PREVALENCE AND ANTIBIOTIC SENSITIVITY PATTERNS OF *SALMONELLA* SPP. IN HOUSEHOLD CATS IN DIFFERENT AREA OF DHAKA CITY

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CERTIFICATE

This is to certify that the thesis entitled **"PREVALENCE AND ANTIBIOTIC SENSITIVITY PATTERNS OF SALMONELLA SPP. IN HOUSEHOLD CATS IN DIFFERENT AREA OF 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 bonafide research work carried out by **MD. AKIB ZABED**, Registration No. **14-05879** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

December, 2021

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DEDICATED TO
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.

Date: 21.07.2021   MD. AKIB ZABED

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
AMC	:	Amoxicillin + Clavulanic acid
AMX	:	Amoxicillin
AZM	:	Azithromycin
℃	:	Degree celsius
CC	:	Cubic centimetre
CFM	:	Cefuroxime
CFU	:	Colony Forming Unit
CIP	:	Ciprofloxacin
COT	:	Co-trimoxazole
CLSI	:	Clinical and Laboratory Standards Institute
CTR	:	Ceftriaxone
CXM	:	Cefixime
DLS	:	DepartmentofLivestockServices
EMB	:	Eosin Methylene Blue
et al.	:	And others
e.g.	:	That is
FAO	:	FoodandAgriculturalOrganization
etc.	:	Etcetra
Fig.	:	Figure
GEN	:	Gentamicin
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen Sulphide
hrs.	:	Hours
IN	:	Intermediate
Lbs.	:	Pound
Ltd.	:	Limited
M. S.	:	Master of Science

LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION		FULL MEANING
MC	:	MacConkey
Mg	:	Milligram
MH	:	Muller Hinton
Ml	:	Millilitre
Mm	:	Milimeter
Min.	:	Minute
MR	:	Methyl Red
NB	:	NuttrientBroth
No.	:	Number
PBS	:	Phosphate buffered solution
R	:	Resistant
S	:	Sensitive
S	:	Streptomycine
SAU	:	Sher-e-Bangla Agricultural University
Spp	:	Species
SS	:	Salmonella Shigella
USA	:	United states of America
μg	:	Microgram
μl	:	Microlitre
VNPC	:	Vet and Pet Care
VP	:	Voges-Proskauer
WHO	:	WorldHealtthOrganization
yrs.	:	Years

ABSTRACT

This study was conducted to isolate, identify and antibiotic profiling of *Salmonella* spp. harbors in cat, which are raised as household pets at Dhanmondi, Mohammadpur, Mirpur and Agargaon in Dhaka city. A total of 40 fecal samples were collected from Vet and Pet Care Clinics to carry out this study. All samples were collected aseptically and inoculated onto various culture media for isolation of bacteria. Identification of bacteria from positive samples were performed by cultural characteristics, Gram's staining and biochemical tests. The overall prevalence of *Salmonella* spp. in fecal samples in cats were found 53%. The study was conducted according to breed, sex, age and area basis where the most prevalence of *Salmonella* were found between 1 to 3 years of age (75%). Agargaon and Mohammadpur areas were subjected to most *Salmonella* infected area (60%). Antibiotic sensitivity profiling of the isolated *Salmonella* spp. was performed by the disc diffusion method against 10 randomly used antibiotics. The highest rate of sensitivity against *Salmonella* was found with Ceftriaxone (77%) and Ciprofloxacin (72%) followed by Cefuroxime (67%), Amoxicillin + Clavulanic Acid (67%), Cotrimoxazole (53%), Cefixime (52%) and Gentamycin (48%). The highest rate of resistance was recorded to Amoxicillin (57%) and Cefuroxime (43%). Among the areas, the most sensitive of *Salmonella* was found in Dhanmondi (60%) and Mirpur (56%). The drug resistance of *Salmonella* spp. among Mohammadpur (28%), Agargaon (25%), Mirpur (20%) and Dhanmondi (17%) area were observed in this study. Data from this study suggest that domestic cats carry multi-drug resistant against *Salmonella* spp. which can be transferred to humans through direct contact or the food chain and can cause a potential public health hazard.

CHAPTER I

INTRODUCTION

Salmonellosis is a worldwide spread infection, it causes considerable economic losses and it is of public health significance (Hoelzer *et. al.* 2011). *Salmonella* is Gram-negative, aerobic or facultative anaerobic bacilli of the family Enterobacteriaceae and has a diverse animal reservoir, including mammals, birds, reptiles, amphibians, and invertebrates (Greene and Saunders, 1998). The organism inhabits the intestinal tract of vertebrate and invertebrate animals worldwide and its excretion results in the contamination of food, water and the environment (Turnbull, 1979).

In humans, infections caused by *Salmonella* spp. are associated with severe food-borne illnesses, especially in the case of acute gastroenteritis, which is caused by consuming contaminated water and food products (Gargano *et. al.* 2021 and Li *et. al.* 2021). *Salmonella* species mostly inhibits the intestinal tract of vertebrate and invertebrate, consequently, they are excreted in feces resulting in contamination of food, water and environment (Hale *et. al.* 2012). Modes of infection of Salmonellosis include fecal-oral transmission, ingestion of contaminated feed, ocular transmission, and possibly aerosolization and transplacental transfer (Greene and Saunders, 1998; Pelzer, 1989; Fox and Beucage, 1979). *Salmonella typhimurium* is most commonly isolated from domestic cats; however, naturally acquired infection is uncommon in this species (Timoney *et. al.* 1978). Clinical syndromes described for cats with salmonellosis include gastroenteritis, bacteremia/endotoxemia, localized infection of extra intestinal organs, conjunctivitis, abortion, and subclinical infection or an asymptomatic carrier state (Greene and Saunders, 1998; Timoney *et. al.* 1978 and Barrs *et. al.* 1999). Despite the disappearance of clinical signs, the infected cats remain carriers for long period; particularly those recovering from acute infection continue to shed *salmonellae* in their feces for 12 weeks (Wall *et. al.* 1995). Moreover, excretion of the organisms may be increased with stress factors and prolonged use of antibiotics (Shane *et. al.* 2003 and Mather *et. al.* 2013). Cats can carry *Salmonella* organisms asymptomatically and the clinical form is uncommon (Callaway *et. al.* 2008).

