**Antimicrobial Resistance Pattern of Fecal *Escherichia coli* Isolated From non-diarrhoeic Pet Dogs at Chittagong Metropolitan Area**

# Veterinary Logo

# 

# A CLINICAL REPORT SUBMITTED

# BY

# 

Intern ID: D-33

Roll No: 08/50

Registration No: 392

***Report presented in partial fulfillment for the degree of Doctor of Veterinary Medicine (DVM)***

**Chittagong Veterinary and Animal Sciences University**

**Khulshi, Chittagong-4225**

**March, 2014**

**Antimicrobial Resistance Pattern of Fecal *Escherichia coli* Isolated from Non-diarrhoeic Pet Dogs at Chittagong Metropolitan Area**

****

# A CLINICAL REPORT SUBMITTED

# BY

# 

Intern ID: D-33

Roll No: 08/50

Registration No: 392

***Approved as to style and content by***

**CONTENTS**

**Chittagong Veterinary and Animal Sciences University, Khulshi-4202**

**…………………………….**

**Signature of Supervisor**

Dr. Mohammad Mahbubur Rahman

Associate Professor

Department of Pathology & Parasitology

**…………………………….**

**Signature of Author**

Name: Nasima Akter

Roll No: 08/50

Reg. No: 392

Intern ID: D-33

|  |  |  |  |
| --- | --- | --- | --- |
| CHAPTER | SERIAL | SUBJECTS | PAGE |
|  |  | **Acknowledgement** | I |
|  |  | **Abstract** | II |
| Chapter: 1 |  | **Introduction** | 1-2 |
| Chapter: 2 |  | **Review of literature** | 3-15 |
|  |  |  |  |
|  | 2.1.1 | Enterobacteriaceae | 3 |
|  | 2.1.2 | *Escherichia coli* | 3 |
|  | 2.1.3 | Taxonomy | 4 |
|  | 2.1.4 | Biology of *E. coli* | 4-5 |
|  | 2.1.5 | Isolation and identification of *E. coli* | 5-6 |
|  | 2.1.6 | Clinical Significance | 6 |
|  | 2.2 | Antimicrobial resistance | 6-7 |
|  | 2.2.1 | Molecular mechanisms | 7-8 |
|  | 2.2.2 | Natural and acquired resistance | 8 |
|  | 2.2.3 | Acquisition by chromosomal mutations | 8-9 |
|  | 2.2.4 | Acquisition by horizontal gene transfer | 9 |
|  | 2.2.5 | Intracellular migration of resistance genes | 10 |
|  | 2.2.6 | Measurement of resistance in bacterial populations | 10-11 |
|  | 2.2.7 | The microbial threat | 11 |
|  | 2.2.8 | Multidrug resistance efflux pumps in bacteria | 11 |
|  |  |  |  |
| Chapter: 3 |  | **Materials and methods** | 16-28 |
|  |  |  |  |
|  | 3.1 | Description of study area | 16 |
|  | 3.2 | Study duration and sample collection | 17 |
|  | 3.3 | Isolation and identification of *Escherichia coli* | 17-20 |
|  | 3.3.1 | Media used | 17 |
|  | 3.3.2 | Isolation of *E. coli* | 18 |
|  | 3.3.3 | Gram’s staining | 19 |
|  | 3.3.4 | Biochemical test | 20 |
|  | 3.4 | Preservation of the culture | 20 |

**CONTENTS**

|  |  |  |  |
| --- | --- | --- | --- |
| CHAPTER | SERIAL | SUBJECTS | PAGE |
| Chapter: 3 | 3.5 | DNA Extraction from bacterial culture for PCR test | 20-24 |
|  | 3.5.1 | Identification of *E.coli* by PCR using 16S rRNA primer | 21-23 |
|  | 3.5.2 | Visualization of PCR Product of *E. coli* through agar gel electrophoresis | 23-24 |
|  | 3.5.2.a | Materials and Reagents required | 23-24 |
|  | 3.5.2.b  3.6  3.7 | Procedure of agar gel electrophoresis  Antimicrobial Sensitivity Test at Muller Hinton Agar  Data analysis | 24  25-27  27 |
|  |  |  |  |
| Chapter : 4  Chapter : 5 |  | **Results**  **Discussions** | 29-33  34-36 |
|  |  |  |  |
| Chapter : 6 |  | **Conclusion and recommendation** | 37 |
|  |  |  |  |
| Chapter : 7 |  | **References** | 38-45 |
|  |  |  |  |
| Chapter : 8 |  | **Appendix** | 46-48 |

**LIST OF TABLE**

|  |  |  |
| --- | --- | --- |
| SL | TITLE OF THE TABLES | PAGE |
| 3.1 | Oligonucleotide primers used in PCR to detect *E. coli* | 21 |
| 3.2 | Contents of each reaction mixture of PCR used to detect *E. coli* | 22 |
| 3.3 | Cycling conditions used for PCR detection of *E. coli*. | 23 |
| 3.4 | Diameter (zone of inhibition) standards for *E. coli* (CLSI, 2007) | 37 |
| 4.1 | Isolation and identification of *E. coli*. on MacConkey Agar | 29 |
| 4.2 | Isolation and identification on *E. coli* onEMB Agar | 29 |
| 4.3 | Microscopic identification of *E. coli* by Gram’s staining | 29 |
| 4.4 | Indole test for identification of *E. coli* | 30 |
| 4.5 | Molecular identification (PCR) of *E. coli* by using 16S rRNA gene primer | 30 |
| 4.6 | Prevalence of E. coli in different breed, age & sex group | 31 |
| 4.8.b | Percentage of different patterns of Antimicrobial sensitivity test for *E. coli* | 32 |

