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Role of Cattle Egrets in the Dissemination of Multi-drug Resistant *Escherichia coli*: A One Health Perspective

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to establish the role of cattle egrets that feed on a nearby animal farm as a possible source of MDR bacteria. A total of 50 faecal dropping samples of cattle egret were collected from cattle farm and processed in the laboratory for isolation of *E. coli*. Total 45 isolates were recovered, of which 20 were identified and characterized as *E. coli* morphologically and biochemically. The antibiogram profile of all *E. coli* isolates recovered from cattle egret revealed 100% resistance to Ampicillin, Cloxacillin and Cefotaxime. Antibiotics such as Imipenem,

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Gentamycin, Trimethoprim, and Triple sulphas were found highly sensitive (100%) followed by Streptomycin (90%). The Sensitivity of Norfloxacin and Kanamycin ranged between intermediate to high degree, while Polymyxin was found sensitive for 55% isolates. Multi antimicrobial resistance index of all the isolates was found to be more than 0.27 indicating a “high risk” for the community. These, MDR positive *E. coli,* were further subjected to phenotypic and genotypic confirmation of ESBL producers by DDST and PCR, respectively. Out of 20, 12 isolates (60%) were confirmed as ESBL producers by DDST and PCR. PCR analysis of ESBL positive isolates revealed the predominance of *bla* $_{CTX}$ gene. All *E. coli* were also evaluated for pathogenicity using in vitro tests and 50% and 40% of the isolates were found to be pathogenic on the Congo red binding and hemolysis test, respectively. In the present study, the occurrence of MDR *E. coli* and ESBL producers in cattle egret is a cause of concern for public health and animal health.

Keywords: E. coli; antimicrobial resistance; ESBL; cattle egret; pathogenicity test.

1. INTRODUCTION

“Antimicrobial resistance (AMR) is one of the top ten threats to public health, as listed by the World Health Organisation” [1]. “Today, antimicrobial resistance has been a major challenge for pharmaceuticals, clinicians and researchers due to their indiscriminate and extensive use in human and veterinary medicine. The emergence of AMR, especially among *Enterobacteriaceae* has been increasing day by day and poses serious threat for both human and animal health with limited antimicrobial therapeutic options” [2]. “Among *Enterobacteriaceae, Escherichia coli* (*E. coli*) is a major environmental contaminant, as it is a natural inhabitant of the GUT microbiota of animals and humans and plays an important role in the transmission of AMR through horizontal gene transfer. Unfortunately, AMR from the environmental sources has received less attention compared to that from the human and animal health spheres despite the significant variation in the geographical spread of AMR bacteria in the environment” [3]. Forces working behind the dissemination of antimicrobial resistant bacteria are uncountable, but one commonly neglected source happens to be the wild avifauna living in close vicinity of animals. The cattle egret (*Bubulcus ibis*, a gregarious species of bird), which is commonly found foraging with grazing livestock, can acquire AMR bacteria through commensalism from the environment. Since no work has been done in this area on cattle egrets, the present study was undertaken with the aim of establishing their role as a potential source of AMR, especially extended-spectrum beta lactamase (ESBL) producing bacteria.

2. MATERIALS AND METHODS

Locale: The study was carried out in the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya. The samples were collected from diverse places of university cattle farm by swab technique and brought to laboratory in ice box.

Sample Processing: Total 50 faecal swab samples were collected from contaminated environment of cattle farm and each sample was homogenized in 5ml of sterile normal saline solution. These suspensions were kept for 2 hours at room temperature to allow larger debris to settle down and bacterial-rich supernatant was utilized for subsequent analyses.

Isolation and Identification: The isolation and identification of *E. coli* was done as per the method of Edward and Ewing [4]. All samples were enriched with 2ml nutrient broth and incubated at 37^0C for 24 hrs. A loopful inoculum was taken and streaked on MacConkey agar plates added with 2 mg/L cefotaxime, then incubated at 37^0 C for 24 h. Colonies showing rose pink colouration were streaked on Eosine Methylene Blue (EMB) agar plates and incubated at 37^0 C for 24 hrs. Green metallic sheen colonies on EMB were tentatively considered as *E. coli* and taken onto a sterilized nutrient agar slant. The further identification of the isolates was performed by Gram’s staining, motility test, and various biochemical tests, *viz.* IMViC pattern, catalase test, nitrate reduction, urease test, triple sugar iron agar and sugar fermentation reaction etc.

