PREVALENCE AND PROFILING OF ANTIBIOTIC SENSITIVITY OF A KEY PUBLIC HEALTH PLAYER *ESCHERICHIA COLI* FROM COMPANION ANIMAL IN DHAKA CITY

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CERTIFICATE

This is to certify that the thesis entitled **"PREVALENCE AND PROFILING OF ANTIBIOTIC SENSITIVITY OF A KEY PUBLIC HEALTH PLAYER *ESCHERICHIA COLI* FROM COMPANION ANIMAL IN DHAKA CITY"** submitted to the Department of Medicine & Public Health, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTERS OF SCIENCE (M.S.)** in **MEDICINE**, embodies the result of a piece of bona fide research work carried out by **KAKOLEE MOHANTO**, Registration No. **14-06191** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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MY
BELOVED PARENTS AND TEACHERS

DECLARATION

I declare that the thesis hereby submitted by me for the MS degree at the Sher-e-Bangla Agricultural University is my own independent work and has not previously been submitted by me at another university/faculty for any degree.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
AMX	:	Amoxicillin
AZM	:	Azithromycin
BG	:	Brilliant Green
CIP	:	Ciprofloxacin
COT	:	Co-trimoxazole
CFM	:	Cefixime
CTR	:	Ceftriaxone
CXM	:	Cefuroxime
CLSI	:	Clinical and Laboratory Standards Institute
CFU	:	Colony Forming Unit
DLS	:	Department of Livestock Services
EMB	:	Eosin Methylene Blue
et al.	:	And others
e.g.	:	That is
E. *coli*	:	*Escherichia coli*
FAO	:	Food and Agricultural Organization
etc.	:	Etcetera
Fig.	:	Figure
G	:	Gentamicin
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen Sulphide
hrs.	:	Hours
HEQEP	:	Higher educational quality enhancement project
IN	:	Intermediate
Lbs.	:	Pound
Ltd.	:	Limited
M. S.	:	Master of Science

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
MC	:	MacConkey
MET	:	Metronidazole
Mg	:	Milligram
MH	:	Muller Hinton
Ml	:	Milliliter
Mm	:	Millimeter
Min.	:	Minute
MR	:	Methyl Red
NB	:	Nutrient Broth
No.	:	Number
PBS	:	Phosphate buffered solution
R	:	Resistant
S	:	Sensitive
S	:	Streptomycin
SAU	:	Sher-e-Bangla Agricultural University
Spp	:	Species
TSI	:	Triple Sugar Iron
USA	:	United states of America
µg	:	Microgram
µl	:	Microliter
VP	:	Voges-Proskauer
WHO	:	World Health Organization
yrs.	:	Years
C	:	Cat
D	:	Dog

LIST OF SYMBOLS

SYMBOLS		FULL MEANING
℃	:	Degree Celsius
&	:	And
+	:	Positive
-	:	Negative
%	:	Percentage
®	:	Registered trademark
<	:	Less than
>	:	Greater than
≥	:	Greater than equal
≤	:	Less than equal

PREVALENCE AND PROFILING OF ANTIBIOTIC SENSITIVITY OF A KEY PUBLIC HEALTH PLAYER *ESCHERICHIA COLI* FROM COMPANION ANIMAL

ABSTRACT

This study was conducted in order to isolate, identify and to profiling the status of antibiotic sensitivity of the important public health organism *Escherichia coli* of companion animal. A total of 30 fecal samples of cats and dogs were collected from two different regions of Dhaka city, aseptically and inoculated onto various culture media for isolation of *E. coli*. The microorganisms were isolated and identified by performing cultural characteristics and biochemical tests. The study had been conducted based on species, age, location and gender of host species. The prevalence of *E. coli* infection in cat was 66.67% and in dog was 60.00%, respectively with same ratio of resistance in male and female in dog whereas in cat female were more resistance than male. To perform the disc diffusion method for profiling the antibiotic sensitivity, 10 commonly used antibiotics were used. The highest rate of resistance was recorded in Metronidazole (57.89%) and Amoxicillin (52.63%) followed by Co-trimoxazole (41.10%), Ciprofloxacin (31.57%), Ceftriaxone (31.57%) and Azithromycin (26.31%). The highest rate of sensitivity was recorded to Cefixime (52.63%) followed by Ciprofloxacin (36.84%), Cefuroxime (36.84%) and Gentamicin (36.84%). Data from this study reveal that companion animal like cat and dog carry multi-drug resistant *E. coli* sp. which can be transferred to human and can cause a public health hazard.

CHAPTER I

INTRODUCTION

Faster pace of urbanization (up to 28%) since the emergence of Bangladesh, more than 130% increment of national per capita income since 1971 and Dhaka being the top urbanized city, pet rearing has been increased manifold. Therefore, socio-economic development paved to acquire the advantages over companionship, playing, policing, guarding children, house, and possessions; and economic purposes by rearing pets, in particular cats and dogs increasingly (Bhowmik P *et al.*, 2020). Much have been talked and reported on the multifarious benefits of human–companion animal interaction (Barker SB & Wolen AR, 2008). Dogs and cats are living in a proximity with human for many centuries. As a result of these contacts, the possibility of transmission of micro-organisms between these diverse host species is quite high (Beutin L, 1999).

Based on commensalism of bacterial communities and the evidence of resistance determinants between bacteria carried by humans and animals, a relationship is presumed to co-exist between antimicrobial resistance (AMR) in bacteria isolated from humans and animals along with the development of AMR in bacteria from humans and animals necessarily go side by side (Boure´ly C *et al.*, 2020). Antimicrobial agents are frequently used for the prevention of disease and as growth promoter in animal production along with their therapeutic use in veterinary and human medicine. Commensal bacteria in the GIT of food animals that are exposed to antimicrobial agents can inevitably select for antimicrobial resistance. The resistant bacterium is supposed to colonize the human GIT via contaminated foods of animal origin and can confer resistance to pathogens in the gut. The emergence of AMR among meat borne pathogens has stood a major issue in meat safety, and widespread antimicrobial use in production sector is considered an important driver to this problem (Aslam M and Service C, 2006). The implications of microbial resistance for public health have increased the interest of the scientific community in the presence and circulation of resistant organisms between pets and the human population. Among pets, dogs share proximity with humans, and proximity is known to increase the possibility of transmitting resistant bacteria between these host species. It is important to

note that the bacterial ability to transfer genetic cassettes, which confer resistance to several classes of drugs, and observations of the spread of resistance have increased in recent years (Carvalho A. C. *et al.*, 2021).

Animal and human beneficial microbiotas, especially intestinal microbiota, are frequently exposed to numerous antimicrobial pressures due to farming practices and veterinary and human medicines. They are playing a major role in disseminating bacterial resistance. Indeed, considerable number of bacterial species is present at high density in environment that allows exchange of gene between bacteria, and these bacteria can disseminate among ecosystems through the contact between humans and animals, the food chain, and the recycling of animal waste as fertilizer. A few studies have concomitantly documented the prevalence of multi-resistant bacteria and integrons. They showed that, although at a lower rate than in clinical isolates, both are clearly present, with an integron prevalence of about 15%. Several authors have documented a high prevalence of integrons in intensive reared farm animals, ranging from 23% to 44%, a level comparable to the prevalence found in human clinical isolates. Some studies have been devoted to compare the level of antimicrobial resistance in bacteria isolated from the faeces of wild animals with the level in animals in contact to humans. The prevalence of acquired antimicrobial resistance and characterization of the integrons in faecal *E. coli* isolated from several animal populations subject to various degrees of exposure to humans were reported (Skurnik D *et al.*, 2006).

The emergence and quick dissemination of AMR continue to be a public health concern in both medicine and agriculture. It is estimated that the global consumption of antimicrobial agents in animal food production will increase by 67% between 2010 and 2030. The tendencies of using raw food for pet animal is very common in various region for improvement of intestinal microbiomes and stool quality which is also a cause of anti-microbial resistance (Davis RH *et. al.,* 2019). Accordingly, this global rapid increase of AMR has been mostly attributed to the overuse, illegitimate use, and misuse of antibiotics in human and veterinary medicine. In the veterinary environment, antimicrobial use is discussed as one of the main drivers of development of antimicrobial

resistance. *Escherichia coli*, a Gram-negative bacterium that normally habits in the gastrointestinal tract of healthy humans and warm-blooded animals, are also an important opportunistic pathogen. This commensal microorganism is known to be an important indicator of the antibiotic resistance evolution as well as an eventual reservoir of virulence genes in different ecosystems. Urinary tract infections (UTIs) are one of the common health problems among companion animals in European countries. Gram-negative bacteria, mainly *E. coli* strains, are responsible for 75% of the cases. Treatment of UTIs caused by *E. coli* strains is becoming difficult due to the antibiotic resistance phenomenon (Lee DS. *et al.,* 2018). *E. coli* can act as a reservoir of antimicrobial-resistant genes that can be transmitted to other pathogenic bacteria. Multidrug-resistant (MDR) microorganisms are transmitted among pets, owners, and veterinary staff, which leads to their spread within the community (Carvalho I *et al.*, 2021; Harada K *et al.*, 2012; Shaheen BW *et al.*, 2013). *Escherichia coli* are characterized by a substantial genetic diversity, broad host range, versatility in pathogenic potential, and distribution between hosts in the environment. *E. coli* isolates, commensal or pathogenic, obtained from humans and other animals, have been extensively studied and characterized in terms of their drug resistance profiles based on their phenotypic sensitivity to various antimicrobial drugs (Carvalho A. C. *et al.*, 2016).

