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Prevalence of Shigatoxigenic, Enteropathogenic and Antimicrobial Drug Resistant *Escherichia coli* from Captive Wild Animals of Alipore Zoo, Kolkata, India

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ABSTRACT

Background: *Escherichia coli* is one of the intestinal microbiota in wildlife as in domestic animals and humans. The prevalent serogroups, pathogenic strains like STEC, EPEC and antimicrobial resistance is less frequently studied in captive wildlife of India. The present study was aimed to study the prevalence of STEC, EPEC and antimicrobial resistant *E. coli* from captive wild animals in a zoo in Kolkata, India.

Methods: This study was done during March 2019 to June 2019 for *E. coli* from faecal samples of captive wild birds and mammals. The pathotypes STEC and EPEC were detected for the presence of *stx1*, *stx2* and *eae* virulence genes, respectively. Extended spectrum beta- lactamases, ampC beta-lactamases production and antimicrobial susceptibility were detected by standard phenotypic methods.

Result: In this study, some *E. coli* isolates were found harbouring genes for ETEC and EPEC. Extended spectrum beta- lactamases and ampC beta-lactamases were produced by some isolates. Most *E. coli* isolates were detected multidrug-resistant but were mostly sensitive to gentamicin and amikacin. This study demonstrated that captive wild animals harbour potentially pathogenic *E. coli* that were resistant for many antimicrobials.

Key words: Captive wild animals, *E.coli*, EPEC, ESBL, Multi-drug resistance, Serogroup, STEC.

INTRODUCTION

The presence of large population of India's wildlife needs attention in prioritizing research on zoonotic pathogens in wildlife, particularly in a multidisciplinary one-world one-health approach (Singh and Gajadhar, 2014). *Echerichia coli* is one of the commonest intestinal flora harboured by wildlife, domestic animals and humans and it is considered one of the indicator pathogen for studying antimicrobial resistance. Currently, there are 186 different *E. coli* serotypes based on outer membrane lipopolysaccharide (O) antigen and 53 H-types. Several serotypes of *E. coli* are non-pathogenic and pathogenic strains of *E. coli* are detected by specific virulence factors and their effect in susceptible species.

Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains belong to the category of diarrheagenic *E. coli* that are important food-borne pathogens associated with diarrhoeal disease in humans. Although ruminants especially cattle are the main reservoirs of STEC, wildlife including game animals also play an important role as carriers of these pathogens (Miko *et al*., 2009).

Antimicrobial resistance of *E. coli* isolates from wildlife origin was reported for the first time from Japanese wild birds in 1978. The production of extended spectrum beta-lactamases (ESBLs) by *Enterobacteriaceae*, specifically by *E. coli*, has caused a major concern in past few decades. The production of the enzyme confers resistance to a variety of beta-lactam antibiotics and monobactams (*e.g*. aztreonam), but usually not the carbapenems or the cephamycins (*e.g*. cefoxitin). ESBL-producing *E. coli* isolates from wild birds and animals was reported first time in Portugal in 2006 (Costa *et al*., 2006), it has now been observed all over the world. AmpC β-lactamase- producing organisms (ACBL) can produce resistance against cephalosporins, penicillins, cephamycins and monobactams in addition to β-lactamase inhibitors such as clavulanic acid.

Although STEC and EPEC *E. coli* were extensively studied in humans, farm animals, birds, food and the environment, it has been less frequently studied in captive wild animals of India (Mishra *et al*., 2016; Milton *et al*., 2019). Antimicrobial resistance of *E. coli* from wildlife also

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contributes eventually to the global problem of ESBL producing pathogens. The present study was designed to study the prevalence of STEC, EPEC and antimicrobial resistance of *E. coli* from captive wild animals in a zoo in Kolkata, India.

MATERIALS AND METHODS

In the present study, seventy five fresh faecal samples were collected during March 2019 to June 2019 from birds and mammals housed at Alipore zoo, Kolkata, India (Table 1). These wildlife species were treated with both *β-lactam* and non-β-lactam antibiotics for therapeutic purpose individually as veterinary care when necessary. This study did not require ethical approval because faecal samples were collected only after defaecation but consent was taken from zoo authority for sampling.