Antibiotic Susceptibility Test: Bauer’s disk diffusion method [5] was used to determine the isolate’s resistance or susceptibility of the isolate to various antibiotics. A uniform lawn of fresh 24 h grown culture of test organism (1.5×10^8 organism/ml) was spread on Muller and Hinton

Agar plates (MHA) and then commercially available discs were placed at 30 mm apart from centre on the surface of MHA plates and incubated at 37^0Cfor 24 hrs. The results were interpreted as per manufacturer's guidelines (Hi Media).

Phenotypic confirmation of ESBL producers: Double disc synergy test (DDST) was used for phenotypic confirmation of ESBL producers. All *E. coli* isolates were subjected to DDST using ESBL kit 1 (Cefotaxime, Cefotaxime/ Clavulanic acid) and Kit 3 (Ceftazidime, Ceftazidime/Clavulanic acid) of Hi-media. A uniform lawn of fresh 24 h grown culture of test organism (1.5 x10^8 organism/ml) was prepared on Muller and Hinton Agar plates. The commercially available discs were placed at 30 mm apart from centre on the surface of plates and incubated at 37^0C for 24 hrs. The results were interpreted as per CLSI guidelines [6].

Molecular Characterization of ESBL producers: The ESBL genes, viz. *bla* $_{\text{TEM}}$ and *bla* $_{\text{CTX-M}}$ were targeted for the molecular characterization of ESBL producers using published primer sequences (Table 1). The DNA templates were prepared using snap- chill method as described by Pandey et al. [7]. The PCR assay was performed in 20 µl final volume comprising 10µl of primer master mix, 2µl of forward and reverse primer (100pmol), 2µl of MgCl2, 2µl of free DNA template and 2 µl of nuclease water. The PCR cycling condition included initial denaturation at 95^0C for 5 min followed by 35 cycles at 94^0C for 30 sec, annealing at 50^0C for 35 sec, elongation at 72^0 C for 40 sec and final extension at 72^0 C for 5 min. Amplified PCR products were run in 1.5% agarose gel and analysed using the gel documentation system (Uvi tech, UK).

Multiple Antibiotic Resistance (MAR) Index: The MAR index is the number of antibiotics to which the test isolate showed resistance divided by the total number of antibiotics to which the test organism has been evaluated for sensitivity. MAR index of each isolate was calculated as per the method of Krumpermann [10].

Congo red binding test: The Congo red binding (CR) test was carried out according to the technique of Berkhoff and Vinal [11]. The isolates were streaked on the plate of Trypticase Soya Agar (TSA) containing 0.03% Congo red dye and incubated at 37^0 C for 24-72 hours. Appearance of brick red coloured colonies within 24 to 72 h was recorded as a positive reaction, while the colonies that didn't bind dye and remained white or grey even after 72 h PI, were considered as negative.

Haemolysis test: *E. coli* isolates were also tested for Haemolysin production by plate as well as tube inoculation method. Enterohaemolysin production on plates was determined as per the method of Joshi and Joshi [12]. The isolates were grown on a blood agar base supplemented with 5% w/v sheep RBCs, 10mM calcium chloride, and incubated at 37^0 C for overnight. Haemolysin production was indicated by the zone of complete lysis of erythrocyte and clearing of medium. The tube method used for alpha-haemolysin production was a slight modification of those of Asnani et al. [13]. The isolates were inoculated in 1 ml of sterilised nutrient broth supplemented with an equal volume of washed sheep RBC (5% V/V) in graduated centrifuge tube. The tubes were incubated at 37 ^{0} C for 4-6 hours with intermittent agitation followed by centrifugation at 6000 rpm for 5 min. All test cultures were observed for the transparent reddish colour of supernatant; the signs of hemolysis [14].