The emergence of antibiotic resistance has also been documented in *Escherichia coli* isolates from human, animal, and environmental sources. Multidrug resistant (MDR) *E. coli* is an emerging health concern. Again, several studies have demonstrated an increase in MDR *E. coli* associated with infections in dogs and cats throughout both the United States and Europe (EPSA J., 2019). Nosocomial infections associated with MDR *E. coli* have been reported in dogs in intensive care units. The extensive use of broad-spectrum antimicrobials is a likely contributing factor for MDR *E. coli*. In addition to the impact on animal health, emergent MDR *E. coli* might have important public health consequences if isolates are transmitted between humans and their pets (Gómez-Beltrán DA *et al.*, 2020; Shaheen B.W. *et al.*, 2010). The emergence of multidrug-resistant bacteria in companion animals is an increasing concern as it narrows the potential use of antimicrobials for the treatment of infections. Because antimicrobial resistance (AMR) is constantly evolving,

studies that monitor AMR regularly are important in order to guide therapeutic decisions and develop up-to-date control strategies. In general, variation in resistance complicates empirical selection of antimicrobial agents, and enhances the need for culturing and testing of susceptibility. In addition, although the organisms in pets that most commonly cause disease to tend to be different from humans, this is not always the case, and there is always the potential for the passage of drug-resistant genes and transmission between humans and pets. Most studies in different countries investigate trends and/or patterns in resistance by focusing on a specific pathogenic bacterium (i.e., *Escherichia coli*) or a specific organ/system i.e., urinary tract infections (Gómez-Beltrán DA *et al.*, 2020).

Escherichia coli is a bacterium with a special place in the microbiological world since it can cause severe infections in humans and animals but also represents a significant part of the autochthonous microbiota of the different hosts. Of major concern is a possible transmission of virulent and/or resistant *E. coli* between animals and humans through numerous pathways, such as direct contact, contact with animal excretions, or via the food chain. *E. coli* also represents a major reservoir of resistance genes that may be responsible for treatment failures in both human and veterinary medicine. An increasing number of resistance genes have been identified in *E. coli* isolates during the last decades, and many of these resistance genes were acquired by horizontal gene transfer. In the enterobacterial gene pool, *E. coli* acts as a donor and as a recipient of resistance genes and thereby can acquire resistance genes from other bacteria but can also pass on its resistance genes to other bacteria. In general, antimicrobial resistance in *E. coli* is considered one of the major challenges in both humans and animals at a worldwide scale and needs to be considered as a real public health concern (Poirel L *et al.*, 2018).

The global challenge for AMR is increasing rapidly and it is essential to study jointly for developing the control operations. Few research has been conducted for the AMR of *E. coli* in human health, but the frequency of AMR in case of companion animal is completely unaddressed in Bangladesh.

Considering those situations, present work is designed with the following objectives –

General objective:

To elucidate prevalence & AMR sensitivity of *E. coli* as a public health concern organism isolated from cats & dogs in Dhaka City.

Specific objectives:

1. To isolate and identify the *E. coli* bacteria from fecal samples of the companion animals.
2. To investigate the AMR sensitivity of isolated bacteria for effective drug choice.

CHAPTER II

REVIEW OF LITERATURE

Following literatures have been reviewed to perform isolation, identification, characterization, and determination of prevalence & drug sensitivity of most important bacteria public health, *E, coli*, from the fecal samples of companion animals of Dhaka City.

2.1 Companion Animal

An animal kept primarily for personal companionship or recreation is a companion animal or pet which provides the owner both physical and mental benefits (ASPCA, 2021). Pet ownership has also been investigated as physiologically beneficial to the owner in terms of cardiovascular ailments (Barker SB & Wolen AR, 2008). In industrialized or urbanized countries, dogs and cats are belongs to those domestic animals which have been living for thousands of years (Beutin L, 1998). People of the urbanized cities are very busy with their works and have a little time to spend with family. Dhaka is one of the most urbanized cities of Bangladesh where people are becoming interested to rear pet to avoid their loneliness (Bhowmik P. *et. al*., 2020).

2.2 Microorganisms and Companion Animal

Animal gut microorganisms including bacteria, fungi, protozoa, viruses, bacteriophages etc. exist in a symbiotic association with animal. The co-existence of various species in microorganism assists each other to maintain bodily function such as digestion, metabolism, developing immunity and protection against invaded pathogens but changes in microbial communities of GIT cause acute and chronic gastrointestinal diseases (Turner, 2018). *Escherichia coli* is a normal inhabitant microorganism of gut of human and animal which colonized from their first day of life, is also an opportunistic pathogen (Trevena *et. al.,* 1996). Beneficial microorganisms in animal and human, in particular intestinal microorganisms face huge antimicrobial pressures due to farming practices and veterinary and human medicines. They have been found to play a pivotal role in descending bacterial resistance. Indeed, an enormous number of bacterial species are

present at high density in an environment allowing gene exchange among bacteria, and these bacteria can disseminate among ecosystems via the contact between humans and animals, the food chain and the recycling of animal waste as fertilizer. A few studies have concomitantly documented the prevalence of multi-resistant bacteria and integrons, highly efficient molecular tools used by the bacteria for antimicrobial resistance acquisition and expression, in human commensal microbiota. The prevalence of acquired antimicrobial resistance and characterization of the integrons in fecal *Escherichia coli* isolated from several animal populations subject to various degrees of exposure to humans have been demonstrated (Skurnik D *et al.*, 2006).

2.3 Bacterial Diseases

According to Suchodolski JS (2010) there have been more than 10 complex bacterial phyla in the gut of animal which may cause detrimental effects on host health including canine and feline inflammatory bowel diseases. Bacterial infections including *Pasteurella, Salmonella, Brucella, Yersinia enterocolitica, Campylobacter, Capnocytophaga, Bordetella bronchiseptica, Coxiella burnetii, Leptospira, Staphylococcus intermedius* and Methicillin resistance *staphylococcus aureus* are the most common in case of dog and cat. Among all the bacterial infections about 60% are related to food poisoning (Buma R, 2006). It has been reported that antibiotic-resistant *Escherichia coli* and *Salmonella* infecting humans have originated from cattle. Although cattle have been implicated as a source of antimicrobial resistant bacteria and resistant bacteria in retail meats have often been documented, a critical link between antimicrobial-resistant bacteria in animals and those on meats has yet to be established. Definitive information concerning the prevalence of resistant bacteria on meat at various steps of beef processing and packaging is unfortunately sparse. Without such information, the efforts to promote prudent and judicious use of antimicrobial agents may lack legitimacy. Furthermore, this information can be helpful in assessing the relative risks associated with excessive use of antibiotics in animal production systems and will facilitate identification of emerging trends in antimicrobial-resistant bacteria arising from the beef production, slaughter, and processing environments (Aslam M and Service C, 2006).

2.4 Antibiotic Resistance

Bacteria, viruses, protozoa, and other microorganisms are always changing to ensure their survival by developing the resistant power against commonly used drugs on them. Antibiotic resistance is a subset of AMR refers specifically resistance to antibiotics that occurs in common bacterial infections. One species acquiring from another by genetic mutation and extended use of antimicrobials is encouraging the natural selection for mutation (Dabour *et. al.*, 2016). The emergence and spread of antimicrobial resistance (AMR) genes in bacterial pathogens have been identified as one of the major threats to human health by the World Health Organization (WHO Global Reports, 2014). Antimicrobial agents are routinely used for disease prevention and growth promotion in animal production in addition to their therapeutic use in veterinary and human medicine. Commensal bacteria in the intestinal tracts of food animals that are exposed to antimicrobial agents can inevitably select for antimicrobial resistance. The resistant bacteria may colonize the human intestinal tract via contaminated foods of animal origin and can confer resistance to pathogens in the gut. The emergence of antimicrobial resistance among meat borne pathogens has become a major issue in meat safety, and widespread antimicrobial use by the beef production sector is considered an important contributor to this problem (Aslam M and Service C, 2006). Based on commensalism of bacterial communities and based on the evidence of resistance determinants between bacteria carried by humans and animals, a relationship is presumed to co-exist between antimicrobial resistance (AMR) in bacteria isolated from humans and animals along with the development of AMR in bacteria from humans and animals necessarily go hand in hand (Boure´ly C *et al.*, 2020).

2.5 *E. Coli* and Antibiotic Resistance

Escherichia coli are the bacterium with a substantial genetic diversity, broad host range, versatility in pathogenic potential, and distribution between hosts in the environment can be a potential threat of infection for human population. A study in Northern Colorado revealed that 185 AMR genes in *E. coli* isolates and confirmed the presence of diverse

AMR gene classes (e.g., beta-lactams and efflux pumps) in isolate genomes (Hannah B *et. al*, 2019).

The emergence and fast dissemination of antimicrobial-resistant bacteria (AMR) continue to be a public health concern in both medicine and agriculture. It was estimated that the global consumption of antimicrobial agents in animal food production will increase by 67% between 2010 and 2030. Accordingly, this global rapid increase of AMR has been mostly attributed to the overuse and misuse of antibiotics in human and Veterinary medicine. In the Veterinary environment, antimicrobial use is discussed as one of the main drivers of development of antimicrobial resistance. *Escherichia coli*, a Gram-negative bacterium that normally habits in the gastrointestinal tract of healthy humans and warm-blooded animals, are also an important opportunistic pathogen. This commensal microorganism is known to be an important indicator of the antibiotic resistance evolution as well as an eventual reservoir of virulence genes in different ecosystems. Urinary tract infections (UTIs) are one of the common health problems among companion animals in European countries. Gram-negative bacteria, mainly *E. coli* strains, are responsible for about 75% of the cases. Treatment of UTIs caused by *E. coli* strains is becoming difficult due to the antibiotic resistance phenomenon. *E. coli* can act as a reservoir of antimicrobial-resistant genes that can be transmitted to other pathogenic bacteria. Multidrug-resistant (MDR) microorganisms are transmitted among pets, owners and Veterinary staff, which lead to their spread within the community (Carvalho A C *et al.*, 2016).

2.6 Antibiotic Resistance and One Health

The overuse of antibiotics in animal health sectors results in antimicrobial resistance (AMR) and causes public health issues through the contact of host species. The organisms in pets that most commonly cause disease to tend to be different from humans but there is always the potential for the passage of drug-resistant genes and transmission between humans and pets. In One-Health perspective surveillance plays an important role of antimicrobial resistance. Monitoring systems is only existed in human and livestock sectors but no monitoring program for companion animal and there is an urgent need to initiate AMR monitoring program nationally and globally (Anjum MF *et al.*, 2021).

