STUDY ON OCCURRENCE AND MOLECULAR DETECTION OF *ESCHERICHIA COLI* HARBOURING EXTENDED SPECTRUM β-LACTAMASES FROM CAPTIVE BIRDS AND ANIMALS

THESIS

BY

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Submitted to

BIHAR ANIMAL SCINCES UNIVERSITY,
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In Partial fulfillment of the requirements
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IN

(Veterinary Microbiology)

Department of Veterinary Microbiology
Master of Veterinary Science

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CERTIFICATE-I

This is to certify that the thesis entitled**, "Study on occurrence and molecular detection of *Escherichia coli* harbouring Extended Spectrum β-Lactamases from captive birds and animals"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the discipline of **Veterinary Microbiology** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the bonafide research work carried out by **Dr. Mrinalini Saini,** daughter of Mrs. Saroj Saini and Dr. Mrigraj Saini, under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

(Signature & Name of the Major Advisor)

Place:

Date:

CERTIFICATE-II

This is to certify that the thesis entitled, **" Study on occurrence and molecular detection of *Escherichia coli* harbouring Extended Spectrum β-Lactamases from captive birds and animals"** submitted by **Dr. Mrinalini Saini**, **BVC/M/VMC/002/2017-18** daughter of Mrs. Saroj Saini and Dr. Mrigraj Saini, to the Bihar Animal Sciences University, Patna, Bihar in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the discipline of **Veterinary Microbiology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Date:

Place: *(Mrinalini Saini)*

CONTENTS

ABBREVIATIONS

AK	Amikacin
AMP	Ampicillin
AMC	Amoxicillin-clavulanic acid
AMR	Antimicrobial Resistance
ABR	Antibiotic Resistance
ABST	Antibiotic sensitivity testing
ß	beta
bp	Base pair
BPW	Buffered peptone water
CTR	Ceftriaxone
CTX	Cefotaxime
Conc	Concentration
CLSI	Clinical and laboratory standard institute
CDC	Centers for Disease Control and Prevention
CTX-M	Cefotaxime
C	Chloramphenicol
CIP	Ciprofloxacin
CZA	Central Zoo Authority
Da	Dalton
°C	Degree centigrade
DNA	Deoxy-ribonucleic acid
dNTPs	Deoxy-nucleotide triphosphates
DW	Distilled water
et al	et alibi
EPEC	Entero pathogenic *E. coli*
ETEC	Entero toxigenic *E. coli*
EIEC	Entero invasive *E. coli*
E. coli	*Escherichia coli*
ESBL	Extended Spectrum Beta Lactamase
EX	Enrofloxacin
g	gram
GEN	Gentamicin
hr	hour
pH	-log H ion concentration
≤	Less than equals to
mg	Milli gram
µg	Micro gram
µl	Micro liter
µ	Micron
ml	Milli liter

mM	Milli mole
Mw	Molecular weight
MHA	Mueller Hinton Agar
≥	More than equals to
MDR	Multi Drug Resistance
M	Molar
NA	Nutrient Agar
O	Oxytetracycline
OXA	Oxacillinase
%	Percentage
pmol	Pico mole
PCR	Polymerase chain reaction
rpm	Revolution per minute
SHV	Sulpha hydrl group
TEM	Temoniera
i.e.	That is
TAE	Tris acetate EDTA
Tris	Tris hydroxyl methyl aminoethane
Taq	*Thermus aquaticus*
UV	Ultra voilet
VTEC	Vero toxigenic *E. coli*
v	Volts
w/v	Weight by volume
WHO	World Health Organization

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Title of the thesis: Study on occurrence and molecular detection of *Escherichia coli* harbouring Extended Spectrum β-Lactamases from captive birds and animals

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Abstract: *Escherichia coli* are the predominant gram-negative bacteria in gastrointestinal tract of animals, including free range and captive wild animals. The incidence of multidrug-resistant (MDR) *E. coli* is escalating across the globe. The present study aims at determining the prevalence of *E. coli* in captive mammals and birds, antibiotic susceptibility, multidrug resistant (MDR) from different accredited zoos of India, including Bihar. Besides, the study focused in determining the frequency of ESBL (extended spectrum beta-lactamase) producing *E. coli*. A total of 73 isolates out of ninety-four obtained from Sanjay Gandhi Biological Park, Patna, Bihar, and Kanpur Zoological Park, Kanpur, U.P were confirmed as *E. coli* by conventional as well as molecular method.

The susceptibility patterns of the *E. coli* isolates were determined against 10 antibiotics belonging to five different antibiotic classes by disc diffusion method. The highest rate of resistance was seen against Ampicillin (100%) followed by Cefotaxime (76.71%) and Oxytetracycline (52.05%). Minimum resistance was observed for Chloramphenicol (2.74%) followed by Enrofloxacin (13.70%), Ciprofloxacin (15.07%) and Amoxycillin-Clavulanic acid (19.18%). None of the isolates was found resistant to aminoglycosides (Amikacin and Gentamicin). 15.07% of the *E. coli* isolates were multi drug resistant.

For the determination of ESBL producing *E. coli* in the preliminary screening, third generation cephalosporins were used and the positive isolates were further confirmed by Hexa G-Minus 24 kit and Combination disc method. 73 isolates examined in the preliminary screening, 54 (73.9%) isolates were confirmed as ESBL producers by Hexa G-Minus 24 kit method. But only 45 (61.64%) were ESBL producers by 45 (61.64%).

The findings of present study thus reveal moderate frequency of ESBL producing *E. coli* in the zoo settings, which might have implications in treating infections caused by this bacterium. The data generated will be useful in the better management of *E coli* infections among wild animals. Regular monitoring and surveillance of ESBL producing *E. coli* strains harboured in wild animals, especially when kept in captivity is, therefore, essentially required to contain the infections particularly in nosocomial settings.

Keywords: *Escherichia coli*, Multidrug resistant, Extended spectrum beta-lactamases producer, captive mammals and birds, Zoo.

Student **Major Advisor**

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