Biomedical Statistics and Informatics
2021; 6(3): 42-46
http://www.sciencepublishinggroup.com/j/bsi
doi: 10.11648/j.bsi.20210603.11
ISSN: 2578-871X (Print); ISSN: 2578-8728 (Online)

Occurrences of Antimicrobial Resistance in *Escherichia coli* Isolated from Intestinal Faecal Contents of *Covurs splendens* (Indian Crow)

Philbert Balichene Madoshi[1, *], Shuli Nkalango Mitemi[2], Abdul Ahmed Selemani Katakweba[3]

[1]Department of Microbiology and Parasitology, ST Francis University College of Health and Allied Sciences, Ifakara, Tanzania

[2]Department of Veterinary Microbiology, Parasitology and Immunology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania

[3]Pest Management Centre, Sokoine University of Agriculture, Morogoro, Tanzania

Email address:

bmadoshi@gmail.com (P. B. Madoshi)

[*]Corresponding author

To cite this article:

Philbert Balichene Madoshi, Shuli Nkalango Mitemi, Abdul Ahmed Selemani Katakweba. Occurrences of Antimicrobial Resistance in *Escherichia coli* Isolated from Intestinal Faecal Contents of *Covurs splendens* (Indian Crow). *Biomedical Statistics and Informatics*. Vol. 6, No. 3, 2021, pp. 42-46. doi: 10.11648/j.bsi.20210603.11

Received: May 6, 2021; **Accepted**: June 18, 2021; **Published**: August 18, 2021

Abstract: *Introduction*: Indian crows have been commented to present significant physical community harm with respect to property damage and agricultural products. However the public health significant of these birds in the developing countries has been underrated. Their scavenging nature might result into spread of pathogens in the public as well as the antimicrobial traits among bacteria. In order to gain the insight into the role of Indian crows as carriers and reservoir of multi-resistant E. coli; broad spectrum antimicrobial agents were tested using a wild E. coli isolated from the birds themselves. *Methods*: The *Escherichia coli* bacteria were isolated and identified from intestinal contents of 30 Indian crows in Morogoro. An 100µl Intestinal contents were spread on MacConkey for *E. coli* isolation. A total of eight (8) antimicrobial agents: STX (25 µg), AMP (10 µg), AMC (30 µg), CN (10 µg), CIP (5 µg), TE (30 µg), S (10 µg) and CTX (30 µg) were used. *E. coli* ATCC 25922 and included as control strains. *Results*: The resistance TE and SXT were the most prevalent (90%), followed by AMP (76.7%) and S (73.3%), CIP (53.3%), and CTX (36.7%). The susceptibility of these isolates was high in CN 96.7%. It also shown among 30 of the *E. coli* isolates; twenty-eight of them presented multiple antibiotic resistances. *Conclusion*: The results have shown that Indian crow can serve as a reservoir of antibiotic-resistant *E. coli*, and potentially transmit such *E. coli* over long distances. This is a hidden public health threat as these crows move from one place to another and is found in residential areas contaminating water and food with resistant bacteria and antimicrobial resistance strains.

Keywords: Indian Crow, AMR, Peri-urban, Morogoro, Antibiotics

1. Introduction

Indian crows (*Coryuss plendens*) are birds native to the Indian sub-continent but are now widely spread throughout the tropical climate. The birds entry in Tanzania dates back 1890s in Zanzibar isles; Long [1], described that the birds were introduced in the island as scavengers of garbage. However the birds are now ubiquitous along the Indian coast of Kenya and Tanzania mainland [2, 3]. This could be explained by the fact that the birds are very good at adapting various habitats, Wium-Anderson and Reid [3] reported that the crows are tremendously increasing in numbers in Tanzania mainland where they have been located in Morogoro since 1997 and to other places such as Mikumi national park and as far as Ifakara.