Escherichia coli is a bacterium with a special place in the microbiological world since it can cause severe infections in humans and animals but also represents a significant part of the autochthonous microbiota of the different hosts. Of major concern is a possible transmission of virulent and/or resistant *E. coli* between animals and humans through numerous pathways, such as direct contact, contact with animal excretions, or via the food chain. *E. coli* also represents a major reservoir of resistance genes that may be responsible for treatment failures in both human and veterinary medicine. An increasing number of resistance genes have been identified in *E. coli* isolates during the last decades, and many of these resistance genes were acquired by horizontal gene transfer. In the enterobacterial gene pool, *E. coli* acts as a donor and as a recipient of resistance genes and thereby can acquire resistance genes from other bacteria but can also pass on its resistance genes to other bacteria. In general, antimicrobial resistance in *E. coli* is considered one of the major challenges in both humans and animals at a worldwide scale and needs to be considered as a real public health concern (Poirel L *et al.*, 2018).

2.7 Isolation, identification, characterization, and determination of drug sensitivity

Isolation of organisms identified from culture of fecal samples & for further culture and to elucidate prevalence, biochemical properties have widely been used (Gómez-Beltrán DA *et al.*, 2020; Poirel L *et al.*, 2018; Carvalho A. C. *et al.*, 2016; Shaheen B.W. *et al.*, 2010) and thus addressed in order to enumerate the prevalence of *E. coli*, the most important public health related microorganism. In a study of Hata A *et al.,* 2022 in animal shelters in Chiba and Kanagawa prefectures, in the Kanto Region, Japan, to assure the AMR status of *Escherichia coli* prevalent in shelter dogs and the detection in the fecal isolates was 61 and 71 with AMR resistance percentage 16.4% and 26.0% of samples from Chiba and Kanagawa exhibited resistance to at least one antibiotic, respectively.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Samples

Samples were obtained from companion animal of two locations of Dhaka city.

Table 01. Fecal samples of cats and dogs with sample number

SL and Name	Species	Sample Source	Gender	Age
C1	Cat	Agargaon	Male	9 months
C2	Cat	Agargaon	Male	1.2 Years
C3	Cat	Agargaon	Female	1.1 Years
C4	Cat	Agargaon	Male	6 Months
C5	Cat	Agargaon	Female	1.5 Years
C6	Cat	Agargaon	Female	1.3 Years
C7	Cat	Agargaon	Male	8 Months
C8	Cat	Mohakhali	Male	7 Months
C9	Cat	Mohakhali	Male	8 Months
C10	Cat	Mohakhali	Male	2.5 Years
C11	Cat	Mohakhali	Female	2 Years
C12	Cat	Mohakhali	Male	1.9 years
C13	Cat	Mohakhali	Female	9 Months
C14	Cat	Mohakhali	Female	11 Months
C15	Cat	Mohakhali	Male	1.2 Year
D1	Dog	Agargaon	Male	1.3 Years
D2	Dog	Agargaon	Male	1.5 Years
D3	Dog	Agargaon	Male	2.2 Years
D4	Dog	Agargaon	Female	2.6 Years
D5	Dog	Agargaon	Female	1.9 Years

Continued Table 01.

D6	Dog	Agargaon	Female	2.1 Years
D7	Dog	Agargaon	Male	2.5 Years
D8	Dog	Agargaon	Male	7 Months
D9	Dog	Mohakhali	Male	5 Months
D0	Dog	Mohakhali	Female	1.5 Year
D11	Dog	Mohakhali	Female	2.7 Years
D12	Dog	Mohakhali	Male	2.1 Years
D13	Dog	Mohakhali	Male	2.4 Years
D14	Dog	Mohakhali	Male	1.5 Years
D15	Dog	Mohakhali	Male	6 Months

3.1.2 Bacteriological Media

3.1.2.1 Agar media

Agar media used for bacteriological analysis were Eosin Methylene Blue (EMB) agar, MacConkey (MC) agar, Brilliant Green (BG) agar, Nutrient agar, and Mueller Hinton (MH) agar.

3.1.2.2 Liquid media (broth)

To pursue this study the liquid media were Nutrient broth, Peptone broth, Methyl-Red and Voges-Proskauer broth (MR-VP broth) and Sugar media (dextrose, maltose, lactose, sucrose, and mannitol).

3.1.2.3 Phosphate Buffered Saline (PBS)

For preparation of phosphate buffered saline, 8 gm. of sodium chloride (NaCl), 2.89 gm. of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), 0.2 gm. of potassium chloride (KCl) and 0.2 gm. of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely, and pH meter was used to adjust the pH. The solution was sterilized by autoclaving and stored at 4℃ for future use.

3.1.3 Chemicals and reagents

The chemicals and reagents used for this study were 0.1% Peptone water, Phosphate buffered saline (PBS), reagents for Gram's staining (Crystal Violate, Gram's iodine, Safranin, Acetone alcohol), 3% Hydrogen peroxide, Phenol red, Methyl red, 10% Potassium hydroxide, Kovac'sindole reagent (4-dimethylamino-benzaldehyde, concentrated HCl), Mineral oil, Normal saline and other common laboratory chemicals and reagents.

3.1.4 Glass wares and other appliances

The following glass wares and appliances were used during the experiment: Test tubes (with or without Durham's fermentation tube and stopper), petridishes, conical flask, pipette (1 ml, 2 ml, 5 ml, 10 ml) & micro-pipettes (1ml, 200µl, 100µl, 10 µl) slides and cover slips, hanging drop slides, immersion oil, compound microscope, bacteriological loop, sterilized cotton, cotton plug, test tube stand, water bath, bacteriological incubator, refrigerator, sterilizing instruments, thermometer, ice carrier, hand gloves, spirit lamp, match lighter, laminar air flow, hot air oven, syringe, needle, tray, forceps, scalpel, scissors etc.

3.1.5 Antimicrobial discs

To interpret the disease potentiality, commercially available antimicrobial discs (OXOID Limited, Canada) were used for the test to determine the drug sensitivity and resistance pattern. This method allowed for the rapid detection of the efficacy of drugs against the test organisms by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the discs inhibiting the growth of the organisms. The following antimicrobial agents with their disc concentration were used to test the sensitivity and resistance pattern of the selected *E. coli* isolated from the fecal samples of dogs and cats.

Table 02. Drugs with their disc concentration

(Source: CLSI= The Clinical and Laboratory Standards Institute, 2020)

Antibiotics	Disc concentration (µg/disc)	Zone Diameter Interpretive Standard (mm)		
		Resistance (%)	Intermediate (%)	Sensitive (%)
AMX	30	≤13	14-17	≥18
AZM	15	≤13	14-17	≥18
CIP	30	≤11	12-14	≥15
COT	25	≤10	11-15	≥16
CTR	30	≤19	20-22	≥23
CFM	5	≤14	15-17	≥18
CXM	30	≤14	15-17	≥18
GEN	10	≤12	13-14	≥15
MET	15	≤14	15-17	≥18
S	10	≤11	12-14	≥15

Here, AMX = Amoxicillin, AZM = Azithromycin, CIP = Ciprofloxacin, COT = Co-Trimoxazole, CTR = Ceftriaxone, CFM = Cefixime, CXM = Cefuroxime, GEN = Gentamicin, MET = Metronidazole S = Streptomycin

3.2. Methods

3.2.1 Experimental Design

The study was conducted by two major steps. The first step included selection of sources, collection of samples, isolation, identification, and characterization of microorganisms based on their colony morphology, staining properties, motility, and biochemical characteristics & molecular identification. In the second step, the status of drug sensitivity and resistance pattern of microorganism isolated from fecal samples were determined.