Pet animals make up an important reservoir of zoonotic diseases as they share the same environment as humans (Kornblatt and Schantz, 1980). Household pets have

been found to play a direct role in transmitting zoonosis (Dada *et. al.* 1979; Kornblatt and Schantz, 1980). Pets are often regarded as members of the family unit and close contact with them increases the likelihood of disease transmission. In addition, there has been a population explosion among pet animals, especially dogs and cats, that has necessitated an increase in the number of shelters and pounds to care for stray pets placed there for adoption. In these facilities, animals may become carriers of *Salmonella* through contact with infected animals (Fox and Beucage, 1979).

The increased popularity of high-protein raw meat-based diets (RMBDs) demonstrates the growing preference for higher protein quantity and quotient in pets diets. Uncooked animal products, such as skeletal muscle, fat, internal organs, cartilage, bones (from ruminants, pigs, poultry, horses, game, or fish), unpasteurized milk, and uncooked eggs are included in these diets (Fredriksson *et. al.* 2017). Feeding raw foods may increase the risk of exposure to potentially pathogenic bacteria for companion animals and human subjects. During infection, pathogenic bacteria compete with and displace the commensal species, resulting in the dysbiosis of the microbial population and gastrointestinal upset (Kerr *et. al.* 2014).

Antimicrobial-resistant *Salmonella* and other zoonotic pathogens originating from companion animals have great public health importance. Antimicrobial resistance is one of the main causes of failure in antimicrobial therapy. This mechanism of survival presented by the microorganisms occurs naturally or can be acquired. However, acquired resistance is more important due to the fact that it limits viable options of drugs. This form is originated from mutation or gene transference, which may be chromosomic or extra-chromosomic (Spinosa *et. al.* 2006). The control of salmonellosis mainly relies on the use of antimicrobial drugs. Recently, the prevalence of antimicrobial resistance has been increasing in major bacterial pathogens (Parry and Threlfall, 2008). Bacteria have developed strategies for survival within the host during an infection and one of these strategies is the resistance of isolates to the antimicrobial drugs. Antimicrobial resistance is a serious problem because it limits the therapeutic possibilities in the treatment of bacterial diseases in domestic animal species in general (Williams and Heymann, 1998).

Because food animals are thought to be the primary source of resistant strains of non-typhoidal *Salmonella*, the majority of studies on antimicrobial resistance in

Salmonella have focused on food animals and farm environments. Several studies, however, have reported antimicrobial-resistant *Salmonella* isolates, including multidrug-resistant (MDR) strains from cats and other companion animals in various parts of the world (Guardabassi *et. al.* 2004; Lloyd, D. H. 2007 and Umber, J. K. & Bender, J. B. 2009).

Salmonella causes an estimated 93.8 million human cases of gastroenteritis and 155,000 deaths worldwide each year, with approximately 80 million cases being food-borne. It is estimated that approximately 9% are caused by direct contact with animals (Hedberg, 1999 and Majowicz *et. al*. 2010). In Bangladesh, the prevalence of infectious diseases and the conditions of domestic animals and birds have already been studied in various regions (Tarafder and Samad, 2010 and Mahmud *et. al.* 2014). However, very few studies on the prevalence of clinical diseases and conditions in pets have been published (Parvez *et. al.* 2014).

The objectives of the investigation:

1. To isolate and identify *Salmonella* spp. harbors in household cats around Dhaka city.
2. To investigate the antibiotic sensitivity pattern of *Salmonella* spp.

CHAPTER II

REVIEW OF LITERATURE

Salmonella has long been recognized as a significant zoonotic pathogen with global economic implications (Humphrey, 2000). Severe gastroenteritis with vomiting, bloody diarrhea, fever, anorexia, dehydration, and depression are among the clinical signs (Rutgers, 1994). Salmonellosis is a serious infectious disease that can infect both humans and animals (Sánchez-Vargas *et. al.* 2011). *Salmonella* is a Gram-negative, aerobic or facultative anaerobic bacilli of the Enterobacteriaceae family with a wide animal reservoir that includes mammals, birds, reptiles, amphibians, and invertebrates (Greene and Saunders, 1998).

2.1. Prevalence of Salmonella

Usmael *et. al.* (2022) conducted a study among 415 diarrheic and non-diarrheic dogs where non-typhoidal *Salmonella* were isolated from 26 (6.3%) of the rectal swab samples, with significantly higher occurrence in diarrheic (15.2%) than non-diarrheic (5.5%) dogs. They concluded that the risk of *Salmonella* harboring was significantly higher in female dogs than in male dogs (OR = 2.5, *p* = 0.027). They added that the fecal shedding of *Salmonella* was relatively higher in households who used offal as a main feed type for their dogs (23.1%; 95% CI = 5–53.8) than those who used leftover food (10.1%; 95% CI = 5.7–16.1) and practiced mixed feeding system (17%; 95%CI= 7.6-30.8).

Degi *et. al.* (2021) undertook a study to investigate the presence of *Salmonella spp.* in the feces of client-owned cats in urban areas and to evaluate the risk that is posed to public health. They collected fresh fecal samples directly from the rectums of 53 diarrhoeic and 32 non-diarrhoeic cats. They found a total of 16 of the samples (18.82%) tested positive for *Salmonella spp.* according to conventional and molecular testing methods. The serotyping of the *Salmonella* isolates showed the presence of three serotypes, namely *S. enteritidis* (n = 9; 56.3%), *S. typhimurium* (n = 4; 25%), *and S. kentucky* (n = 3; 18.8%).

Wei *et. al.* (2020) conducted a study to determine the prevalence and antimicrobial susceptibility of fecal *Salmonella* were investigated in pet dogs and cats in Xuzhou,

Jiangsu Province, China. Fecal samples from 243 dogs and 113 cats, at seven pet clinics were tested between March 2018 and May 2019. They concluded that the prevalence of *Salmonella* was 9.47% in dogs and 1.77% in cats.