The faecal samples of 2-10 g were collected in the morning using sterile swabs in peptone water (HiMedia, India), kept in chilled box at 4°C, transported to the laboratory of Department of Veterinary Microbiology at West Bengal University of Animal and Fishery Sciences, Kolkata and processed immediately. One gram of each sample was suspended in 9 ml of peptone water broth (HiMedia, India) and incubated overnight at 37°C. After enrichment, *E. coli* were isolated as per standard procedure and identified using Gram-staining and biochemical tests (Quinn *et al*., 1999). *E.coli* isolates were serogrouped at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, India. Pathotypes of *E. coli* isolates were detected by PCR assays for STEC (*stx1*, *stx2* genes) and EPEC (*eae* gene) respectively (Vidal *et al.,* 2004).

For phenotypic confirmation of the presence of ESBL, cefotaxime (30 µg) and ceftazidime (30 µg) disks were used with and without clavulanate (HiMedia, India) in disc diffusion method (CLSI, 2015). Cefoxitin-cloxacillin double disc synergy test was used for AmpC β-lactamase production (ACBL) as per Tan *et al.* (2009).

Antimicrobial susceptibility was also determined by the disk-diffusion method (CLSI, 2015) using fourteen standard discs (Himedia, India). *E. coli* isolates showing resistance to three or more classes of antimicrobials were recognised as multidrug-resistant (MDR). Determination of multiple antibiotic resistance (MAR) index was performed as per the procedure described by Krumperman (1983).

RESULTS AND DISCUSSION

From seventy five samples, sixty (80.00%) bacterial isolates were identified as *E. coli*, 33 isolates belonged to captive wild birds and 27 isolates to captive wild mammals (Table 1). Overall sixteen different O-serogroups of *E. coli* were detected. Twenty one *E. coli* isolates were untypeable. Some strains of *E. coli* were rough or autoagglutinating, making these cultures O-untypeable (DebRoy *et al.*, 2016). Prevalent serogroups in captive wild birds seemed to partially differ from earlier studies in wildlife. Awadallah *et al*. (2013) detected *E.coli* serogroup O119 in cattle egrets birds which was observed in Blue and Yellow Macaw, White Ibis and Goffin's Cockatoo in the current study. None of the serogroups was observed in Bengal tiger by the study of Satpute *et al*. (2010) who reported serogroups O17, O103, O147 in Maharastra. Four *E. coli* serogroups O26, O118, O121 and O157 isolated in the current study, were among the most clinically relevant STEC serotypes in human illness (Sánchez *et al*., 2015).

Overall, 7 isolates (11.67%) were found to be positive for *stx1* gene and 21 isolates (35.0%) were found to be positive for *eae* gene (Table 1). No isolate was found positive for *stx2* gene and most *E. coli* isolates from birds (n=20) and mammals (n= 18) harboured none of the three virulence genes studied. EPEC was found to be the predominant pathotype (Table 1). Similar study was done by Milton *et al*. (2019). In contrast, captive wild mammals were found as the main reservoir of STEC and no isolate from birds harboured STEC in Chile (Marchant *et al*., 2016). Prevalence of EPEC and STEC strains were low among captive wild birds in Sau Paulo, Bazil (Sanches *et al.,* 2017) and among zoo animals and birds in Spain (Alonso *et al*., 2017). Prevalence of STEC at Alipore zoo was low compared to wild animals (23.87%) in Spain (Sánchez *et al*., 2009). Prevalence of STEC in wildlife was found variable in different studies (Marchant *et al*., 2016; Sanches *et al.,* 2017; Alonso *et al*., 2017), probably because of the difference in animal population studied and diagnostic methods used.

In the present study, STEC belonged to serogrup O157 and non-O157 serogroups such as O2, O26, O118 and O149. Similarly STEC serogroup O157 and non- O157 serogroup were also reported previously from wild birds (Foster *et al*., 2006), petting zoo animals such as jaguar (Hamzah *et al*., 2013) and from free ranging wild animals (Sánchez *et al*., 2009).

Overall, ESBL production by *E. coli* was found in 23 (38.33%) isolates and AmpC β-lactamase production in 35 (58.33%) isolates (Table 1). A similar observation was reported by Wang *et al.* (2012) from primates in six different zoos in China. Dobiasova *et al.* (2013) reported a high percentage (71%) of wild birds and animals colonized by ESBL producing *E. coli* in a zoo of Czech Republic. Recently, prevalence of multi-drug resistant ESBL and *AmpC* producing *E. coli* was observed in domestic birds such as ducks and chicken (Banerjee *et al.* 2019; Samanta *et al.* 2015). Presence of antimicrobial resistance genes from clinically ill and farm animals might have impact in antimicrobial resistance of organisms harboured by wild animals.