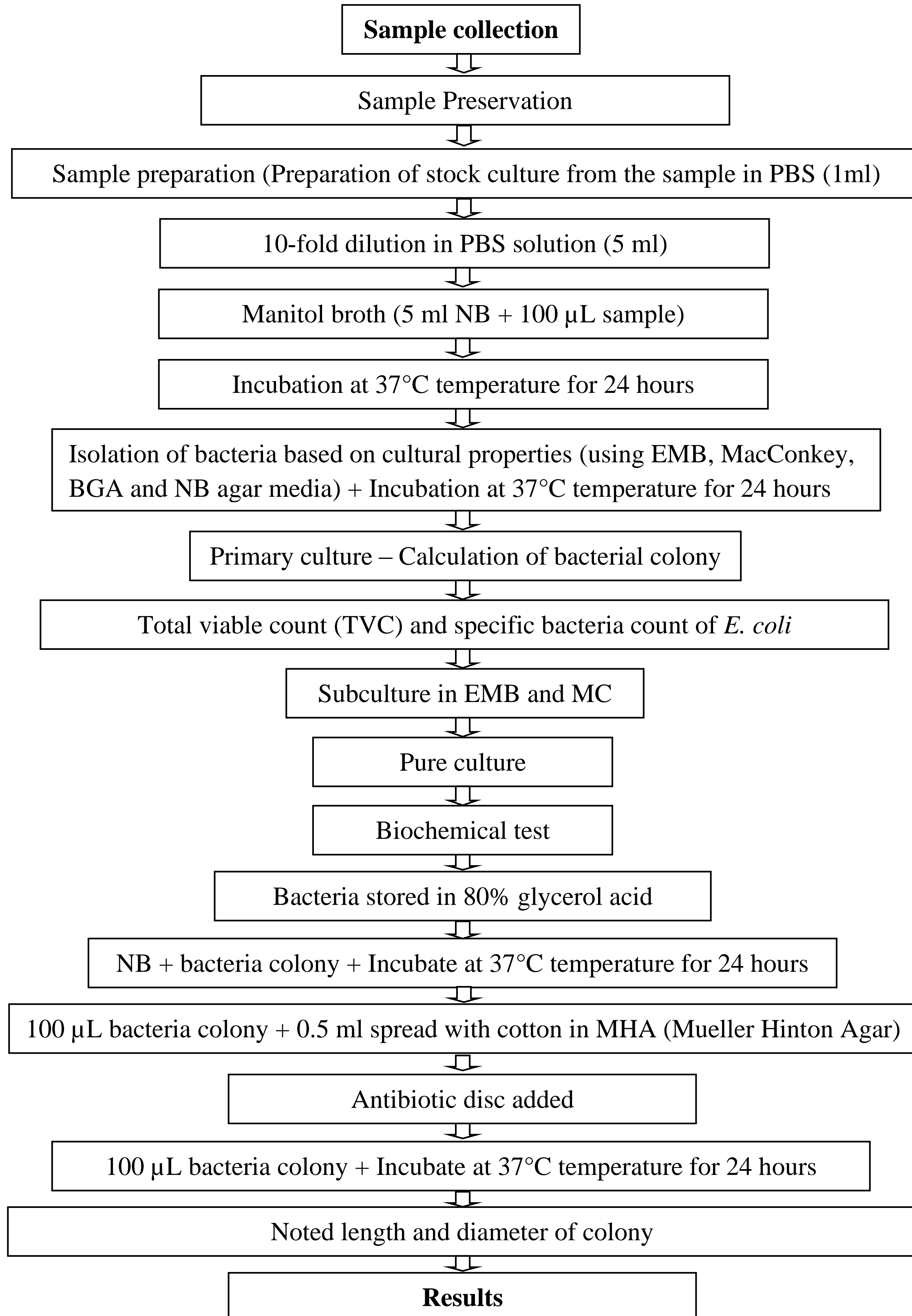

Figure 01. Experimental design of the study

3.2.2.1 Nutrient Broth

Nutrient Broth was prepared by Suspended 25 grams in 1000 ml purified/distilled water. Heated to dissolve the medium completely. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 30 minutes. The broth was filled in test tubes & incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator for future use.

3.2.2.2 Nutrient Agar

Nutrient agar was prepared by dissolving 28 grams of dehydrated nutrient agar (Hi Media, India) in to 1000 ml of distilled water and was sterilized by autoclaving at 121ºC under 15 lbs. pressure per square inch for 15 minutes. Then the agar was dispensed into Petridis (90 mm and 100 mm) and was incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.2.2.3 MacConkey's agar

A quantity of 49.53 grams of Bacto MacConkey agar (Hi Media, India) was suspended in to 1000 ml of cold distilled water and was heated for boiling to dissolve the medium completely. It was then poured in to sterile petridishes and allowed to solidify. After solidification of the medium in the plates, the plates were then incubated at 37ºC for overnight to check their sterility.

3.2.2.4 Eosin Methylene Blue (EMB) agar

36 grams powder of EMB agar base (HI Media, India) was suspended in 1000 ml of distilled water. The suspension was heated to boil few minutes to dissolve the powder completely with water. The medium was autoclaved for 30 minutes to make it sterile. After autoclaving the medium was put into water bath at 45ºC to cool down its temperature at 40ºC. From water bath 10-20 ml of medium was poured in to small and medium sized sterile petridishes to make EMB agar plates. After solidification of the medium in the plates, the plates were incubated at 37ºC for overnight to check sterility.

3.2.2.5 Brilliant Green (BG) agar

According to the direction of manufacturer (HI Media, India) 58 grams of dehydrated medium was suspended in 1000 ml distilled water and heated for boiling to dissolve the medium completely. The medium was sterilized by autoclaving. After autoclaving the medium was put into water bath of 45ºC to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.2.2.6 Mueller Hinton Agar

A quantity of 38 grams in 1000 ml distilled water and heated to dissolve the medium completely. After the sterilization by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Cooling was done from 45-50°C. Then it was mixed well and poured into sterile Petridishes. After solidification of the medium in the petridishes were allowed for incubation at 37ºC for overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.2.2.7 Triple Sugar Iron (TSI) agar

A quantity of 65 grams of dehydrated medium (Difco, USA) was mixed with 1000 ml cold distilled water in a flask and heated for boiling to dissolve the medium completely. The solution was distributed in tubes which were plagued with cotton. The tubes were then sterilized by autoclaving and slanted in such a manner as to allow a generous butt. After solidification tubes were incubated at 37ºC for overnight to check sterility. The sugar solutions were sterilized in Arnold's steam sterilizer at 100ºC for 30 minutes for three consecutive days. An amount of 0.5 ml of sterile sugar solution was added aseptically in each culture tubes containing sterile peptone water. The sugar solutions were incubated at 37ºC for 24 hours to check sterility. These solutions were used for biochemical test.

3.2.2.8 Methyl Red and Voges–Proskauer (MR-VP) broth

A quantity of 3.4 gm. of MR-VP medium (HI Media, India) was dissolved in 250 ml of distilled water, distributed in 2 ml quantities in test tube and then autoclaved. After

autoclaving, the tubes containing medium were incubated at 37ºC for overnight to check their sterility and then stored at 4ºC for future use.

3.2.3 Isolation of bacteria

3.2.3.1 Collection, transportation, and preparation of sample

A number of 30 fecal samples were collected by using sterile swab stick in 2ml Eppendorf tube filled with PBS from Vet and Pet Care clinic, Sher-E-Bangla Agricultural University Campus, Mirpur, Mohakhali DOHS, Dhaka. The collected samples were carried to university laboratory immediately in a cool box with ice and processed for isolation and characterization of bacteria for studying AMR sensitivity.

3.2.3.2 Serial dilution for bacterial culture (10-fold dilution method)

To lower the bacterial count for total viable count (TVC) and total coliform count (TCC) 10folds serial dilution was needed. A total 8(1-8) of Eppendorf tubes were taken with 900µl of PBS and 100µl stock samples were transferred to the tubes from stock sample tube. Then 100µl of diluted sample is transferred from the first Eppendorf tube to the next. Successive dilution should be made in the same way till the last tube and from the last tube 100µl of diluted sample should be discarded. From the last tube 25µl of liquid sample should be transferred to the nutrient Agar media and MacConkey agar to elucidate the total viable count & total coliform count.

3.2.3.3 Primary culture of microorganism

Primary growth of all kinds of bacteria present in the collected samples was performed in nutrient broth. The samples were inoculated in nutrient broth and incubated for overnight at 37ºC for the growth of the organisms.

3.2.3.4 Isolation in culture media

When primary cultures of the organism were done, a small number of inoculums from Nutrient Broths were streaked on MacConkey Agar and Brilliant Green Agar to notice the colony morphology of the isolates. Characteristic colony morphology of the organisms indicating E. coli was selected for subculture on selective media such as EMB

agar. Morphological characteristics (shape, size, surface texture, edge and elevation, color, opacity etc.) of the suspected colonies on different agar media developed within 18 to 24 hours of incubation were carefully recorded.

3.2.3.5 Motility test

According to the method described by Cowan, 1985 the motility test was performed to differentiate the motile bacteria from the non-motile one. Prior to the test, a pure culture of the test organism was allowed to grow in nutrient broth. One drop of cultured broth was placed on the cover slip and was placed inverted condition over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover slip to prevent air current and evaporation of the fluid. The hanging drop slide was then examined carefully under 100X power objective of a compound microscope using immersion oil. By observing motility in contrasting with to and from movement of bacteria, the motile and non-motile bacteria were identified.

3.2.4 Identification of isolated *E. coli* using specific biochemical tests

Several biochemical tests were performed for confirmation of *E. coli* bacteria from the collected samples.

3.2.4.1 Carbohydrate fermentation test

The carbohydrate fermentation test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing different sugar media (five basic sugars such as dextrose, maltose, lactose, sucrose, and mannitol) and incubated for 24 hours at 37ºC. Acid production was indicated by the color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 2006).

3.2.4.2 Catalase test

For this test 3 ml of catalase reagent (3% H_2O_2) was taken in a test tube. Single colony from the pure culture of *E. coli* was taken with a glass rod and merged in the reagent. The

tube was observed for bubble formation. All the isolates were catalase positive; formation of bubble within few seconds was the indication of the positive test, while the absence of bubble formation indicated negative result (Cheesbrough, 2006).

3.2.4.3 Methyl Red test

The test was conducted by inoculating single colony from the pure culture of the test organism in 5 ml sterile MR-VP broth. After 5 days incubation at 37ºC, 5 drops of methyl red solution was added and observed for color formation. Development of red color was positive and indicated an acid pH of 4.5-6 resulting from the fermentation of glucose. Development of yellow color indicated negative result (Cheesbrough, 2006).

3.2.4.4 Voges-Proskauer (V-P) test

The test *E. coli* organisms were grown in 3 ml of sterile MR-VP broth at 37ºC for 48 hours. Then 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture of the test organism. After shaking well and allowed to stand for 5-10 minutes to observe the color formation. Positive case was indicated by the development of a bright orange red color. In negative cases there was no development of pink color (Cheesbrough, 2006).

3.2.4.5 Indole test

The test organisms were cultured in test tubes having 3 ml of peptone water containing tryptophan at 37ºC for 48 hours. Then 1 ml of diethyl ether was added. Shaked well and allowed to stand until the ether rises to the top. 0.5 ml of Kovac's reagent was gently run down the side of the test tube so that it forms a ring in between the medium and the ether layer and observed for the development of color of the ring. Development of a brilliant red colored ring indicated indole production. In negative case there is no development of red color (Cheesbrough, 2006).

3.2.5 Maintenance of stock culture

By adding 1ml of 80% sterilized glycerol in 1 ml of pure culture in nutrient broth, stock culture was prepared, and it was stored in -200°C.