Botha *et. al.* (2018) conducted an investigation to find out the prevalence of *Salmonella* in juvenile dogs affected with parvoviral enteritis in South Africa. They investigated a group of 74 dogs with canine parvovirus from 2015 to 2017 and concluded the prevalence of fecal *Salmonella* shedding was 22% and 31% for the affected and apparently healthy dogs, respectively.

Gelaw *et. al.* (2018) was conducted a retrospective laboratory-based surveillance on *Salmonella* serotypes isolated from various animal species from 2007 to 2014 at the Agricultural Research Council, Onderstepoort Veterinary Research Institute, South Africa and reported a prevalence of *Salmonella* 0.5% (six cats).

Kiflu *et. al.* (2017) conducted a study was to determine the prevalence, serotype distribution and antimicrobial resistance of *Salmonella* from feces of apparently healthy dogs in Addis Ababa, Ethiopia. They examined a total of 360 dogs and results revealed that 11.7% (42 dogs) were positive for *Salmonella*. They also reported that fourteen serotypes were detected and the predominant ones were *S. bronx* (n = 7; 16.7%), *S. newport* (n = 6; 14.3%), followed by *S. typhimurium*, *S. indiana*, *S. kentucky*, *S. saintpaul* and *S. virchow* (n = 4; 9.5%) each.

Koenig *et. al.* (2017) experimented on an adult feline blood donor, group-housed in a closed colony with other blood donor cats in a laboratory animal facility, developed anorexia, abdominal pain, and abdominal mass effect hemorrhagic diarrhea. The study showed that the index cat and 2 additional cats in the closed colony had clinical signs consistent with *Salmonella* and yielded *Salmonella* serotype 4,12: i: – in fecal cultures. They noticed the implementation of individual housing and additional barrier precautions, combined with antibiotic treatment of the index case, all the cats survived and subsequently had multiple, negative *Salmonella* PCR test results. This case report highlights the potential for unlikely infections to occur, even in a closed colony of research animals, as well as the important role of sanitation in the elimination of this enteric pathogen.

Reimschuessel *et. al.* (2017) revealed the prevalence of *Salmonella* in cats (3 of 542) was 1%. The study revealed that only four isolates were resistant to one or more antibiotics by testing an antimicrobial susceptibility test of 66 isolates.

Sultana *et. al.* (2016) conducted a cross-sectional study among 1070 pet animals in a private pet clinic in Dhaka where they reported the overall prevalence of Salmonellosis is 18% in cats.

Jay-Russell *et. al.* (2014) conducted a prevalence survey of Shiga toxin-producing E. coli (STEC) and *Salmonella* presence in stray dogs and coyote feces. *Salmonella* was cultured from 33 (9.2%) dog and 33 (32%) coyote samples comprising 29 serovars with 58% from dogs belonging to *Typhimurium*. Their study suggested that stray dogs and coyotes in the desert southwest may not be significant sources of STEC, but are potential reservoirs of other pathogenic *E. coli* and *Salmonella*.

Zenad *et. al.* (2014) cultured rectal swabs of 59 healthy cats with tetrathionate broth and Salmonella–Shigella agar. They detected a high isolation rate of *Salmonellae* (10.16%). The study reported that stray cats have greater chances of getting intestinal infections than house cats due to their living style. In conclusion asymptomatic (carriers) stray cats were considered a dangerous source of infection with *Salmonellae*, besides their significant role in contamination of the environment; they will threaten public and animal health particularly in cities.

Salehi *et. al.* (2013) conducted a study of 38 clinically healthy mixed breed shepherd dogs from Garmsar, Iran and results revealed a prevalence of *Salmonella* 10.5% (nine dogs).

Thomas *et. al.* (2013) conducted a studyt o investigate the diversity and seasonality of the *Salmonella* serotype in urban and rural cats. The study results showed that urban cats had a higher prevalence of salmonellosis than rural cats.

Sabshin *et. al.* (2012) conducted a cross-sectional study to determine the frequency of enteropathogens in cats entering an animal shelter with normal feces or diarrhea. Study results concluded that enteropathogens identified in cats with and without diarrhea included *Clostridium perfringens* enterotoxin A (42% and 50%, respectively), *Cryptosporidium* spp (10% and 20%, respectively), *Giardia* spp (20% and 8%, respectively), *Cystoisospora* spp (14% and 10%, respectively), hookworms

(10% and 18%, respectively), ascarids (6% and 16%, respectively), *Salmonella* spp (6% and 4%, respectively), astrovirus (8% and 2%, respectively), feline panleukopenia virus (4% and 4%, respectively), calicivirus (0% and 2%, respectively), and *Spirometra* spp (0% and 2%, respectively).

Hoelzer *et. al.* (2011) reported that the prevalence of salmonellosis in domestic cats was between 1-5% in the United States.

Gow *et. al.* (2009) investigated stools of clinically healthy 57 cats at 9 to 20 weeks of age in the United Kingdom, and isolated *Salmonella* spp. from one cat. They emphasized that *Salmonella* spp. can be detected without clinical symptoms in kittens.

Leonard *et. al.* (2011) conducted a study to determine pet-related management factors that may be associated with the presence of *Salmonella* spp. in the feces of pet dogs from volunteer households. Results revealed that 23.2% (32/138) had at least one fecal sample positive for *Salmonella*, and 25% (21/84) of the households had at least one dog shedding *Salmonella.* They also reported that 12 serotypes of *Salmonella enterica subsp. enterica* were identified, with the predominant serotypes being *Typhimurium* (33.3%; 13/39), *Kentucky* (15.4%; 6/39), *Brandenburg* (15.4%; 6/39) and *Heidelberg* (12.8%; 5/39).

Bagcigil *et. al.* (2007) examined rectal swabs of 200 dogs and isolated agents from two dogs as *S.typhimirium*and *S. enteritidis*.

Tsai *et. al.* (2007) collected rectal swabs from 437 households and 491 stray dogs in northern Taiwan from May 2003 to June 2005 to investigate the prevalence and antimicrobial susceptibilities of *Salmonellae* and *Campylobacter*s. The results revealed that 2.1% of household dogs and 6.3% of stray dogs were positive for *Salmonellae* with *Salmonella duesseldorf* being the most dominant serotype in both.