3. RESULTS AND DISCUSSION

Antimicrobial resistance is a multifaceted global health problem and its containment requires a multi-pronged approach like One Health concept. This concept rightly recognizes the interrelatedness of human, animal and environment health and fosters collaborative and multisectoral efforts to tackle the challenge [3]. The present study was conducted with aim to establish the role of egret found in cattle farm as potential spreader of Multi Drug Resistant (MDR) bacteria. Keeping in view, total 50 faecal droppings of cattle egret were collected from the environment and processed in the laboratory for isolation and identification of *E. coli*. Based on morphological, growth and biochemical characteristics, 20 isolates (66.66%) were identified as *E. coli* out of 45 isolates recovered from fecal swabs. These findings were found to be in agreement with the reports of previous workers [7,15,16]. All *E. coli* isolates were subjected to an antibiogram study against 11 antibiotics, viz. Ampicillin (AMP), Cloxacillin (COX), Norfloxacin (NX), Kanamycin (K), Streptomycin (S), Gentamycin (GEN), Polymyxin B, Triple Sulfa (S3), Cefotaxime (CTX) and Imipenem (IMP). The results of antibiotic susceptibility test (ABST) are mentioned in Table

2. In this study, all *E. coli* isolates were found resistant to Ampicillin (100%), Cloxacillin (100%) and Cefotaxime (100%) followed by Polymyxin (45%), Kanamycin (33%), Norfloxacin (22%). Only two isolates exhibited resistance to Streptomycin (10%) and Trimethoprim (10%). Similar findings were reported by Yadav et al*.* [16] for *E. coli* of bovine origin against cefotaxime, ampicillin, norfloxacin and trimethoprim from the same area. It reveals that sharing of environment could result in transmission of AMR bacteria in wild bird also. Antibiotics like Gentamycin, Triple Sulpha and Imipenem were found sensitive against all isolates (100%) followed by Streptomycin (90%) and Trimethoprim whereas Norfloxacin (55%), Polymyxin (55%) and Kanamycin (30 %) were found moderately sensitive. Likewise, Hasan [17] also recorded the highest resistance for Cloxacillin, moderate resistance for Kanamycin, and the highest sensitivity for Imipenem for *E. coli* isolated from UTI patients. The Multi antimicrobial resistance index of all the isolates was found to be more than 0.27 in this study (Table 3). MARI $\geq$ 0.2 indicates that pathogen is in a “high risk environment” where antibiotics are used indiscriminately without antibiogram study [10]. In similar study, Ahmed and Gulhan [18] also reported occurrence of MDR *E. coli* in Gulls and Pigeon showing a varying degree of resistance to Tetracycline, Kanamycin, Streptomycin, Ampicillin, Chloramphenicol, Trimethoprim, Gentamicin, Enrofloxacin and Ciprofloxacin.

All *E. coli* isolates were also assessed for their propensity to produce ESBL using Cefotaxim and Ceftazidime and 18 isolates (91%) were selected as producers of ESBL [6]. In phenotypic testing, 12 (60 %) isolates were confirmed to be positive for ESBL by DDST, which coincided with the observations of Vibha et al*.* [19]. The study of the gene distribution of phenotypically positive ESBL isolates revealed that *bla*$_{\text{CTX}}$ was present in 12 (60 %) isolates, while *bla*$_{\text{TEM}}$ was detected in 8 (40 %) isolates. Previous studies conducted in this area of eastern UP have also shown predominance of the *bla* $_{\text{CTX}}$ gene [15,19,20]. There was little difference in the prevalence between phenotypic and genotypic method and these findings corroborated with the observation of Vibha et al*.* [19], Prajapati et al*.* [20] and Badri et al*.* [21].