3.2.6 Antimicrobial sensitivity pattern of *E. coli*

A total of 19 *E. coli* isolates collected from 15 fecal samples of cat and 15 fecal samples of dogs were used for disc sensitivity testing. The antimicrobial sensitivity testing of each isolate was carried out by the Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) procedures. Antibiotic sensitivity discs used were amoxicillin (AMX), azithromycin (AZM), ciprofloxacin (CIP), co-trimoxazole (COT), ceftriaxone (CTR), cefixime (CFM), cefuroxime (CXM), gentamicin (GEN), metronidazole (MET), streptomycin (S). This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent in the medium surrounding the disc. The suspension of the test organism was prepared in a test tube containing 5 ml nutrient broth by overnight incubation in shaking incubator. By micropipette 100μl of broth culture of the test organism was poured on Muller-Hinton agar plate. Sterile glass spreader was used to spread the culture homogenously on the medium. Inoculated plates were closed and allowed to dry for approximately 3-5 minutes. Then the antibiotic discs were applied aseptically to the surface of the inoculated agar plates at a special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37ºC for 24 hours. After incubation the plates were examined and the diameter of the zone of complete inhibition was measured by mm scale. The zone diameters for individual antimicrobial agents were translated in to sensitive, intermediate, and resistant categories by referring to an interpretation table.

3.2.7 Antibiotic Sensitivity analysis

The antibiotic sensitivity of the isolated strain at different concentration was performed by using standard paper disc diffusion method described by NCCLS (2009). Antibiotics selected for susceptibility testing included a panel of antimicrobial agents of interest to the veterinary and human public health authorities. From the range of antimicrobial drugs, 10 were selected based on the range of activity against enterobacteria and on their use in small animal medicine.

Human antibiotics were selected based on their use and /or importance in human medicine.

The following antibiotics and disc potencies were used:

1. AMX: Amoxicillin
2. AZM: Azithromycin
3. CIP: Ciprofloxacin
4. COT: Co-trimoxazole
5. CTR: Ceftriaxone
6. CFM: Cefixime
7. CXM: Cefuroxime
8. GEN: Gentamicin
9. MET: Metronidazole
10. S: Streptomycin

The antibiotic susceptibility tests were performed in Mueller-Hinton agar by micro-disc diffusion techniques. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2ml of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 minutes and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB agar to obtain uniform inoculum.

The plates were then allowed to dry for 3-5 minutes. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from

center to center were ensured. Five discs were placed in each petridish. Within 15 minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16-18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate, and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA. Measurement of the growth inhibition zone permitted the classification of each isolate as susceptible, intermediate, and resistant according to data provided by CLCI. The result of antibiotic sensitivity test was then recorded, analyzed, and discussed.

CHAPTER IV

RESULTS AND DISCUSSION

The results presented below demonstrated the isolation and identification of bacteria isolates from the fecal samples of cats and dogs of Dhaka city. Of the 30 samples, 15 were collected from dog and 15 were from cat from two separates areas.

4.1 Total viable count and coliform count from the isolated samples

Table 03. Total viable and coliform count from the fecal samples

SL & Name of the Sample	Total Viable Count (TVC)(CFU/ml)	Total Coliform Count (TCC)(CFU/ml)
C1	1.8×10^{5}	Nil
C2	2.3×10^{5}	3.2×10^{5}
C3	1.6×10^{5}	2.1×10^{5}
C4	1.1×10	Nil
C5	2.8×10^{5}	$.8\times10^{5}$
C6	3.2×10^{7}	1.7×10^{9}
C7	2.7×10^{9}	4.1×10^{9}
C8	3.6×10^{9}	Nil
C9	3.4×10^{9}	Nil
C10	3.0×10^{5}	3.5×10^{6}
C11	1.3×10^{5}	6.8×10^{9}
C12	1.4×10^{5}	1.75×10^{9}
C13	1.4×10^{7}	Nil
C14	3.7×10^{7}	1.22×10^{9}
C15	2.5×10^{7}	3.1×10^{7}
D1	3.6×10^{5}	3.9×10^{6}
D2	2.0×10^{7}	2.9×10^{6}
D3	2.2×10^{9}	3.3×10^{7}
D4	2.5×10^{7}	3.1×10^{6}

Continued Table 03.

D5	2.8×10^5	3.6×10^7
D6	2.6×10^7	2.96×10^9
D7	3.1×10^7	5.7×10^7
D8	3.9×10^5	Nil
D9	1.4×10^5	Nil
D10	2.9×10^9	Nil
D11	3.8×10^9	1.75×10^9
D12	4.7×10^5	Nil
D13	1.1×10^9	4.1×10^9
D14	1.8×10^5	Nil
D15	2.7×10^7	Nil

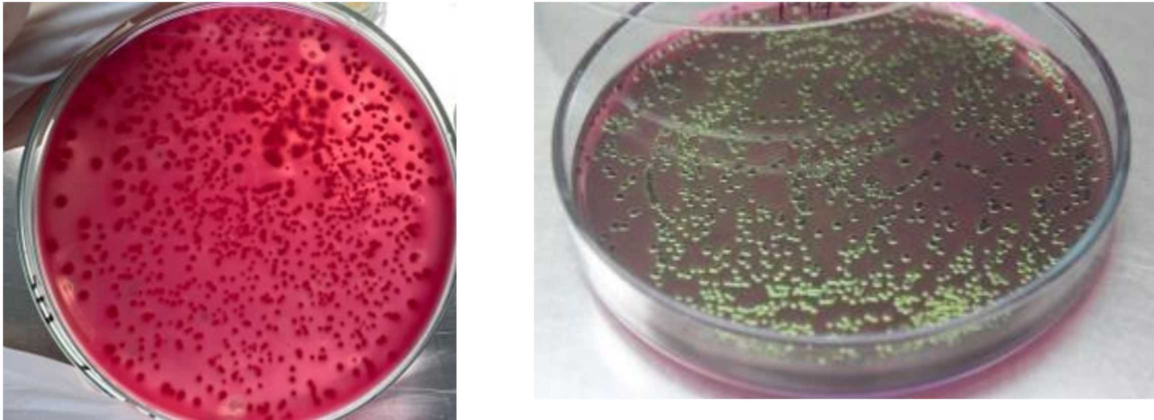

Figure 2: Total coliform count by 10-folds methods

4.2 Prevalence of *E. coli* in fecal samples

Table 04. Prevalence of *E. coli*

Number of collected samples	***E. coli* containing samples**	**Percentage (%) of prevalence**
30	19	63.34%

Table 05. Prevalence of *E. coli* in according to host species

E. coli containing total samples	*E. coli* in cat samples	*E. coli* in dog samples	Percentage (%) of prevalence in cat	Percentage (%) of Prevalence in dog
19	10	9	66.67	60.00

Table 06. Prevalence of *E. coli* according to age

Age	Total samples	*E. coli* containing sample	Percentage (%) of prevalence
Less than one year	10	2	20
More than one year	20	17	85

4.3 Isolation and identification of *E. coli*

E. coli is identified and isolated through cultural examination performed in SAU Medicine and Public Health Laboratory.

4.3.1 Cultural examination

E. coli is cultured in Nutrient broth and then sub-cultured in EMB agar media, MacConkey (MC) agar media and BG agar media for isolation of pure bacteria.

4.3.1.1 Culture in nutrient broth

All the *E. coli* isolates produced turbidity in nutrient broth.

4.3.1.2 Culture on Eosine Methylene Blue (EMB) agar

Greenish colonies with metallic sheen produced by the organisms on EMB agar after overnight incubation were tentatively confirmed as *E. coli.* (Table 7, Fig. 4).

4.3.1.3 Culture on MacConkey (MC) agar

Bright, pink-colored colonies on MacConkey agar produced by the organisms after overnight incubation were presumptively selected as *E. coli* (Table 7, Fig. 3).

4.3.1.4 Culture on Brilliant green (BG) agar

Yellowish green colonies surrounded by an intense yellow green zone on BG agar produced by the organisms after overnight incubation were tentatively chosen as *E. coli* (Table 7, Fig. 5).

Table 07. Demonstration of the cultural characteristics of *E. coli* isolated from fecal samples in different agar media

Sources of *E. coli*	Colony characteristics in different agar media		
	MC agar	EMB agar	BG agar
Samples from cat	Bright, pink-colored colonies	Greenish colonies with metallic sheen	Yellowish green colored colonies
Samples from dog	Bright, pink-colored colonies	Greenish colonies with metallic sheen	Yellowish green colored colonies

Figure 3: *E. coli* in MC agar

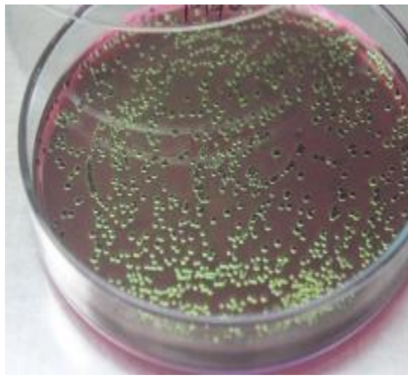

Figure 4: *E. coli* in EMB agar

Figure 5: *E. coli* in BG media

4.3.1.5 Culture on nutrient agar

Negative reaction was found by the organisms on nutrient agar after overnight incubation was tentatively confirmed as *E. coli*.

4.3.1.6 Sugar fermentation test

All the *E. coli* isolates fermented five basic sugars with the production of acid and gas. Decreased production of acid and gas was observed during sucrose fermentation.

Table 08. Demonstration of the biochemical reactivity pattern of *E. coli* isolated from fecal samples

Sources of *E. coli*	Fermentation properties with five basic sugars					M R test	V-P Test	Catalase test
	DX	ML	L	S	MN			
Cat fecal sample	AG	AG	AG	A↓G↓	AG	+	-	+
Dog fecal sample	AG	AG	AG	A↓G↓	AG	+	-	+

Legends: DX = Dextrose; ML = Maltose; L = Lactose; S= Sucrose; MN=Mannitol.
A = Acid production; G = Gas production; A↓ = Less acid production; G↓ = Less Gas production; + = Positive reaction; - = Negative reaction.

Acid production was indicated by the color change of the sugar media from reddish to yellow and the gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Table 8, Figure. 6).