Kocabıyık *et. al.* (2006) received rectal swab specimens from 82 dogs and found a positive rate of *Salmonella* spp. 11%.

Seepersadsingh *et. al.* (2005) conducted a study to determine the prevalence of *Salmonella* spp. in non-diarrhoeic cats across Trinidad. Study results concluded that of the 94 cats sampled 2 (2.1%) were positive for *Salmonella* spp.

Van Immerseel *et. al.* (2004) experimented with rectal swab specimens taken from 278 healthy house cats, from 58 cats that died or were euthanized because of incurable diseases and from 35 group-housed cats that were kept in 1 room with 3 cat trays and common water and feed tray. They enlisted eighteen (51%) of 35 group-housed kittens, 5 (9%) of 58 diseased cats with incurable diseases and 1 (0.4%) of 278 healthy house cats excreted *Salmonella*. The authors conclude that cats that excrete *Salmonella* could pose a public health hazard to people who are highly susceptible to *Salmonella*, such as children, the elderly and immune compromised persons.

Stiver *et. al.* (2003) investigated that the prevalence of salmonellosis is higher in cats feeding raw meat-based food rather than home-prepared food.

Zenad and Ali, (2003) reported a total rate of *Salmonella* infection in stray cats was 13.5%, which was near to that value reported in stray dogs (15%) in Mosul.

Sanchez *et. al.* (2002) reported that the prevalence of *Salmonella* in fecal samples from healthy dogs was between 1% and 36% and that in samples from healthy cats, it ranged from 1% to 18%.

Cantor *et. al.* (1997) conducted a study to determine *Salmonella* shedding in racing sled dogs. They isolated *Salmonella* from 26 asymptomatic dogs and 30 diarrhoeal dogs, with rates of 69% and 63%, respectively.

Clyde *et. al.* (1997) detected *Salmonella* spp. in more than 90% of exotic cats in two different groups eating raw chicken meat and horse meat.

Weber *et. al.* (1995) unleashed 39 strains of *salmonella* representing a total of 17 different serovars from the fecal sample of cats. They noticed the most frequently detected serovar was *S. typhimurium* including *var. copenhagen* with 49% in the cats.

Ikeda *et. al.* (1986) documented that the fecal *Salmonella* carrier state in most cats is clinically inapparent and the prevalence of carriers was found to be variable, with isolation rates of *Salmonella* serovars ranging from 0.0% to 14.0%.

Fox and Beaucage, (1979) studied over 18 months while they received 142 cats from commercial vendors for use in the research were screened for enteric *Salmonella*. *Salmonella* was isolated from 15 animals, with an incidence of 10.6%. Five (29%) of the 17 shipments contained animals that were positive for *Salmonella*. It was also

found that the serotypes isolated were *Salmonella* der by, *Salmonella typhimurium*, *Salmonella anatum*, *Salmonella enteritidis* and *Salmonella bredeney.*

Stucker *et. al.* (1952) investigated the prevalence and distribution of salmonellosis in dogs in greyhounds in Florida in 1951. 1,741 strains of *Salmonella* were isolated from 3,072 samples from hunting dogs in Florida, with a prevalence rate of 45.25%.

Ball, (1951) detected *Salmonella* spp. in 4 (2.3%) of 175 shelter cats compared to a zero prevalence in household cats.

2.2. Antibiotic Sensitivity of Salmonella

Usmael *et. al.* (2022) conducted a study among 415 diarrheic and non-diarrheic dogs where they found 41.7% *Salmonella* isolates showed higher resistance to ampicillin, while all isolates were fully susceptible to gentamicin. They added that 58.3% of *Salmonella* isolates showed resistance to at least one of the tested antimicrobials. Majorities (72.7%) of the dog owners had no awareness on the risk of zoonotic salmonellosis from dog and all of the respondents use bare hand to clean dog kennel.

Degi *et. al.* (2021) undertook a study to investigate the presence of *Salmonella* spp. in the feces of client-owned cats in urban areas and to evaluate the risk that is posed to public health. The research stated that all of the tested strains showed strong resistance towards cefazolin, cefepime, ceftazidime, and ceftriaxone; intermediate resistance (listed in descending order of strength) was observed to trimethoprim/sulfamethoxazole (11/16; 68.8%), ampicillin (10/16; 62.5%), ampicillin/sulbactam (9/16; 56.3%), gentamicin (9/16; 56.3%), nitrofurantoin (8/16; 50.0%), and amikacin (5/16; 31.3%). While they found no resistance against ciprofloxacin, ertapenem, imipenem, levofloxacin, piperacillin/tazobactam, and tobramycin.

Wei *et. al.* (2020) conducted the antibiotic susceptibility test against *Salmonella* and found the highest resistance rates for tetracycline (92%), azithromycin (88%), cefazolin (84%), nalidixic acid (80%), ampicillin (80%), ceftriaxone (80%), and streptomycin (76%). They also stated that resistance to three or more antimicrobial agents was detected in 24 (96%) isolates. Most of the *S. kentucky* and *S. indiana* isolates were multi-drug resistant to more than 11 agents.

Gebremedhin *et. al.* (2019) studied among 438 dogs in selected towns of West Shoa

Zone, Oromia, Ethiopia where they found *Salmonella* isolates have developed the highest level of resistance for ampicillin (100%), tetracycline (93.3%) and streptomycin (80.0 %), however, all isolates were susceptible to Norfloxacin. They stated that the occurrence of *Salmonella*, most of which resistant to commonly used antimicrobials, in rectal swab samples show the potential threat dogs may pose to public health in the study area.

Andersen *et. al.* (2018) experimented with 482 fecal samples of cats, approximately half of the cats with normal fecal consistency and half with diarrhoea, were tested by zinc sulfate centrifugation and by real-time PCR for a panel of entero-pathogens. They reported that at least one entero-pathogen of feline or zoonotic importance was detected in a majority of cats, regardless of the management model. They also reviewed that management practices for unowned cats are inadequate for the control of entero-pathogens and that the presence of diarrhea is a poor indicator of entero-pathogen carriage. Risk-management strategies to reduce transmission to people and other animals should focus on sanitation, housing, compliance with preventive care guidelines, periodic surveillance, response to specific enteropathogens, humane population management of free-roaming community cats, public health education, and minimizing the duration and number of cats in mass confinement.