The *E. coli* isolates from cattle egrets were further tested for pathogenicity traits by a C R binding test and a haemolytic assay. In this study, C R binding activity was revealed by 10 isolates (50%) showing brick red colonies. This marker can differentiate pathogenic strains from commensal ones and has been used as an epidemiological marker of APEC by some

Table1. Primer sequence used for identification of ESBL genes

Gene	Sequence (5’ 3’) ⟶	Product size	Reference
bla$_{\text{TEM}}$	F-ATGAGTATTCAACATTTCCG R-TTAATCAGTGAGGCACCTAT	851bp	Grimm et al*.* [8]
bla$_{\text{CTX}}$	F-CGCTTTGCGATGTGCAG R-ACCGCGATATCGTTGGT	551bp	Paterson et al*.* [9]

Table 2. Antimicrobial resistance pattern of *E. coli* isolates of Cattle Egret

S. No.	Antibiotic	Disc Conc.	Number of *E. coli* Isolates (%)		
			Resistant	Intermediate Sensitive	Sensitive
1.	Ampicillin (AMP)	10 mcg	20(100)	-	-
2.	Cloxacillin (COX)	10 mcg	20(100)	-	-
3.	Norfloxacin (NX)	10 mcg	5 (22)	4 (20)	11 (55.0)
4.	Kanamycin (K)	30 mcg	6 (30)	8 (40)	6 (30)
5.	Streptomycin (S)	10 mcg	2 (10)	-	18 (90)
6.	Gentamycin (GEN)	10 mcg	-	-	20 (100)
7.	Polymyxin B (PB)	300 unit	9 (45)	-	11(55.0)
8.	Triple Sulpha (S3)	300 mcg	-	-	20 (100)
9.	Trimethoprim (TR)	10mcg	2 (10)	-	18 (90)
10.	Cefotaxime (CTX)	30mcg	20(100)	-	-
11.	Imipenem (IMP)	10 mcg	-	-	20(100)

Table 3. Multi Antimicrobial Resistance Index (MARI) of *E. coli* isolates of Cattle Egret

Isolates	AMP	COX	NX	K	S	GEN	PB	S3	TR	CTX	IMP	MARI
E1Aa	R	R	S	S	S	S	R	S	S	R	S	0.36
E1	R	R	S	S	S	S	S	S	S	R	S	0.27
E2	R	R	S	S	S	S	S	S	R	R	S	0.36
E1Ab	R	R	R	S	S	S	R	S	S	R	S	0.45
E3	R	R	S	S	S	S	S	S	S	R	S	0.27
E1Ba	R	R	IS	R	S	S	R	S	S	R	S	0.45
E4	R	R	IS	R	S	S	S	S	S	R	S	0.36
E1Bb	R	R	S	IS	S	S	R	S	S	R	S	0.36
E5	R	R	S	IS	S	S	S	S	S	R	S	0.27
E12a	R	R	R	IS	S	S	R	S	S	R	S	0.45
E6	R	R	R	IS	S	S	S	S	S	R	S	0.36
E12b	R	R	IS	IS	S	S	R	S	S	R	S	0.45
E7	R	R	IS	IS	S	S	S	S	S	R	S	0.27
E1Bb1	R	R	S	R	S	S	R	S	S	R	S	0.45
E8	R	R	S	R	S	S	S	S	S	R	S	0.36
E1Bb2	R	R	S	IS	R	S	R	S	S	R	S	0.45
E9	R	R	S	IS	R	S	S	S	S	R	S	0.36
E12a1	R	R	R	R	S	S	R	S	S	R	S	0.54
E10	R	R	R	R	S	S	S	S	S	R	S	0.54
E11	R	R	S	S	S	S	S	S	R	R	S	0.36