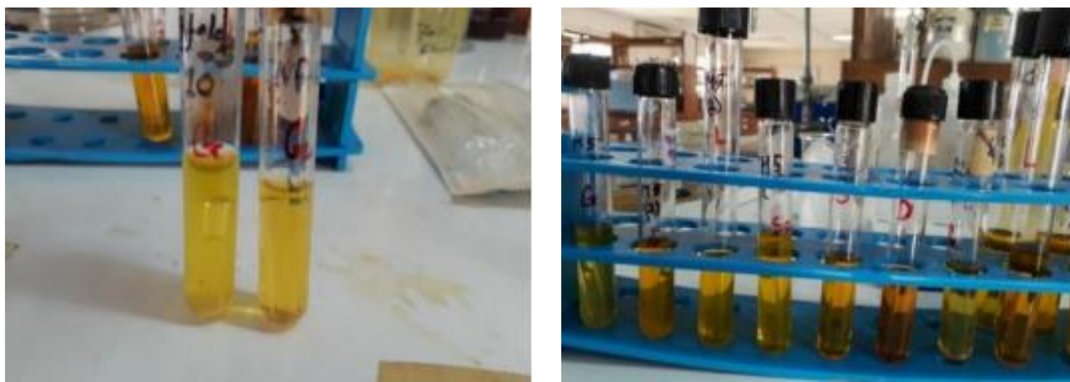

Figure 6: Production of acid and gas in sugar fermentation test for *E. coli*

4.4 Drug sensitivity and resistance pattern of *E. coli*

A total 19 of *E. coli* isolates have been collected from total 30 samples of cat and dog. After performing the sensitivity test by disc diffusion method, we had found a sensitivity pattern against different antibiotics.

Table 09. Demonstration of the sensitivity and resistance pattern of different *E. coli* isolates to different drugs in percentage

Samples	Antibiotic Disc										% Of resistance to different drugs		
	AMX	AZM	CIP	COT	CTR	CFM	CXM	GEN	MET	S	R	I	S
C2	R	I	S	S	R	S	I	S	R	I	30	30	40
C3	R	R	S	R	R	S	I	R	I	R	60	20	20
C5	I	R	S	R	I	S	S	I	R	S	30	30	40
C6	R	I	S	I	R	I	R	R	R	I	50	40	10
C7	S	S	I	I	S	I	I	S	R	I	10	40	50
C10	R	I	R	I	I	R	S	S	R	R	50	30	20
C11	I	S	I	I	R	I	S	S	I	I	10	60	30
C12	I	I	R	R	R	S	I	I	I	R	40	50	10
C14	R	R	I	I	S	S	R	I	S	S	30	30	40
C15	R	I	I	I	S	S	S	I	S	S	10	40	50
D1	R	R	S	S	S	R	I	S	I	I	30	30	40
D2	S	I	S	I	I	I	R	I	R	R	30	50	20
D3	R	I	R	R	I	I	S	R	R	S	50	30	20
D4	I	S	S	R	I	S	R	R	R	I	40	30	30
D5	R	S	I	R	R	R	I	I	R	R	60	30	10
D6	I	R	R	I	I	I	S	S	I	S	20	50	30
D7	S	S	I	S	I	S	S	I	I	I	0	50	50
D11	S	I	R	R	I	S	I	R	R	S	40	30	30
D13	R	I	R	R	S	S	R	S	R	S	50	10	40

Here, R= Resistance; I= Intermediate; S= Sensitive; AMX = Amoxicillin, AZM = Azithromycin, CIP = Ciprofloxacin, COT = Co-Trimoxazole, CTR = Ceftriaxone, CFM = Cefixime, CXM = Cefuroxime, GEN = Gentamicin, MET = Metronidazole S = Streptomycin

Table 10. Resistant pattern of *E. coli* in different samples

Antibiotics	Resistant		Intermediate		Sensitive	
	Cat	Dog	Cat	Dog	Cat	Dog
AMX	6	4	3	2	1	3
AZM	3	2	5	4	2	3
CIP	2	4	4	2	4	3
COT	3	5	6	2	1	2
CTR	5	1	2	6	1	2
CFM	3	1	3	3	5	5
CXM	2	3	2	3	4	3
GEN	2	3	4	3	4	3
MET	5	6	3	3	2	0
S	3	2	4	3	3	4

Here, R= Resistance; I= Intermediate; S= Sensitive; AMX = Amoxicillin, AZM = Azithromycin, CIP = Ciprofloxacin, COT = Co-Trimoxazole, CTR = Ceftriaxone, CFM = Cefixime, CXM = Cefuroxime, GEN = Gentamicin, MET = Metronidazole S = Streptomycin

Table 11. Prevalence of Antimicrobial resistance pattern against *E. coli* isolates

Antibiotics	Percentage (%) of resistance to different drugs		
	Resistance (R)	Intermediate (I)	Sensitive (S)
AMX	52.63	26.31	21.05
AZM	26.31	47.36	21.05
CIP	31.57	31.57	36.84
COT	41.10	42.10	15.78
CTR	31.57	42.10	26.31
CFM	15.78	31.57	52.63
CXM	26.31	36.84	36.84
GEN	26.31	36.84	36.84
MET	57.89	31.57	10.52
S	26.31	36.84	36.84

Legend, AMX = Amoxicillin, AZM = Azithromycin, CIP = Ciprofloxacin, COT = Co-Trimoxazole, CTR = Ceftriaxone, CFM = Cefixime, CXM = Cefuroxime, GEN = Gentamicin, MET = Metronidazole S = Streptomycin

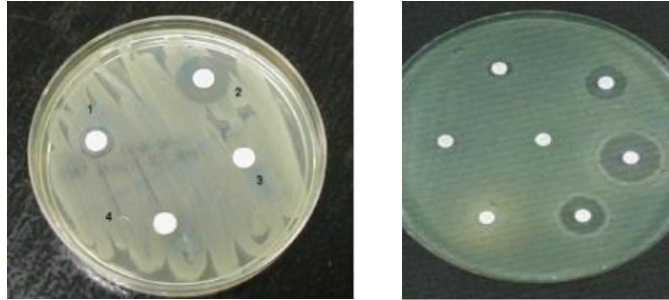

Figure 7: Antibiotic sensitivity test for *E. coli*

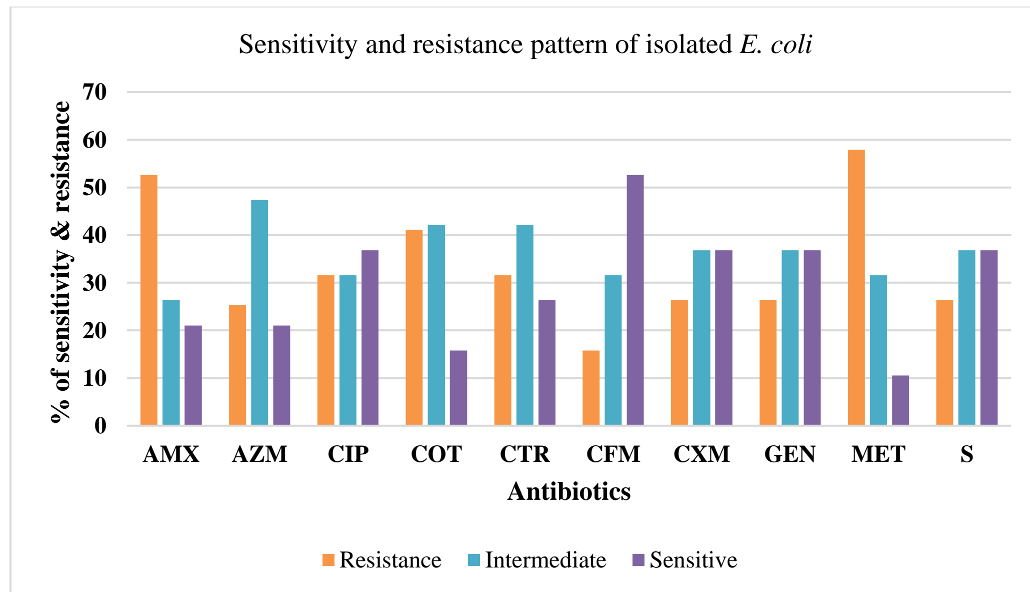

Figure 8: Prevalence of Antimicrobial resistance pattern against *E. coli* isolates

A total 19 number of *E. coli* isolates from fecal samples were found sensitive to MET (57.89%), AMX (52.6%), COT (41.10%) and CTR (31.57) (Table 11 and Figure 8). A few numbers were sensitive to CFM (52.63%) and CIP (36.84%), CXM and GEN (36.84%). The highest resistance of *E. coli* was against MET (57.89%). The lowest resistance of *E. coli* was against CFM (15.78%) & S (26.31%). Comparatively lower

resistance was showed by them against AZM, CIP, CFM, GEN, and S were 26.31%, 31.57, 15.78%, 26.31% and 26.31%, respectively. Most of the isolates showed intermediate sensitivity against CXM, GEN, S (36.84%). Against COT and CTR they have shown the equal intermediate sensitivity (42.10%) and CIP, MET, and CFM (31.57%) also showed equal intermediate sensitivity which was lowest (Table 11 and Figure 8).