|  |  |  |  |
| --- | --- | --- | --- |
| No. | CHAPTER | TITLE OF THE FIGURES | PAGE |
| 1  2 | Chapter : 2  Chapter : 2 | Diagrammatic figure of *Escherichia coli*  Diagram showing the difference between non-resistant bacteria and drug resistant bacteria. | 5  7 |
| 3 | Chapter : 2 | Molecular mechanisms of antibiotic resistance | 8 |
| 4 | Chapter : 2 | Mechanism of bacterial genetic transfer | 9 |
| 5 | Chapter : 3 | Study area map | 16 |
| 6 | Chapter : 3 | Preparation and inoculation of agar plate for isolation and identification of the isolates | 18 |
| 7 | Chapter : 3 | Colony features of E. coli on EMB and MacConkey agar | 19 |
| 8 | Chapter : 3 | Gram staining and features of isolated *E. coli*, under microscope | 19 |
| 9 | Chapter : 3 | Indole test for *E. coli* by using Kovac’s reagent | 20 |
| 10 | Chapter : 3 | Extracted DNA putting into PCR thermo cycler | 22 |
| 11 | Chapter : 3 | Preparation for visualization of PCR Product through gel electrophoresis technique | 24 |
| 12 | Chapter : 3 | Resistance pattern of *E. coli* isolates to tested antimicrobials. | 24 |
| 13 | Chapter : 4 | Results of PCR for 16s rRNA gene of*E. coli* ; Lane M: 100 bp ladder; Lane N: Negative control; Lane 1-9: 16S rRNA gene-sized (585bp) amplicon | 26 |
| 14 | Chapter : 4 | Resistance pattern of *E. coli* positive isolates against different antimicrobials | 31 |

**LIST OF FIGURE**

**LIST OF ABBREVIATION AND SYMBOL USED**

|  |  |
| --- | --- |
| Abbreviation and symbol | Elaboration |
| % | Percent |
| / | Per |
| +ve  -ve | Positive  Negative |
| ±  ˂  ˃  ≤  ≥  χ2  β  = | plus-minus  Less than  Greater than  Less than or equals to  Greater than or equals to  Chai square  Beta  Equals to |
| 0C | Degree Celsius |
| CDDEP | Center for Disease Dynamics, Economics & Policy |
| CLSI | Clinical and Laboratory Standards Institute |
| Cm | Centimeter |
| CS  CVASU | Culture Sensitivity  Chittagong Veterinary and Animal Sciences University |
| *E. coli* | *Escherichia coli* |
| EMB  ESBLs | Eosin Methylene Blue  Extended Spectrum β-lactamases |
| FAO | Food and Agriculture Organization |
| Hrs | Hours |
| Ltd. | Limited |
| µg | Microgram |
| mg | Milligram |
| ml | Milliliter |
| mm | Millimeter |
| NIAD | National Institute of Allergy and Infectious Diseases |
| NO. | Number |
| PRTC | Poultry Research and Training Center |
| SAQTVH  SL | Shahidul Alam Quadery Teaching Veterinary Hospital  Serial |
| WHO  WWTP | World Health Organization  Waste Water Treatment Plant |
| www | World Wide Web |

**ACKNOWLEDGEMENT**

The author tales the privilege to acknowledge to the almighty Allah, who has given the opportunity to accomplish of the report.

The author would like to express her deep sense of gratitude and thanks to Professor Dr. A. S. Mahfuzul Bari, Vice Chancellor of Chittagong Veterinary and Animal Sciences University (CVASU) for his courage to do this work.

The author would like to express her deep sense of gratitude and heartfelt appreciation to Professor Dr. Kabirul Islam Khan, Dean, Faculty of Veterinary Medicine, CVASU, for giving her a chance to accomplish this report.

The author expresses her sincere gratitude, heartfelt respect and immense indebtness to her supervisor Dr. Md. Mahbubur Rahman, Associate professor, Department of Pathology & Parasitology, CVASU, for his endless help and support to complete this report.

The author highly expresses her sincere gratitude and greatfulness to Dr. Bibek Chandra Sutradhar, Director, External affairs, CVASU, for his support and help.

The author highly gratitude to DR. MD. Shafiqul Islam, Lecturer, Department of Pathology and Parasitology, CVASU, for his cordial co-operation and help.

The author extends her gratefulness Dr. Inkeyas Uddin, SO, Poultry Research and Training Centre.

Finally the author expresses her good wishes and warmest sense of gratitude to all her well wishers, friends and families.

**The Author**

**March, 2014**

Page: I

**ABSTRACT**

Antimicrobial resistance has become an increasingly pressing problem in many countries in human and animal. The study was designed with the aim of isolation and identification of *Escherichia coli* and estimation of its antimicrobial resistance to ascertain the prevalence of *E. coli* in dog. A total of 24 *Escherichia coli* isolates recovered from 40 rectal swab samples of non diarrhoeic pet dogs in SAQTVH, Chittagong. Antimicrobial resistance was determined with 9 antimicrobial agents by means of disc diffusion assay. 100% resistance was observed in Ampicillin which was followed by Amoxicillin (95.83%), Colistin Sulfate (79.16%), Oxytetracycline (75%), Cotrimoxazole (75%), Ciprofloxacin (70.83%) and Ceftriaxone (62.5%). Conversely, 58.33% sensitivity was shown by Gentamicin and 91.66% intermediately sensitive as Doxycyclin. All *E. coli* isolates were reported as resistant to more than five antibiotics (multidrug-resistant). Therefore, more attention should be paid to the indiscriminate use of antimicrobials, and companion animals should be treated with sensitized drugs on the basis of result of the sensitivity study in the specific areas/regions.

**Key words:** *Escherichia coli*, rectal swab, non-diarrhoeic pet dogs, antimicrobial resistance.

Page: II