These groups of birds are good at invading and affecting ecological pattern of the indigenous birds by feeding on the eggs and chickens [3, 4]. Furthermore they affect agricultural productivity activities especially in orchards, decimating grain crops and pecking eyes of pigs and sheep [5, 6]. The crows have also invaded human settlements such that they can be located along houses, damping sites, restaurants and other

public places. Due to this behaviour the crows present hidden public health hazards which need to be dealt before it results into irreparable scenario. The likelihood hazard could be mechanical or biological transmission of pathogens among human or livestock and incubation of pathogenic organisms which could mutate or change their pathogenicity status [1, 6].

The ubiquitous nature of the crows goes in tandem with the likelihood of harbouring pathogens such *Escherichia coli* and *Salmonella* [7, 8]. These could be either pathogenic or non-pathogenic but presenting the hazard of transmitting the resistance genes among members in the same genus or those which are closely related. However the studies to determine the prevalence of such bacteria with respect to antimicrobial resistance in wild birds such as Indian crows in Tanzania have not been conducted intensively [21, 22]. In this regard there is poor information on the hazard which could be presented by the crow in families as well as the public in general.

This study intended to isolate *E. coli* as one of the antimicrobial indicator bacteria in the intestinal contents of the Indian crow and carry out antimicrobial profiling. It can be further argued that crows present a public health problem in the sense that they interact with both human and livestock, thus the likelihood of transmitting pathogenic or organisms with traits with significant disease causation is high. Antimicrobial resistance pattern are among of these traits which are now affecting the health sector because most of the curative doses in patients are not effective [12, 21, 22]. However the potential of the crows to transmit such traits and strains have been undermined in many or developing countries like Tanzania. It is expected that description of antimicrobial patterns in *E. coli* isolated from crows will produce baseline data for further research on antimicrobial resistance in other pathogens and public intervention on the rational control of the birds.

2. Materials and Methods

The study was conducted in Morogoro municipality around Morogoro slaughter house and waste deposit sites at Sokoine University of Agriculture (SUA) where Indian crows were found. While the laboratory activities were done at the department of Veterinary Microbiology, Parasitology and Immunology at Sokoine University of Agriculture. A cross sectional study design was carried where the birds were randomly trapped from the study area using an approved trap. A total of 30 birds were collected and euthanized using an approved animal welfare protocol, thereafter lower intestinal contents were retracted for bacteriological culture.

2.1. Sample Processing

Intestinal content of caught Indian crow were scrapped using sterile blade and caped tubes while observing aseptic conditions. Primary culture for isolation was done in MacConkey agar where the inoculated plates were incubated at 37°C for 24hr. The grown bacteria were identified by colony morphology, Gram stain, Indole and Citrate reaction. The grown bacteria on primary culture were then sub-cultured in blood agar using the same conditions.

2.2. Antimicrobial Susceptibility Profiling

Phenotypic drug sensitivity test was done on E. coli isolates which were grown on blood agar after inoculation in Muller Hinton Agar as described previously by Bauer et al [8] and antimicrobial discs applied on media inoculated using the CLSI [9] guidelines. This study utilized Sulphamethaxazole/Trimethoprim (STX 25µg), Ampicillin (AMP 10µg), Amoxycillin-clavulanic acid 2:1 (AMC 30µg), Gentamicin (CN 10µg), Ciprofloxacin (CIP 5µg), Tetracycline (TE 30µg), Streptomycin (S 10µg) and Cefotaxime (CTX 30µg). The zones of inhibitions were recorded after 18 – 24hrs on plates with clear margins of the zones; however the plates with unclear and occluded zones were re-cultured. The interpretation of the zones of inhibition was based on CLSI [9].

2.3. Data Analysis

The analysis was done using the SPSS for descriptive statistics where Chi-square was used to describe the association of resistance of bacteria to respective drugs. The paired sample T – test was used to test the statistical inference of location with respect to antimicrobial resistance; the p-value of *0.05* was regarded as significant statistical difference.

3. Results

3.1. Demographic Presentation of the Indian Crow

This study used 30 birds which were trapped and caught from two different places in Morogoro Municipality: (1) 19 (63.3%) birds from Morogoro municipal slaughter house and (2) 11 (36.7%) birds from waste deposit sites at Sokoine University of Agriculture – Main campus. Thus more birds were easily caught at Morogoro slaughter house than at the waste deposits.