The resistance of *E. coli* isolates from captive wild birds and mammals were observed against penicillins and cephasporins groups; and less frequently against gentamicin and amikacin (Table 2).Twenty (60.60%) isolates from birds and 17 (62.96%) isolates from mammals were detected exhibiting multiple drug resistance (MDR). Except the three isolates from wild birds (Eurasian spoonbill, Grey peacock-pheasant, Chinese silver pheasant), all 57 (95.0%) *E.coli* isolates exhibited MAR index

Table 1: Serogroups, virulence types, ampC and ESBL production by *Escherichia coli* from captive wild animals of Alipore zoo, Kolkata, India.

Host species	No. of samples tested	No. of *E.coli* isolated	Sero groups	Virulence types STEC		EPEC	Antimicrobial resistance phenotype	
				Positive for *stx1*	Positive for *stx2*	Positive for *eae*	ESBL positive	ACBL positive
Captive wild birds								
White Ibis (*Threskiornis aethiopica*)	2	2	O119, O84	0	0	2	0	2
Blue and Yellow Macaw (*Ara ararauna*)	4	4	O7, O8, O120, Untyped (UT)	0	0	2	1	3
Scarlet Macaw *(Ara macao*)	2	2	O2,O157	1	0	1	1	2
Bare-eyed cockatoo (*Cacatua sanguinea*)	2	1	O121	0	0	1	1	1
Citron-crested cockatoo (*Cacatua sulphurea citrinocristata*)	2	1	O8	0	0	0	0	1
Goffin's cockatoo (*Cacatua goffiniana*)	2	2	O119,UT	0	0	1	1	1
Moluccan cockatoo (*Cacatua moluccensis*)	2	1	O157	1	0	1	0	1
Grey Parrot (*Psittacus erithacus*)	2	2	O120, UT	0	0	1	0	2
Amazon Parrot (*Amazona oratrix*)	1	1	UT	0	0	0	0	1
Rose ringed parakeet (*Psittacula krameri*)	2	2	UT	0	0	0	2	0
Eurasian Spoonbill (*Platalea leucorodia*)	3	2	O118, UT	1	0	1	1	1
Rosy pelican (*Pelecanus onocrotalus*)	1	1	O120	0	0	1	1	1
Green Imperial Pigeon (*Ducula aenea*)	2	1	UT	0	0	0	1	0
White Dove (*Streptopelia alba*)	2	1	UT	0	0	0	0	0
Grey Peacock-Pheasant (*Polyplectron bicalcaratum*)	1	1	UT	0	0	0	0	0
Indian peafowl (*Pavo cristatus*)	1	1	UT	0	0	0	1	0
Chinese Silver pheasant (*Lophura nycthemera*)	2	2	UT	0	0	0	1	0
Keel-billed toucan (*Ramphastos sulfuratus*)	2	2	UT	0	0	0	0	1
Painted Stork (*Mycteria leucocephala*)	2	1	UT	0	0	0	1	1
Lesser adjutant stork (*Leptopilos javanicus)*	2	1	UT	0	0	0	0	1
Bar-headed goose (*Anser indicus*)	2	1	O20	0	0	1	1	0
Hill Mynah (*Gracula religiosa*)	2	1	UT	0	0	0	0	1
Total	43	33		3	0	12	13	20
Captive wild mammals								
Brow-antlered deer(*Cervis eldii eldii*)	2	2	O20, O49	0	0	0	1	2
Barking deer (*Muntiacus muntjak*)	2	1	UT	0	0	0	1	0
Black buck (*Antilope cervicapra*)	1	1	O135	1	0	0	1	1
Mouse deer (*Moschiola meminna*)	1	1	O149	1	0	1	0	1
Sambar deer (*Rusa unicolor*)	1	1	O101	0	0	0	1	0
Swamp deer *(Rucervus duvaucelii*)	3	2	O137, UT	0	0	0	0	1
Spotted deer (*Axis axis*)	2	1	O134	0	0	0	0	1
Four horned antelope (*Tetracerus quadricornis*)	1	1	O149	0	0	0	0	0
Nilgai (*Boselaphus tragocamelus*)	1	1	UT	0	0	0	1	0
Plains zebra (*Equus quagga*)	1	1	O49	0	0	1	0	1
Bengal tiger (White) *(Panthera tigris tigris)*	1	1	O20	0	0	1	0	1
Bengal tiger	2	2	O120, UT	0	0	0	0	1
Jaguar (*Panthera onca onca*)	1	1	O157	1	0	1	1	1
Striped Hyena (*Hyaena hyaena)*	1	1	O8	0	0	1	0	1
Jungle cat (*Felis chaus*)	1	1	O157	1	0	1	1	0
Sloth bear (*Melursus ursinus*)	2	1	O26	0	0	0	0	0
Fishing cat (*Prionailurus viverrinus)*	2	1	O8	0	0	1	0	0