Note: S= sensitive, IS= Intermediate sensitive, R= Resistant

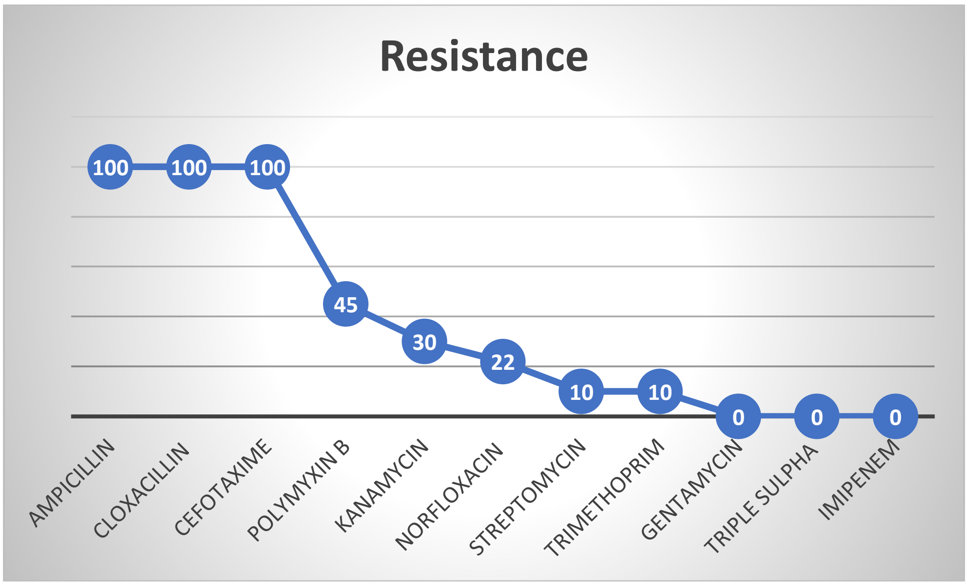

Fig. 1. AMR profile of *E. coli* isolated from cattle egrets

researchers. Binding of Congo Red is associated with presence of virulence genes such as *ompA*, *iss* and *fimH* and genes for multiple resistance to antibiotics as reported by Abdel et al. [22]. Haemolytic activity was exhibited by 8 isolates (40%) by both plate as well as tube method. This finding was in agreement with the observation of Pandey et al*.* [7]. All these HA and CR positive isolates were considered enteropathogenic. Both tests have been used previously by various researchers [12,22,23] to discriminate invasive and noninvasive strains of *E. coli* of bovine and poultry origin.

This study illustrated that antibiotics are widely used in the treatment of human and animal infection leading to proliferation of multidrug resistant (MDR) bacteria which are distributed in environments. The polluted environment serves as a source of antimicrobial-resistant (AMR) bacteria for wild birds, or migratory birds which acts as potential spreaders of resistant elements through migration. The findings of the present study can have implications in framing Public health policies to combat MDR in the country.