3.2. E. coli Antimicrobial Resistance Profiling

The isolates were subjected to phenotypic antimicrobial sensitivity test and it was observed that most of the drugs used showed resistance to one or several drugs. Tetracycline (TE) was the drug that most of the isolates were not affected; 90% of the isolates were not susceptible to TE, 80% for AMP and CTX, 60% for CIP however the resistance was lower in CN (13.3%) (Table 1).

Table 1. *Phenotypic antimicrobial resistance of the isolates.*

Drug	Resistance (n, %)	Susceptible (n. %)
TE	27 (90%)	3 (10%)
STX	27 (90%)	3 (10%)
AMC	10 (33.3%)	20 (66.7%)
AMP	24 (80%)	6 (20%)
CTX	24 (80%)	6 (20%)
CIP	18 (60%)	12 (40%)
S	24 (80%)	6 (20%)
CN	4 (13.3%)	26 (86.7%)

3.3. E. coli Antimicrobial Resistance Patterns with Respect to Location

The resistance profile of the isolates was matched to the location where the birds were trapped. It was found that resistance was high in isolates from the slaughter house than in the waste deposit. The resistance was highly observed in TE, STX, AMP, S and CIP however the isolates were more susceptible in CN and CTX. In the isolates from the waste deposit; the resistance was 100% in TE and STX, followed with S and AMP however the isolates were more susceptible for CN (90.9%) as shown on Table 2.

Table 2. *Phenotypic antimicrobial profiling of the isolates with location.*

Location	AMR Status	Tested Drug							
		TE	STX	AMC	AMP	CTX	CIP	S	CN
Slaughte	Resistant	16 (84.2)	16 (84.2)	8 (42.1%)	16 (84.2)	7 (36.8%)	12 (63.2%)	15 (78.9%)	3 (15.8)
r House	Susceptible	3 (17.8)	3 (15.8)	11 (57.8%)	3 (15.8)	12 (63.2)	7 (36.8%)	4 (21.1%)	16 (84.2%)
Waste	Resistant	11 (100%)	11 (100%)	2 (18.2%)	8 (72.7%)	7 (63.6%)	6 (54.5%)	9 (81.8%)	1 (9.1%)
deposit	Susceptible	0 (0%)	0 (0%)	9 (81.8%)	3 (27.3%	4 (36.4%)	5 (45.5%)	2 (18.2%)	10 (90.9%)

3.4. Statistical Presentation of E. coli Isolates and Location

The statistical analysis was carried out using cross tabulation and T – test for paired two sample for means. It was noted that the resistance of the TE, STX and AMC was statistically associated with the location where the bacteria was isolated (*p=0.03*). Furthermore there was a significant difference on the resistance patterns with respect to where the birds were isolated *(p=0.03)*; however there was no significant difference on the susceptibility of the isolates with respect to isolation *(p=0.178)*.

4. Discussion

This study involved trapping of Indian crows from two different sites in Morogoro municipality, Tanzania. The birds were trapped and collected for the purpose of quantifying the level of antimicrobial resistance profile of one of the faecal indicator bacteria (E. coli) from the intestinal contents.

4.1. Study Population of the Birds

Thirty (30) birds were caught and isolation of the bacteria was done according to the recommended protocols. The study presents a total of 28 (93.3%) out of 30 *E. coli* isolates which had multiple drug resistance (MDR) to most of antimicrobial agents. The resistance of these faecal indicator bacteria has been reported in different avian species, although there is poor information on the resistance of isolates from the Indian crows in Tanzania. Nevertheless this study presents a public health hazard such as disease causation in avian as well as human [10, 11, 13].

4.2. Occurrence of MDR in E. coli in Wild Birds

Although the occurrence of antimicrobial resistance in Indian crows is not extensively studied, it can be further argued that these birds are prototype of the migratory birds as well as the wild birds. Thus they can be categorized and discussed as other wild birds which have been reported to contain different antimicrobial resistant traits elsewhere in the world. Foti et al. [15] reported high resistance of different bacteria isolated from migratory Passeriformes. These authors concluded that migratory birds play an important role in the ecology, circulation and dissemination of potentially pathogenic antimicrobial resistant organisms. Ong et al. [16] described occurrence of antimicrobial traits of *E. coli* isolated from wild birds and rodents, the authors concluded that underscoring the necessity of environment management and close monitoring on AMR bacteria in the wild birds jeopardizes the public health safety. Shobrak & Abo-Amer [17] described the role of wild birds as carriers of multi-drug resistant *E. coli* and *E. vulneris*; the author as well described multidrug resistance in the isolates and concluded that this scenario could create a potential health impact to the human health.