Kiflu *et. al.* (2017) conducted a study was to determine the prevalence, serotype distribution and antimicrobial resistance of *Salmonella* from feces of apparently healthy dogs in Addis Ababa, Ethiopia. They examined a total of 360 dogs and results revealed that the highest resistance rates were found for oxytetracycline (59.5%), neomycin (50%), streptomycin (38.1%), cephalothin (33.3%), doxycycline (30.9%), ampicillin (30.9%) and amoxicillin + clavulanic acid (26.2%). They also reported that 38 (90.5%), 30 (71.4%) and 19 (45.2%) of the isolates were resistant or intermediately resistant to at least one, two or more and three or more of the 16 antimicrobials tested.

Tadee *et. al.* (2015) reported that among 86 strains of *Salmonella* from pigs, the resistance rates to tetracycline and ampicillin were more than 81% in Thailand.

Jay-Russell *et. al.* (2014) conducted a prevalence survey of Shiga toxin-producing E. coli (STEC) and *Salmonella* presence in stray dogs and coyote feces. They reported that 4 (6.1%) of 66 *Salmonella* isolates were resistant to two or more antibiotic

classes.

Tsai *et. al*. (2007) collected Rectal swabs from 437 households and 491 stray dogs in northern Taiwan from May 2003 to June 2005 to investigate the prevalence and antimicrobial susceptibilities of *Salmonellae* and *Campylobacter*s. The results revealed that the resistance rates of *Salmonella* strain from dogs to sulfamethoxazole/ trimethoprim (37.5%), tetracycline (77.5%), chloramphenicol (52.5%), and ampicillin (50%).

Seepersadsingh *et. al*. (2005) concluded that the isolation of *Salmonella spp*. from apparently healthy cats poses a health hazard to their owners since most serovars are known to be potentially pathogenic. Furthermore, their study revealed that the existence of resistance to antimicrobial agents amongst *Salmonella* isolates from cats could cause chemotherapeutic consequences to their human owners.

Van Immerseel *et. al.* (2004) experimented with rectal swab specimens taken from 278 healthy house cats, from 58 cats that died or were euthanized because of incurable diseases and from 35 group-housed cats that were kept in 1 room with 3 cat trays and common water and feed tray. The study found serotype *Typhimurium* resistance to ampicillin, chloramphenicol and tetracycline.

Mathewson *et. al.* (1981) worked on six isolations of *Salmonella krefeld* where they reported that all of these isolates were resistant to several antimicrobial agents commonly used in the treatment of salmonellosis. These cultures of *S. krefeld* were recovered from infectious processes of several animal species' urinary and gastrointestinal tracts. All isolates were resistant to chloramphenicol, gentamicin, kanamycin, tobramycin, and trimethoprim-sulfa. Four of the six cultures were resistant to ampicillin, carbenicillin, and streptomycin.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Samples

Table 1. Fecal sample of cats

Sample no	Collection area	Breed	Gender	Age
S-01	Dhanmondi	Persian	Male	1.5 yr
S-02	Mirpur	Mixed	Male	3 yr
S-03	Mohammadpur	Persian	Female	5 month
S-04	Dhanmondi	Local	Male	9 month
S-05	Mohammadpur	Local	Female	7 yr
S-06	Mohammadpur	Mixed	Male	6 month
S-07	Mirpur	Persian	Female	2 yr
S-08	Dhanmondi	Mixed	Female	6 month
S-09	Dhanmondi	Local	Male	2 yr 8 month
S-10	Mohammadpur	Mixed	Male	4 month
S-11	Agargoan	Mixed	Female	5 month
S-12	Agargoan	Local	Male	3 yr
S-13	Dhanmondi	Persian	Male	2 month
S-14	Mirpur	Mixed	Female	8 yr
S-15	Dhanmondi	Persian	Female	2.5 month
S-16	Agargoan	Local	Male	3 yr
S-17	Dhanmondi	Persian	Female	6 month
S-18	Dhanmondi	Persian	Male	7 yr 3 month
S-19	Mohammadpur	Mixed	Male	6 yr

Continued Table 1.

S-20	Mohammadpur	Persian	Male	7 month
S-21	Dhanmondi	Mixed	Female	2 month
S-22	Mirpur	Local	Male	3 yr
S-23	Mohammadpur	Persian	Male	2 yr 4 month
S-24	Agargoan	Mixed	Female	6 month
S-25	Mohammadpur	Local	Male	6 yr
S-26	Agargoan	Local	Female	7 yr
S-27	Mirpur	Persian	Male	2.5 month
S-28	Agargoan	Mixed	Female	10 month
S-29	Mirpur	Persian	Male	3 month
S-30	Dhanmondi	Mixed	Male	2 month
S-31	Agargoan	Local	Male	2.5 month
S-32	Mirpur	Mixed	Female	7 month
S-33	Mirpur	Local	Female	1.5 month
S-34	Agargoan	Mixed	Male	2.5 month
S-35	Mohammadpur	Mixed	Female	3 month
S-36	Agargoan	Local	Male	5 yr
S-37	Mirpur	Local	Male	3 yr
S-38	Agargoan	Mixed	Male	5 month
S-39	Mirpur	Persian	Female	2 month
S-40	Mohammadpur	Local	Female	3 yr

Legend: S (1-40) = Fecal samples of cat

3.1.2 Bacteriological media

3.1.2.1 Agar media

The agar media used for bacteriological analysis were MacConkey (MC) agar, Eosin Methylene Blue (EMB) agar, Salmonella shigella (SS) agar, and Muller Hinton (MH) agar.

3.1.2.2 Liquid media (broth)

Liquid media as nutrient broth, Peptone broth, Methyl-Red and Voges-Proskauer broth (MR-VP broth), and Sugar media (dextrose, maltose, lactose, sucrose and mannitol) were used in this study.