4. CONCLUSION

In this study, occurrence of MDR positive *E. coli* with MARI ≥ 0.2 from cattle egrets poses a serious risk to human and animal health by contaminating the environment with their faeces. It shows that antibiotics are used indiscriminately in both, human and animal healthcare system leading to the contamination of the environment. These cattle egrets could play a dynamic role in the transmission of AMR bacteria from one place to another. Hence, a largescale epidemiological study is needed to determine possible transmission of AMR bacteria between wild bird, environment, animal and human for investigating genetic relationship between the strain from one health point of view.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thangaraju P, Venkatesan S. WHO Ten threats to global health in 2019: Antimicrobial resistance. Cukurova Med. J. 2019;44:1150–1151.
2. World Health Organization. Critically Important Antimicrobials for Human Medicine-3rd Revision [online]. Geneva, Switzerland: 2013;(9789241504485).
3. Taneza, Neelam and Sharma, Megha. Antimicrobial resistance in the environment: The Indian scenario. Indian J. of Med. Res. 2019;148(2):119-128. DOI: 10.4103/ijmr.IJMR_331_18.
4. Edward PR, Ewing WH. Identification of Enterobacteriaceae (3rd edn.). Burges publicity Co. Minneapolis, Minnesota. 1972;55:415.
5. Bauer AW, Kirby WMM, Sherris JS, Turk M. Antibiotic susceptibility testing by a standardized single disc method. American J of Clin. Path. 1966;45:493.
6. Wayne PA. Performance Standards for Antimicrobial Susceptibility Testing. Twenty- Ninth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI) document. 2019; M100-S29.
7. Pandey A, Joshi N, Joshi RK, Prajapati R, Singh A. Virulence attributes and antibiotic resistance pattern of E. coli isolated from human and animals. Asian J of Ani and Vet. Adv. 2015;11(1):67-72.
8. Grimm V, Ezaki S, Susa M, Knabbe C, Schmid RD, Bachmann TT. Use of DNA Microarrays for Rapid Genotyping of TEM Beta-Lactamases that Confer Resistance. Clin. Microbiol. 2004;42: 3766-3774.
9. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA. Antimicrobial agents. Chemotherapy. 2003; 47:3554-3560.
10. Krumpermann PH. Multiple antibiotics resistance indexing of E. coli to identify high risks sources of faecal contamination of food. Applied Env. Microbiol. 1983;46 (1):165-170.
11. Berkhoff HA, Vinal AC. Congo red medium to distinguish between invasive and noninvasive Escherichia coli pathogenic for poultry. Avian Dis. 1986;30:117-121.
12. Joshi N, Joshi RK. Detection of ESBL production, hemagglutination, and Haemolytic Ability in Escherichia coli. J. Vet. Pub. Hlth. 2017;14 (1):54-57.
13. Asnani PJ, Bhatnagar M, Bhandari S. Production and purification of Escherichia coli hemolysin. Folia Microbiolgic. 1988; 33:393-400.
14. Ibrahim EI, Sayed FH, Ashraf M, Abd EI, Wahab SAK, Helmy AT. Prevalence of Enterobacteriaceae producing ESBL isolated from bovine mastitis milk. Alexandria J of Vet Sci. 2018;58(1):102-108.
15. Yadav A, Joshi N, Joshi RK. Occurrence of Extended spectrum β lactamase producing Enterobacteria in animal products and their environment. Int. J of Curr. Microbiol. & App. Sci. 2019;8(5):2255-2264.
16. Yadav V, Joshi RK, Joshi N, Kumar A, Singh SV. Status of multidrug resistance among E. coli and Klebsiella spp. isolates of buffalo origin from ESBL in Eastern Plain Zone of Utter Pradesh. Haryana Veterinarian. 2021;60(2):208-212.

17. Hasan, Thualfakar Hayder. Extended spectrum β lactamase producing E. coli isolated from UTI patients in Najaf Province, Iraq. Int J of Pharma Res. 2020; 12(4):673-67.
18. Ahmed, Nejash A, Gulhan, Timur. Determination of antibiotic resistance patterns and Escherichia coli isolated from wild birds. Microbiome. 2024;12(1): 8.
    DOI 10.1186/s40168-023-01729-1.
19. Yadav V, Joshi RK, Joshi N, Kumar A, Singh SV, Niyogi D. Detection and molecular characterization of extended spectrum beta lactamase producing E. coli and Klebsiella spp. isolates of cattle origin in Eastern Plain Zone of Utter Pradesh. Indian J of Ani Res. 2022;56(11):1369-1376.
    DOI: 10.18805/IJAR.B-4762
20. Prajapati, Rajeev, Joshi N, Joshi RK. Isolation and Identification of Extended-Spectrum Beta-Lactamases Producing E. coli and Klebsiella from Human. Int J of Curr Microbiol & App Sci. 2020;9(2).
    DOI:https://doi.org/10.20546/ijcmas.2020.902.xx
21. Badri AM, Ibrahim TI, Mohamed SG, Garbi MI, Kabbashi AS, Arbab MH. Prevalence of ESBL producing E. coli and K. peumoniae isolated from raw milk samples in Al Jazirah State, Sudan. Mol. Biol. 2017;7(1)
    DOI:10.4172/2168-9547.1000201.
22. Abdel Ameer H. Zahid, Muna Turkey Mossa AL-Mossawei, Aaisha B. Mahmood. In vitro and In vivo pathogenicity test of local isolates of APEC from naturally infected broiler from Baghdad. Int. J. Adv. Res. Biol. Sci. 2016;3(3):89-100.
23. Sharma, Kishan Kumar, Soni, Shanti Swaroop, and Meharchandani, Sunil. Congo red dye agar test as an indicator test for the detection of invasive bovine Escherichia coli. Veterinarski arhiv. 2006;76(4).

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