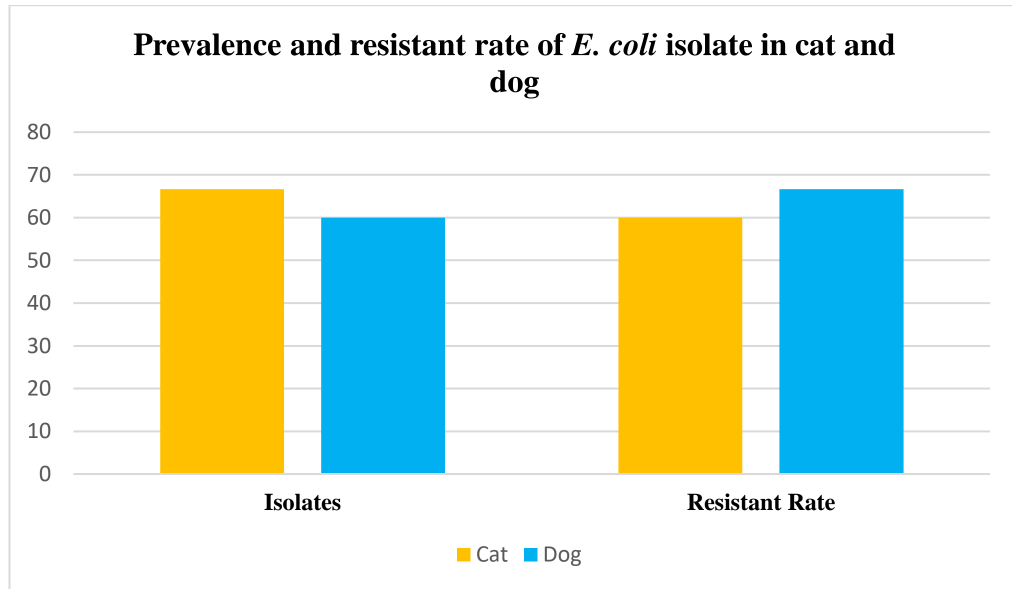

Figure 9: Prevalence and resistant rate of cat and dog

From total 15 fecal samples of cat, it had been found that 10 samples contained *E. coli* sp. whereas in dog 9 samples containing the organism. The resistant pattern of the organisms in cat and dog were nearly similar. The percentage of resistance in dog was 66.67 and the percentage of resistance in cat was sixty (Table 5, Figure 9). The percentages of total isolates in cat and dog were 66.67 and 60 respectively.

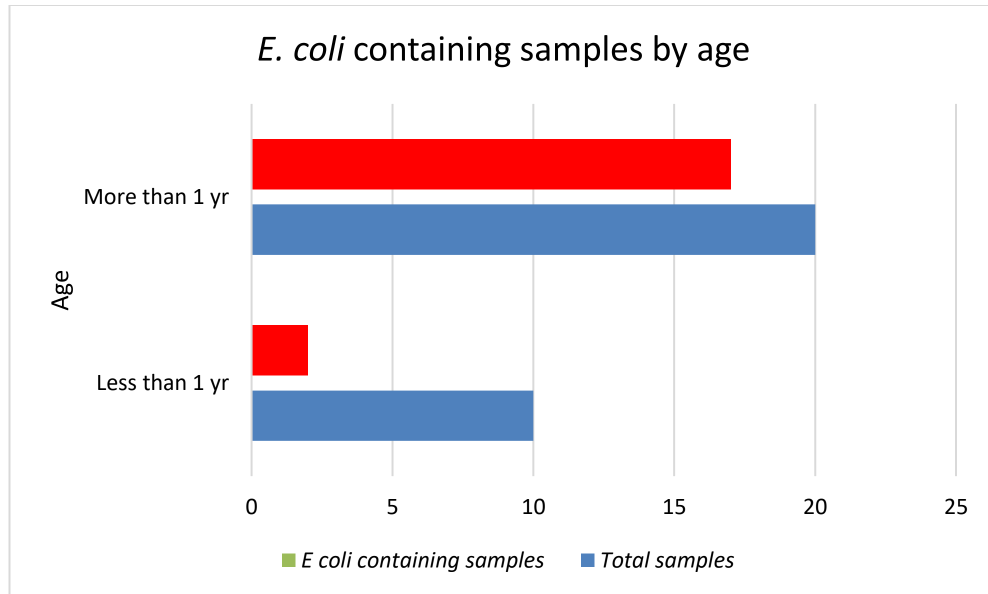

Figure 10: Prevalence of *E. coli* according to ages

Collected samples were also studied according to ages. It had been revealed in the study that the samples collected from the dogs and cats less than one year of age contained a few numbers of *E. coli* spp with the percentage of 20. On the other hand, samples collected from cats and dogs more than one year of ages containing many microorganisms and the percentage is about 90.

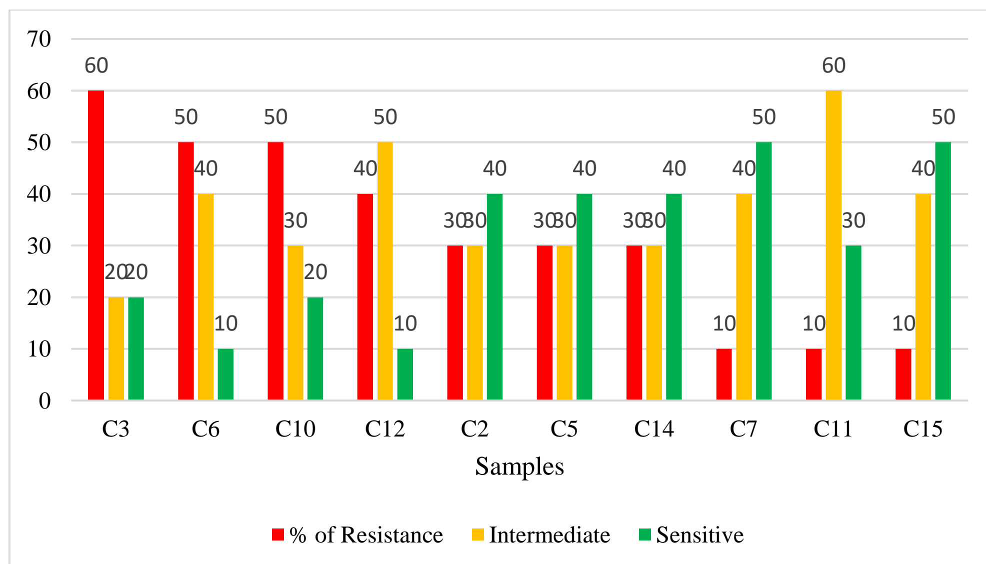

Figure 11: Resistance pattern of isolates from cat

Among the fecal samples collected from cat, highly resistance sample was collected from Agargaon which was male with more than one year old (Table 9, Figure 11). The lowest resistance of antibiotics had been found in the samples of Mohakhali with higher sensitivity of drug in comparison to the samples from Agargaon.

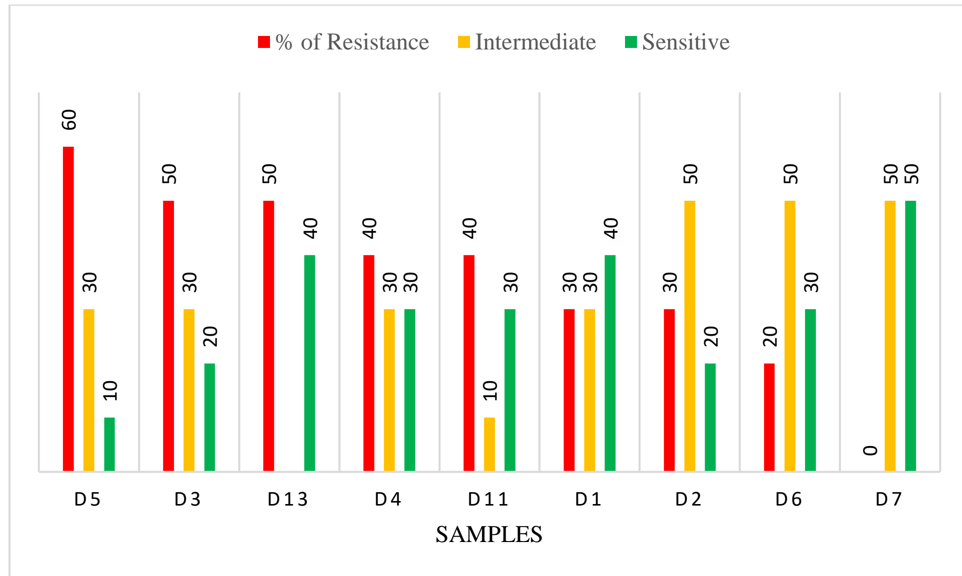

Figure 12: Resistance pattern of isolates in dog

In case of dogs, the scenario was different from cat. There had been highest and lowest resistance pattern in the same region with highest amount of sensitivity of antibiotics. The highest frequency of antibiotic resistance was 60% with 30 % intermediate and only 10% sensitivity. The sample was from Agargaon region. In the same region the sensitivity of drug was highest in different samples with some intermediate and low resistance pattern (Figure 11). According to the study, there were lower isolates in Mohakhali region than Agargaon, but the isolates of *E. coli* found there were quite resistance to different drugs (Table 9, Figure 12). The sensitivity of the isolates to different antibiotics were higher at Mohakhali region than Agargaon. Among seven samples of dog from Mohakhali only two samples contained *E. coli* isolates whereas seven samples from Agargaon contained isolates among eight (Table 1 and 3).

4.5 Discussion

The present study was conducted primarily for the isolation and identification of one of the public health concern bacteria, *E. coli* spp., isolated from fecal samples of companion animal in two locations of Dhaka city and to determine the status of drug sensitivity and resistance pattern of the isolates to determine the drug of choice for therapeutic use against infection caused by *E. coli.*

Colony characteristics of *E. coli* in five different agar media and fermentation ability with five basic sugars were similar with a bit exception. Interesting findings of the colony characteristics of the isolates were also observed. All the *E. coli* isolates were able to produce characteristic greenish metallic sheen colony on the EMB agar, bright pink colony on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on BG agar.

Another fundamental basis for the identification of *E. coli* organism was determining the ability or inability of fermentation of five basic sugars with acid and gas production. All the *E. coli* isolates revealed a complete fermentation of five basic sugars as stated by different scientists (Mckec *et al*., 1995; Shandhu *et al.,* 1996 and Beutin *et al*., 1997).