3.1.2.3 Phosphate Buffered Saline (PBS)

To prepare phosphate buffered saline, 1000 ml of distilled water was mixed with 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). In order to dissolve the solution properly, it was heated, and a pH meter was used to adjust the pH. Then, the solution was autoclaved to sanitize it and kept chilled at 4ºC for later use.

3.1.3 Chemicals and reagents

The chemicals and reagents were used in this study included 0.1% Peptone water, Phosphate buffered saline (PBS), Gram's staining reagents (Crystal Violate, Gram's iodine, Safranin, Acetone alcohol), and 3% hydrogen peroxide, phenol red, methyl red, 10% potassium hydroxide, Kovac's indole 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 course of 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, centrifuge tubes and machine, syringe and needle, tray, forceps, scalpel, scissors etc.

3.1.5 Antimicrobial discs

In order to evaluate the antimicrobial discs' drug sensitivity and resistance patterns and to assess their disease potential, commercially available antimicrobial discs (OXOID Limited, Canada) were used. 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 the diffusion of the agent into the medium surrounding the discs inhibiting the growth of the organisms. The following antibiotic agents with their disc concentration were used to test the sensitivity and resistance pattern of the selected *Salmonella* isolates from cats.

Table 2. Drugs with their disc concentration for the *Salmonella*

Name of drugs	Disc concentration (µg /disc)	Zone Diameter Interpretive Standard (mm)		
		Resistant	Intermediate	Susceptible
Gentamycin (GEN)	10	≤13	14--17	≥18
Azithromycin (AZM)	15	≤13	14--17	≥18
Cefixime (CXM)	5	≤14	15--17	≥18
Cefuroxime (CFM)	30	≤14	15--17	≥18
Ceftriaxone (CTR)	30	≤19	20--22	≥23
Amoxicillin (AMX)	10	≤13	14--17	≥18
Amoxyclav (AMC)	10	≤13	14--16	≥17
Cotrimoxazole (COT)	25	≤10	11--15	≥16
Ciprofloxacin (CIP)	5	≤15	16--20	≥21
Streptomycin (S)	10	≤11	12--14	≥15

Legend: µg = micro gram

3.2 Methods

3.2.1 Brief description of the experimental design

The entire study was divided into two major steps: The first step included the selection of sources, collection of samples, isolation, identification and characterization of microorganisms on the basis of their colony morphology, staining properties, motility and biochemical characteristics. In the second step, the current status of drug sensitivity and resistance pattern of a total of 40 isolates of microorganisms isolated from cats was determined.

Experiment Design

Selection of the cat according to age, sex, breed, area

⬇

Collection of fecal samples

⬇

Preparation of stock culture from fecal samples in PBS (2ml)

⬇

Multiplication of bacteria in the Nutrient Broth by overnight incubation at 37°C for 24 hrs.

⬇

Isolation of the bacteria on the basis of cultural properties (using EMB, SS, MC agar media)

⬇

Isolation of pure colony from the single colony culture in Nutrient Broth

⬇

Antibiotic sensitivity profiling of isolated bacteria

Figure 1: Layout of the Experiment Design

3.2.2 Preparation of various bacteriological culture media

3.2.2.1 Nutrient Agar

To prepare nutrient agar, 28 grams of dehydrated nutrient agar (HiMedia, India) was dissolved into 1000 ml of distilled water and was sterilized by autoclaving at 121ºC under 15 lb pressure per square inch for 15 minutes. Then the agar was dispensed into Petri dish (90 mm and 100 mm) and was incubated at 37ºC overnight to check their sterility and stored at 4ºC in the refrigerator for further use.

3.2.2.2 Nutrient Broth

Preparation of nutrient broth was done by 25 grams were dissolved in 1000 cc of distilled or filtered water. If heat is required to entirely dissolve the medium, do so. Then, 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 further use.

3.2.2.3 MacConkey's agar

For preparation of MacConkey agar media, 49.53 grams of BactoMacConkey agar (HiMedia, India) was suspended in to 1000 ml of cold distilled water and was heated for boiling to dissolve the medium completely with water. It was then poured into sterile Petri dishes 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 Eosine Methylene Blue (EMB) agar

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

3.2.2.5 Salmonella-Shigella agar

According to the direction of the manufacturer (HiMedia, India), 60 grams of the dehydrated medium was suspended in 1000 ml distilled water and heated for boiling to dissolve the medium completely. Then the medium was sterilized by autoclaving. After autoclaving, the medium was put into a water bath of 50ºC to decrease its temperature. After solidification of the medium in the Petri dishes, the Petri dishes were allowed for incubation at 37ºC overnight to check their sterility and then stored at 4ºC in a refrigerator for future use.

3.2.2.6 Mueller Hinton Agar

38.0 grams of Mueller Hinton agar (HiMedia, India) was dissolved in 1000 ml distilled water and heated to boiling to dissolve the medium completely with water. After the sterilization by autoclaving at 15 lbs pressure (121°C) for 15 minutes, cooling was done to 45-50°C. Then it was mixed well and poured into sterile Petri dishes. After solidification of the medium in the Petri dishes, the Petri dishes were allowed for incubation at 37ºC overnight to check their sterility and then stored at 4ºC in a refrigerator until used.

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

According to the direction of the manufacturer (HiMedia, India), 3.4 gm of MR-VP medium 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 until used.

3.2.2.8 Sugar solutions

The medium consists of 1% peptone water to which fermentable sugars were added. Peptone water was prepared by adding 1 gram of Bacto peptone (Difco, USA) and 0.5 grams of sodium chloride in 100 ml distilled water, boiled for 5 minutes, adjusted to pH 7.6 by phenol red (0.02%) indicator, cooled and then filtered through filter paper. The solutions were then dispensed in 5 ml amount into cotton plugged test tubes containing invertedly placed Durham's fermentation tubes. Then the sugars, dextrose (MERCK, India), maltose (s.d.fiNE-CHEM Ltd.), lactose (BDH, England), sucrose

(MERCK, India) and mannitol (PETERSTOL TENBEG) used for fermentation were prepared separately as 10 percent solutions in distilled water (10 grams sugar was dissolved in 100 ml of distilled water). A little heat was necessary to dissolve the sugar. These were then sterilized by autoclaving for 15 minutes. The sugar solutions were sterilized in Arnold's steam sterilizer at 100ºC for 30 minutes for three consecutive days. 0.5 ml of sterile sugar solution was added aseptically in each culture tube containing sterile peptone water. The sugar solutions were incubated at 37ºC for 24 hours to check sterility. These solutions were used for biochemical test.