4.3. Antimicrobial Resistance Pattern Transmission

This study has shown that Indian crows harbour *E coli* strains which present MDR which is a global concern. Despite lack of comparative data and the study presenting AMR phenotypic results, it is worth reporting to present the public health concern if the crows are not adequately prevented to accord with human settlement. It can be argued that although these birds rarely come in contact with antimicrobial agents, they could serve as reservoirs and disseminators of resistant bacteria because they can access human and domestic excreta [17, 18, 22]. Furthermore these birds access contaminated water and waste deposits; these seem to be major source of transmission of resistant bacteria of human and veterinary origin.

4.4. The Need for Indian Crows Control Strategies

As noted area the Indian crows are invaders and scavengers in most of the avian habitats causing massive damage in other avian species. In addition the study has demonstrated a relatively small portion of the crows can harbour significant *E. coli* isolates which have multiple drug resistance, this is of paramount basing on the fact that such birds move haphazardly without boundaries like other birds [15, 16, 19, 22]. Table 2 shows antimicrobial resistance pattern of the isolates; it can explained the distribution of resistance of such *E. coli* was not limited to either location which reflects the ecological nature of the birds; they lack borders and domination with respect to their feeding behaviour.

It can be argued that the control of these birds could safeguard emergence and distribution of MDR bacteria in the environment to human and livestock. Foti et al. [15] further argued that migratory birds are sentinel species and environmental health indicator; thus integration of epidemiological surveillance networks might be pertinent tool to manage such public health hazard. Khan et al. [20] described that the public should undertake steps to control antibiotic release and environmental disposal from all use should be immediate and obligatory. This study concludes that Indian crows are leading to public hazard which is awaited; thus ecological measures could be undertaken so that the birds are managed to the carrying capacity to alleviate public health impacts.

5. Conclusion

The study shows that Indian crows harbour resistant strains of *E. coli*. Antimicrobials commonly used in humans and animals; TE and SXT (Katakweba et al., 2012) in the study area had highest resistances compared to other antimicrobials. Efforts should be made to control Crows as they play role in the spread of resistant bacteria from one area to another. They are leading to public hazard which is awaited; thus, ecological measures could be undertaken so that the birds are managed to the carrying capacity to alleviate public health impacts. This should be used as baseline data in Tanzania for further research on antimicrobial resistance in other pathogens and public intervention on the rational control of wild birds including Indian crows.

Author Contributions

Madoshi P. B.: The author worked on obtaining the relevant permits for conducting research in live and wild birds, worked on the laboratory to provide guidance to the technician during antimicrobial sensitivity test and interpretation, designed the data analysis strategy as well as analysed the obtained data. He reviewed the manuscript and presented obtained results to the communities which were involved in the research.

Shuli M.: Conducted sample collection and preliminary sample processing which include humane killing of the crows by euthanizing using carbon dioxide gas, intestinal contents scrapping and laboratory work. He also made the first version of the manuscript before submitting to the co-author

Katakweba A. A. S.: Designed the study, determined the sample size and organised the meeting with the local leaders where the crows could be trapped. He furthermore developed the survey and developed the protocol for isolation of the targeted bacteria. He as well guided the other research on following the recommended proposal for isolating the bacteria.

Competing Interest

The authors declare that they have no competing interest.

Acknowledgements

The authors acknowledge the publication and research review committee of Sokoine University of Agriculture for permitting the research activities. The College of Veterinary Medicine and Biomedical Sciences for allowing the processing of the laboratory work in the respective laboratories. We further extent the appreciation to the Municipal executive director of Morogoro for the permission to trap and collect Indian crows from different areas.

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