Table 1: Continue...

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Golden Jackal (*Canis aureus)*	1	1	O120	0	0	0	1	1
Chimpanzee (*Pan troglodytes*)	2	2	O26, UT	0	0	1	1	1
Bonnet macaque (*Macaca radiata*)	1	1	O26	0	0	1	0	1
Bengal Slow Loris (*Nycticebus bengalensis*)	1	1	UT	0	0	0	0	0
Eastern Hoolock Gibbon (*Hoolock leuconedys*)	1	1	UT	0	0	0	1	0
Rhesus Macaque (*Macaca mulatta*)	1	1	UT	0	0	0	0	1
Total	32	27		4	0	9	10	15

Table 2: Antimicrobial resistance of *Escherichia coli* isolates from Captive wild animals of Alipore zoo, Kolkata, India.

Antimicrobial agents (concentration)	Captive wild birds (n=33)		Captive wild mammals (n=27)	
	No. sensitive(%)	No. resistant (%)	No. sensitive (%)	No. resistant (%)
Ampicillin (10 µg)	1 (3.03)	32 (96.97)	1 (3.70)	26 (96.29)
Amoxicillin/Clavulanic acid (20/10 µg)	1 (3.03)	32 (96.97)	1 (3.70)	26 (96.29)
Cefotaxime (30 µg)	3 (9.09)	30 (90.91)	2 (7.40)	25 (92.59)
Cefotaxime/ Clavulanic acid (30/10 µg)	3 (9.09)	30 (90.91)	3 (11.11)	24 (88.89)
Ceftazidime (30 µg)	4 (12.12)	29 (87.88)	3 (11.11)	24 (88.89)
Ceftazidime/Clavulanic acid (30/10 µg)	5 (15.15)	28 (84.84)	3 (11.11)	24 (88.89)
Ceftriaxone (30 µg)	12 (36.36)	21 (63.64)	7 (25.93)	20 (74.07)
Tetracycline (30 µg)	20 (60.60)	13 (39.39)	10 (37.04)	17 (62.96)
Norfloxacin (10 µg)	20 (60.60)	13 (39.39)	19 (70.37)	8 (29.63)
Nalidixic acid (30 µg)	20 (60.60)	13 (39.39)	21 (77.78)	6 (22.22)
Co-Trimoxazole (25 µg)	22 (66.66)	11 (33.33)	22 (81.48)	5 (18.51)
Chloramphenicol (30 µg)	19 (57.57)	14 (42.42)	22 (81.48)	5 (18.51)
Amikacin (30 µg)	31 (93.94)	2 (6.06)	26 (96.30)	1 (3.70)
Gentamicin (10 µg)	30 (90.90)	3 (9.09)	26 (96.30)	1 (3.70)

value ≥ 0.2. Similar studies with high resistance was reported in *E.coli* isolates of leopard (*Panthera pardus*) and black buck (Vinodh Kumar *et al*., 2021), captive Nilgiri Langur (*Trachypithecus johnii*) (Balaji *et al.,* 2018). The importance of this study lies with the fact that wild animals provide a biological source for dissemination of antibiotic resistance genes and zoonotic diseases (Radhouani *et al.,* 2014).

CONCLUSION

Most *E.coli* isolates from captive birds and mammals that harboured none of the three virulence genes probably were commensals. Although limited samples were studied from each wildlife species, the occurrence of STEC and EPEC pathotypes in captive wildife warrants attention as they are potential reservoir. High antimicrobial resistance was also observed in captive wildlife in Alipore zoo, Kolkata, India. Continous monitoring is required to assess their impact on animal and public health.

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Conflict of interest

All authors decalre that they have no conflict of interest.

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