3.2.3 Isolation of bacteria

3.2.3.1 Collection, transportation and preparation of sample:

40 fecal samples of cat were collected from Vet and Pet Care (VNPC) located at centre of the capital city Dhaka. All the cats are from four selected area (Dhanmondi, Mohammadpur, Mirpur and Agargoan). Swabbing technique was used to collect the fecal sample. A clean cotton bud moistening with glycerin was inserted gently into the rectum. Then the cotton bud containing rectal swab were kept into a eppendorf tube. After that the eppendorf tube were marked and preserved into the deep refrigerator (-18°C) for future use. The collected samples were carried to the laboratory in an ice box containing ice and processed for isolation and characterization of bacteria.

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

Serial dilution of the stock sample was done to lowering the bacterial count for the Total Salmonella Count (TSC). It was done by taking 8 (1-8) Eppendorf tube filled with 900µl of PBS. 100µl of stock sample was transferred from the stock tube (2ml) to the Eppendorf tube next to the stock 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 to 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 Salmomella –Shigella agar to elucidate the total Salmonella 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 37°C for the growth of the organisms.

3.2.3.4 Isolation in culture media

Once the organism had undergone primary culture, a small quantity of inoculums from nutrient broth was streaked on the MacConkey agar, EMB, and Salmonella-Shigella agar to examine the colony morphology of the isolates. The bacterium was chosen for culturing on Salmonella-Shigella agar selective media based on its distinctive colony shape. The suspected colonies' morphological properties (form, size, surface texture, edge and elevation, color, opacity, etc.) that emerged on various agar media within 18 to 24 hours of incubation were meticulously noted.

3.2.3.5 Microscopic study for identification of *Salmonella* spp. from the suspected colonies by Gram's staining method

To ascertain the size, shape, and arrangement of bacteria, Gram's staining was carried out in accordance with Merchant and Packer's (1976) guidelines. The steps were as follows: Using a bacteriological loop, a little colony was picked up, spread out on a glass slide, and gently heated to fix it. The smear was then stained for two minutes with crystal violet solution, and rinsed with running water thereafter. The next step was to apply Gram's iodine to function as a mordant for one minute, followed by another rinse under running water. Then acetone alcohol which serves as a decolorizer was added. Safranine was added as a counter stain after washing with water, and it was left to stain for two minutes. The slide was then washed with water, blotted and air dried and then examined under microscope with high power objective (100X) using immersion oil.

3.2.4 Identification of isolated *Salmonella* spp. by using specific biochemical tests

Several biochemical tests were performed for confirmation of *Salmonella* isolates.

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

Three milliliters of catalase reagent (3% H_2O_2) were used in this study. Using a glass rod and one colony from the *Salmonella* pure culture, the reagent was added to the sample. We looked for bubble development in the tube. All of the isolates tested positive for catalase; the test was positive when a bubble formed within a few seconds, and the test was negative when a bubble did not develop (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 *Salmonella* organisms were grown in 3 ml of sterile MR-VP broth at 37ºC for 48 hours. Then 0.6 ml of 5% alpha-napthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine was added per ml of broth culture of the test organism. Then 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. Then 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

Stock culture was prepared by adding 1ml of 80% sterilized glycerol in 1 ml of pure culture in nutrient broth and it was stored in -20°C.

3.2.6 Antimicrobial sensitivity pattern of *Salmonella spp.* isolates

A total of 21 *Salmonella spp.* were isolated from 40 fecal samples of cat which 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 Gentamicin (GEN), Azithromycin (AZM), Cefuroxime (CFM), Cefixime (CXM), Ceftriaxone (CTR), Amoxicillin (AMX), Amoxicillin + Clavulanic Acid (AMC), Co-Trimoxazole (COT), Ciprofloxacin (CIP) and 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. 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. The diameter of the zone of complete inhibition was measured using a mm scale after the plates had been incubated. By using an interpretation table, the zone widths for various antimicrobial drugs were converted into sensitive, moderate, and resistant categories.

CHAPTER IV

RESULTS AND DISCUSSIONS

The results presented below demonstrated the isolation and identification of bacteria isolates from fecal samples of cats from four different areas around Dhaka city. The results also presented the sensitivity and resistance pattern of the isolates to different drugs.

4.1 Total Viable Count from the isolated sample

Table 3. Total Viable count from the isolated sample

Sample no	Total Viable Count (TVC)(CFU/ml)
S-01	1.8×10^7
S-02	1.4×10^7
S-03	2.2×10^9
S-04	1.5×10^9
S-05	1.4×10^5
S-06	3.7×10^9
S-07	3.7×10^7
S-08	2.5×10^9
S-09	4.6×10^7
S-10	3.4×10^9
S-11	2.9×10^9
S-12	1.1×10^9
S-13	0.7×10^5
S-14	3.2×10^7
S-15	1.3×10^5
S-16	3.5×10^9
S-17	1.1×10^9
S-18	3.2×10^7
S-19	2.7×10^7
S-20	3.8×10^9
S-21	3.1×10^7
S-22	2.9×10^9

Continued Table 3.

