the microbiology laboratory of Kampala international university teaching hospital western campus, Ishaka. The samples were then placed in buffered peptone water at 37oC for 24hrs.

2.2. Culturing and Isolation of E. coli Strains

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Samples were cultured for *E. coli* on Mac-Conkey agar and incubated at 37^{0}C overnight. Each colony was sub-cultured for purity on secondary Mac-Conkey plates; Gram stain was conducted on each colony and biochemical confirmation tests which included an Indole spot reagent test, TSI, Urea and citrate utilization test were done according to the methods ascribed by Walter (Traub, Raymond, & Linehan, 1970).

2.3. Susceptibility Testing

A sterile swab was used to pick a single colony of each plate positive for *E. coli* and the swab was used to inoculate the entire surface of Müeller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum was allowed to dry for 10min before the antibiotic discs were placed on the plates. The following antibiotic discs were used in susceptibility testing: Augmentin (30µg), amoxicillin (10µg), chloramphenicol (30µg), ceftazidime (30µg), Gentamicin (10µg), ciprofloxacin (10µg), Erythromycin (15µg), Nalidixic acid (30µg), imipenem (10µg), streptomycin (10µg) and ofloxacine (10µg). The plates containing the disks were incubated at 35^{o}C for 24hrs in a microbiology incubator. All procedures were followed in accordance with the Clinical and Laboratory Standards Institute. After the incubation period, the zones of inhibition were measured to the nearest millimeter using the metric ruler, and the isolates were classified as sensitive, intermediately sensitive and resistant based on recommended guidelines (Kiehlbauch et al., 2000).

3. Results

3.1. Isolation and Identification of Escherichia coli

Thirty three Barn swallow fecal droppings from Bushenyi town and twenty five from Sheema town were collected for isolation of *E. coli*. Pure colonies of the bacteria were isolated from the fecal droppings on MacConkey agar and biochemically identified as E. coli.

The distribution pattern of E. coli isolates were as summarized in Table 1

Table 1. E.coli isolates from Sheema and Bushenyi towns.

	SHEEMA	**BUSHENYI**	**TOTAL**
NO. OF SAMPLES TESTED	25	33	58
NO. OF SAMPLES POSITIVE FOR *Escherichia coli.*	9	12	21
%POSITIVE FOR *Escherichia coli*	36	36.36	36.21

3.2. Antimicrobial Susceptibility of the Escherichia coli Isolates

Antimicrobial susceptibility of 21 Escherichia coli isolates From Bushenyi and Sheema town are shown in Table 2.

Table 2. Antimicrobial susceptibility of 21 Escherichia coli isolates From Bushenyi and Sheema towns.

	% RESISTANT		**% INTERMEDIATE**		**%SUSCEPTIBLE**	
ANTIBIOTICS	SHEEMA	BUSHENYI	SHEEMA	BUSHENYI	SHEEMA	BUSHENYI
NALIDIXIC ACID (30µg)	44.44	33.33	25	22.22	33.33	41.67
CEFTAZIDIME (30µg)	88.88	58.33	16.67	11.11	0	25
ERYTHROMYCIN (30µg)	77.78	91.67	8.33	22.22	0	0
IMIPENEM (10µg)	44.44	8.33	0	0	0	91.67
GENTAMICIN (30µg)	0	0	0	0	100	100
OFLOXACIN (10µg)	0	0	8.33	11.11	88.87	91.67
AMPICLOX (30µg)	100	100	0	0	0	0
STREPTOMYCIN (10µg)	55.56	41.67	8.33	22.22	22.22	50

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CIPLOFLOXACIN (30µg)	44.44	41.67	58.33	55.56	0	0
CHLORAMPHENICOL (30µg)	66.67	58.33	0	0	33.33	41.67
AMOXYCILLIN (10µg)	100	100	0	0	0	0
AUGUMENTIN (30µg)	100	100	0	0	0	0

3.3. Overall Resistance Patterns of the E. coli Isolates from Barn Swallow Droppings from Both Sheema and Bushenyi Towns

The isolates obtained from the fecal samples were resistant to more than one antibiotic. High levels of resistance was evident in Erythromycin, Ampiclox, Amoxycillin and Augmentin. There was good levels of susceptibility to Imipenem and Ofloxacin. The isolates were fully susceptible to Gentamycin.

Table 3. Overall resistance patterns of the *E. coli* isolates from Barn swallow droppings from both Sheema and Bushenyi towns.