The isolated *E. coli* organisms fermented dextrose, maltose, lactose, sucrose, and mannitol with the production of both acid and gas. Less production of acid and gas during sucrose fermentation was found by *E. coli* organisms. Ewing and associates (1973) and Ali and associates (1998) also studied the biochemical characteristics of *E. coli* isolated from different sources. They reported a little or no difference in these biochemical characters.

In this study, prevalence of microorganism in all the samples was 100%. Among 30 samples (cat = 15 and dog = 15) all were infected with different microorganisms. Among 30 samples, 19 samples were infected with *E. coli*, 11 samples were unidentified. The prevalence of infection with *E. coli* was (63.34%). Prevalence of infection with *E. coli* for cat was 66.67% and prevalence in dog was 60%, respectively. This result was similar with the finding of Shaheen BW and associates (2010) who reported the prevalence was 51% in canine and feline.

Most of the *E. coli* isolates from fecal samples were found sensitive to MET (57.89%), AMX (52.6%), COT (41.10%) and CTR (31.57%). A few numbers were sensitive to CFM (52.63%) and CIP (36.84%), CXM and GEN (36.84%). The highest resistance of *E. coli* was against MET (57.89%). A similar result was reported by Muloi D and associates (2019) in human and livestock with 21.1% and 47.7% resistance. Intermediate Sensitivity drugs could not be compared due to lack of relevant literature.

To perform this study, samples were drawn from companion animal whereas it had studied the species, age, location, and gender. There were two species and two locations with several ages of animal. From this study the prevalence of *E. coli* isolates in fecal samples of cat was higher than the fecal samples of dogs, but in the scenario of antibiotics resistance it was revealed that the isolates collected from dogs had higher resistance percentage than cat (Figure 8). In the study of Boothe D and associates (2012), in cats and dogs' urinary isolates were most common with greatest percentage of resistance in the isolates from the respiratory tract, urine, and skin compared with the ear. When the data of ages revealed from the study, it was clear that the isolates collected from more than one year ages animal fecal samples were more susceptible for *E. coli* infection than the younger with age of under one year old. The percentage of susceptibility to *E. coli* infection in younger animal was only 20.00% and the percentage in more than one year old was 85.00% from the collected samples in present study.

In the isolates of cat, there was a higher prevalence of multiple drugs resistant *E. Coli* than expected prevalence. Most of the isolates were resistance to AMX, CTR and MET with highest sensitivity to CFM where a study of Saputra S and associates (2017) revealed that the resistance rate of third generation cephalosporin was 11.7% in cat in Australia. According to other study (Moyaert H. *et. al.,* 2005), most of the isolates from cat were resistance to gentamicin a commonly used human antibiotic when in this study we had seen the resistance rate of gentamicin was a bit lower than other antibiotics.

In the same study of Saputra S and associates (2017), they published the resistance rate of cephalosporin to the isolates of dog was 18.1% whereas we found the highest resistance drug for the isolates collected from dogs MET and COT in this study. The highest sensitive drugs for the isolates of *E. coli* from the fecal sample of dog was CFM and

second highest sensitive one was S with the resistance rate of 26.31%. In a similar study (Costa D. *et. al.,* 2008), streptomycin resistance rate was 15% in dog. The resistance rate of AMX in the isolates from dog was 44.45% where a study of KuKanich K and associates (2020) showed that the isolates from dog had a high susceptibility to AMX with a percentage of 92%.

The significance of occurrence of antibiotic resistant pathogens have been increased sharply and probably linked with the extensive use of antimicrobial agents in Veterinary medicine and human (Bronzwaer *et al*., 2002). Indiscriminate use of antimicrobial agents should be avoided to eliminate health hazards in human and animals caused by *E. coli* spp to prevent the development of multi-drug resistant microorganism in nature. Human and companion animal have a close bond that creates way to exchange of microorganisms, including multidrug resistance and for the approach of "One Health" program it is necessary of more representative surveillance efforts and infection control strategies with specific of animal-species (Walther B. *et. al*., 2016). The world has changed, with the rising population of India and China with the percentage of 33% world's population and there is a big challenge of antimicrobial resistance (Livemore DM, 2012).

The aim of this study was to investigate the prevalence of acquired antimicrobial resistance of companion animal and to identify the effective antibiotics for infectious diseases. We detected the multidrug resistant *E. coli* in the fecal samples of cats and dogs. The microorganisms of create diseases in animal and human are not different. So, there could be simultaneous presence of antibiotics resistance commensal in both companion animal and their owners. There may be a possibility that the companion animal and their owner sharing multiple drug resistance bacteria. The data of this study provide a guideline prevalence of multidrug resistance *E. coli* presence in companion animal in different location of Dhaka city. The contributing information can be useful in understanding of epidemiology of antimicrobial resistance in companion animal and the impact of public health hazard can be studied.

CHAPTER V

SUMMARY AND CONCLUSION

The present study was conducted for isolation and identification of *E. coli* spp. from cats and dogs in two different locations of Dhaka city and to perform a comparative study to determine the sensitivity and resistance pattern of the isolates to different antibiotics along with the identification of drug choice. As a most important and readily available bacteria, *E. coli* was pin pointed due to its public health concern.

After collection, the samples were subjected to various tests and experiments for isolation and identification of *E. coli* spp. in companion animal. Primary isolation was performed by propagating the organisms in nutrient broth followed by culture on different agar media such as MacConkey agar, EMB agar, and BG agar for the determination of their colony characteristics. A total of 30 fecal samples were collected from the cats and dogs of two different location of Dhaka city. Total Viable Count (TVC) and Total Coliform Count (TCC) were done by 10-fold dilution method. 19 isolates were found as *E. coli spp.* and the rest 11 couldn't be identified in this study. They were identified based on colony morphology. Gram's staining technique was also performed to determine the size, shape, and arrangement of bacteria. Biochemical properties of the isolates were studied by fermentation test with five basic sugars and by Catalase test, MR test, V-P test, and Indole production test.

The study was conducted according to species, sex, age, and area basis where the most prevalence of *E. coli* was found between 1 to 3 years of age and more in Agargaon region than Mohakhali area.

The study was also extended to investigate in vitro sensitivity and resistance pattern of the *E. coli* spp. isolates to different drugs. Study revealed that there were considerable variations among the isolates of different sources in respect of drug sensitivity and resistance pattern.

It was found that isolated *E. coli* spp. were good sensitivity against CFM, CIP, & S followed by CTR, CXM, and COT while highest resistance was found to MET & AMX.

It can be assumed that one or more drug resistant clones have gradually acquired resistance to other drugs by conjugation with multi-drug resistant strains.

From this study it may be concluded that

(a) Fecal samples collected from the companion animal of Dhaka city are infected with multidrug resistant *E. coli* spp.

(b) *E. coli* infections of different animals and birds and of human being may be treated effectively with CFM, CIP, & GEN.

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APPENDIX

Appendix I. Composition of different media

1. Nutrient broth

Peptic digest of animal tissue	:	5.0 gm.
Sodium chloride	:	5.0 gm.
Beef extract	:	1.5 gm.
Yeast extracts	:	1.5 gm.
Distilled water	:	1000 ml
Final pH (at 25ºC)	:	7.4 ± 0.2

2. Nutrient Agar

Peptone	:	5.000 gm.
Sodium chloride	:	5.000 gm.
HM peptone B#	:	1.500 gm.
Yeast extracts	:	1.500 gm.
Agar	:	15.000 gm.
Final pH (at 25°C)	:	7.4±0.2

3. MacConkey Agar

Peptones (meat and casein)	:	3.000 gm.
Pancreatic digest of gelatin	:	17.000 gm.
Lactose monohydrate	:	10.000 gm.
Bile salts	:	1.500 gm.
Sodium chloride	:	5.000 gm.
Crystal violet	:	0.001 gm.
Neutral red	:	0.030 gm.
Agar	:	13.500 gm.
pH after sterilization (at 25°C)	:	7.1±0.2

4. Eosin Methylene Blue Agar

Peptic digest of animal tissue	:	10.000 gm

Dipotassium phosphate	:	2.000 gm.
Lactose	:	5.000 gm.
Sucrose	:	5.000 gm.
Eosin - Y	:	0.400 gm.
Methylene blue	:	0.065 gm.
Agar	:	13.500 gm.
Final pH (at 25°C)	:	7.2±0.2

5. Brilliant Green Agar Medium

Peptone	:	5.000 gm.
Tryptone	:	5.000 gm.
Yeast extracts	:	3.000 gm.
Lactose	:	10.000 gm.
Sucrose	:	10.000 gm.
Sodium chloride	:	5.000 gm.
Phenol red	:	0.080 gm.
Brilliant green	:	0.0125 gm.
Agar	:	20.000 gm.
pH after sterilization (at 25°C)	:	6.9±0.2 gm.

6. Salmonella-Shigella agar

Protease peptone	:	5.000 gm.
Lactose	:	10.000 gm.
Bile salts mixture	:	8.500 gm.
Sodium citrate	:	8.500 gm.
Sodium thiosulphate	:	8.500 gm.
Ferric citrate	:	1.000 gm.
Brilliant green	:	0.00033 gm.
Neutral red	:	0.025 gm.
Agar	:	13.500 gm.
Final pH (at 25°C)	:	7.0±0.2

7. Mueller Hinton Agar

HM infusion B from	:	300.000
Acicase	:	17.500
Starch	:	1.500
Agar	:	17.000
Final pH (at 25°C)	:	7.4±0.1

8. Methyl Red Indicator

Methyl red	:	0.200 gm.
Ethyl alcohol	:	60.000 ml
Distilled water	:	40.000 ml

9. Voges–Proskauer (MR-VP) broth

Buffered peptone	:	7.000
Dextrose	:	5.000
Dipotassium phosphate	:	5.000
Final pH (at 25°C)	:	6.9±0.2

10. Phosphate buffer saline

Sodium chloride	:	8.0 gm
Disodium hydrogen phosphate	:	2.8 gm
Potassium chloride	:	0.2 gm
Potassium hydrogen phosphate	:	0.2 gm
Distilled water to make	:	1000 ml