ANTIBIOTICS DISCS USED	SAMPLES TESTED	% RESISTANT	% INTERMEDIATE	% SUSCEPTIBLE
NALIDIXIC ACID (30µg)	21	38.10	23.81	38.10
CEFTAZIDIME (30µg)	21	71.43	14.29	14.29
ERYTHROMYCIN (30µg)	21	85.71	14.29	0
IMIPENEM (10µg)	21	23.81	0	76.19
GENTAMICIN (30µg)	21	0	0	100
OFLOXACIN (10µg)	21	0	9.524	90.48
AMPICLOX (30µg)	21	100	0	0
STREPTOMYCIN (10µg)	21	47.62	14.29	38.10
CIPLOFLOXACIN (30µg)	21	42.86	57.14	0
CHLORAMPHENICOL (30µg)	21	61.90	0	38.10
AMOXYCILLIN (10µg)	21	100	0	0
AUGUMENTIN (30µg)	21	100	0	0

4. Discussion

Presence of *E. coli* was determined using morphological characteristics, gram stain and biochemical tests which included TSI test, citrate utilization test, Indole test and Urease test. Samples where *E. coli* was isolated appeared as pink colonies on MacConkey agar which indicates lactose fermenting ability of *E. coli*. Presumptive *E. coli* isolates on MacConkey agar subjected to other tests showed yellow slant/yellow butt plus gas production with no H_2S production on TSI test, indicating glucose and lactose utilization. On Indole test, a red ring was formed indicating ability of E. coli to form Indole from tryptophan, in addition to negative citrate and urease test. This confirmed presence of *E. coli* bacteria in the fecal samples that were cultured. In another study done in Canada and U.S, similar biochemical tests were considered important in the isolation of *E. coli* from baked meat and water respectively (Stiles & Ng, 1981; U.S. Environmental Protection Agency, 2010).

Out of 58 samples tested, 21 samples were positive for E. coli which is equivalent to 36.2%, indicating that the prevalence of *E. coli* in barn swallow droppings was low in Sheema and Bushenyi town. This is comparable to another study done in Ishaka town, Uganda where out of 116 fecal samples of barn swallow droppings collected, only 23.3% of the samples were positive for *E. coli* (Okullu *et al*., 2016).

All isolates were found resistant to Augmentin, amoxicillin and Ampiclox. This indicates that the *E. coli* isolates are resistant to penicillins. This may be due to complex surface structure of gram negative bacteria with outer membrane lipopolysaccharide which acts as an impenetrable barrier for

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some penicillin. Resistance to other antibiotics starting from the most resistant bacteria were as follows; erythromycin (85.7 %,), ceftazidime (71.4%) and chloramphenicol (61.9%) indicating that *E. coli* is developing resistance to macrolides and cephalosporins in Sheema and Bushenyi which may be due to their frequent use/misuse in these areas (Kariuki, 2024). This is in agreement with a previous study done in Ishaka town which showed that resistance was high in ampicillin (100%) and ceftazidime (55%) (Okullu *et al*., 2016). According to the results, it was noted that *E. coli* is still susceptible to gentamicin (100%), ofloxacine (90.5%) and imipenem (76.2%) in Sheema and Bushenyi which shows that these antibiotics can still be used in these areas. There was no difference in resistance patterns between isolates from Bushenyi and Kabwohe Sheema indicating that susceptibility profiles in these two areas are the same. Resistance to ciprofloxacin (57.1%) and Nalidixic acid (23.9%) is still intermediate; this requires that their use be controlled to prevent further development of resistance. All isolates were resistant to one or more of the tested antibiotics. 76% and 81.8% of isolates from Sheema and Bushenyi respectively were resistant to at least three or more classes of antibiotics tested (Multi-drug resistance). These classes of antibiotics were majorly macrolide (erythromycin), cephalosporin (ceftazidime), penicillins (Ampiclox, Augmentin) and chloramphenicol. This is contrary to another research done in America which showed that 13% of *E. coli* isolates from domestic and wild animal fecal samples were resistant to at least three or more antibiotics (Raida, Kaneene, Johnson, & Miller, 2005).

Percentage number of isolates resistant to antibiotics tested differed from to isolates that were sensitive and intermediately resistance. This indicated that a greater percentage of isolates were resistant to most antibiotics tested than they were sensitive. This also shows that there is a narrow range of antibiotics still effective in these areas making treatment difficult. Acquisition of resistant *E. coli* from barn swallows (*Hirrundo rustica*) in these areas could be due to their interaction with the environment like livestock, hospital waste water and sewage where they pick resistant bacteria. In interaction of these birds with humans through their droppings, an undesirable end result is transferring of resistant bacteria to humans.

5. Conclusions and Recommendation

There is ample evidence to suggest that wild birds can carry antibiotic resistant bacteria and indicators for transmission between wild birds and humans have been characterized. This study has shown that isolated E. coli from bird droppings is resistant to most antibiotics that were used; this can potentially impose a risk of spread of resistance from wild birds to humans in these areas. Much attention is needed to reduce the risks of transmission of antimicrobial resistance from sympatric and wild birds to humans. Studies should be done to determine the pattern of antimicrobial resistance in clinical isolates of *E. coli* in order to compare them to the pattern of resistance observed in the current isolates. This can in turn help us to better understand the dissemination of antibiotic resistance in the environment within the two towns.

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