S-23	1.3×10^5
S-24	1.9×10^5
S-25	3.7×10^7
S-26	1.8×10^5
S-27	3.5×10^9
S-28	2.5×10^9
S-29	2.7×10^9
S-30	2.6×10^7
S-31	2.3×10^7
S-32	3.5×10^9
S-33	2.2×10^9
S-34	2.8×10^7
S-35	3.9×10^9
S-36	3.2×10^7
S-37	1.8×10^5
S-38	3.2×10^7
S-39	2.9×10^5
S-40	2.9×10^9

Legends: S (1-40) = Fecal samples of cat, TVC= Total Viable Count, CFU= Colony Forming Unit
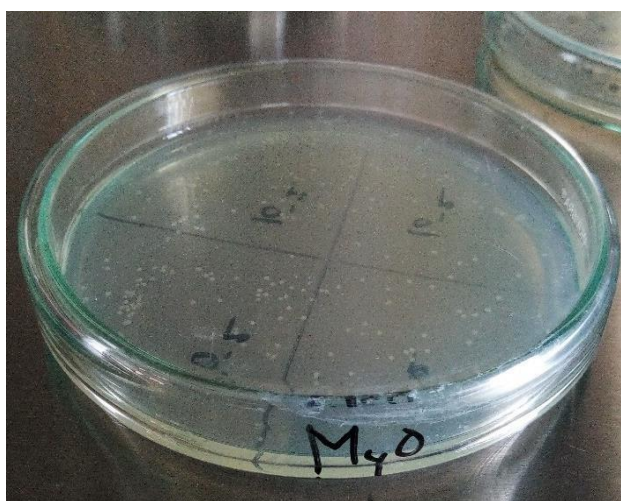
Figure 2: Total Viable Count by 10 fold dilution method

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4.2 Prevalence of microorganisms in cat

Table 4. Prevalence of microorganisms in cat

No. of sample investigated	No. of sample containing organism	Percentage (%) of prevalence
40	40	100

4.3 Total Coliform Count (TCC) (CFU ml^{-1}) from isolated samples

Total 40 cat fecal samples were investigated for identification of *Salmonella* in different areas around Dhaka city (Table 3). Total Coliform Count (TCC) from the collected samples of cat fecal are presented in Table 5. Among the 40 samples, 33 samples (82.5 %) showed the presence of Coliform bacteria.

Table 5. Results of Total Coliform Count (TCC) (CFU ml^{-1}) from isolated samples.

No.	Name of the samples	Total Coliform Count (TCC) (CFU ml^{-1})
1	S-01	1.7×10^5
2	S-02	2.7×10^7
3	S-03	1.6×10^7
4	S-04	2.8×10^6
5	S-05	2.5×10^9
6	S-06	1.7×10^5
7	S-07	2.6×10^9
8	S-08	Nil
9	S-09	1.9×10^5
10	S-10	2.8×10^6
11	S-11	2.2×10^5
12	S-12	1.6×10^6

Continued Table 5.

13	S-13	3.6×10^8
14	S-14	2.9×10^7
15	S-15	Nil
16	S-16	2.7×10^6
17	S-17	2.6×10^7
18	S-18	1.9×10^8
19	S-19	2.6×10^9
20	S-20	Nil
21	S-21	2.8×10^5
22	S-22	3.4×10^6
23	S-23	1.3×10^8
24	S-24	1.5×10^8
25	S-25	1.4×10^4
26	S-26	Nil
27	S-27	1.9×10^8
28	S-28	2.1×10^7
29	S-29	3.7×10^7
30	S-30	2.2×10^7
31	S-31	1.8×10^5
32	S-32	Nil
33	S-33	Nil
34	S-34	2.2×10^6
35	S-35	1.7×10^8
36	S-36	1.2×10^6
37	S-37	Nil
38	S-38	2.6×10^8
39	S-39	1.7×10^5
40	S-40	1.3×10^7

Legends: S (1-40) = Fecal samples of cat

4.4 Prevalence of Coliform bacteria in cat

Table 6. Prevalence of Coliform bacteria in cat

No. of sample investigated	No. of sample containing Coliform bacteria	Percentage (%) of prevalence
40	33	82.5

4.5 Results of isolation of *Salmonella* spp.

Table 7. Results of isolation of *Salmonella* spp.

No	Name of the sample	Total *Salmonella* Count (TSC) (CFU ml^{-1})
1	S-03	2.2×10^9
2	S-06	3.7×10^9
3	S-07	3.7×10^7
4	S-09	4.6×10^7
5	S-11	2.9×10^9
6	S-13	0.7×10^5
7	S-16	3.5×10^9
8	S-17	1.1×10^9
9	S-19	2.7×10^7
10	S-22	2.9×10^9
11	S-23	1.3×10^5
12	S-24	1.9×10^5
13	S-25	3.7×10^7
14	S-28	2.5×10^9
15	S-29	2.7×10^9
16	S-30	2.6×10^7
17	S-31	2.3×10^7
18	S-33	2.2×10^9
19	S-36	3.2×10^7
20	S-39	2.9×10^5
21	S-40	2.9×10^9

4.5.1 Identification of *Salmonella* spp.

The isolated samples are arranged as follows.The total Salmonella count was the same as the total viable count

Figure 3: Total *Salmonella* Count (TSC) in SS agar media

4.5.2 Results of isolation and identification of *Salmonella* spp.

For isolation and identification of *Salmonella*, different cultural examinations were performed in Medicine and Public Health Laboratory, SAU.

4.5.3 Results of cultural examination

Salmonella is cultured in nutrient broth and then sub-cultured in MacConkey (MC) agar media, EMB agar media and Salmonella-Shigella (SS) agar media for isolation of pure bacteria.

4.5.3.1 Culture in nutrient broth
All *Salmonella* spp. isolates produced turbidity in nutrient broth.

4.5.3.2 Culture on MacConkey agar

Red to pink-white colonies surrounded by brilliant red zones after overnight incubation were presumptively selected as *Salmonella* spp. (Table 8, Figure 4).

4.5.3.3 Culture on Eosin Methylene Blue (EMB) agar

Grey color colonies produced by the organisms on EMB agar after overnight incubation were tentatively confirmed as *Salmonella* spp. (Table 8, Figure 5).

4.5.3.4 Culture on Salmonella-Shigella(SS) agar

Colonies with black centers produced after overnight incubation the *Salmonella* spp. isolated from fecal samples (Table 8, Figure 6)

Table 8. Demonstration of the cultural characteristics of *Salmonella* Isolated from cats in different agar media

Sources of *Salmonella* spp.	Colony characteristics in different agar media		
	MC agar	EMB agar	SS agar
Fecal samples (3,6,7,9,11,13,16,17,19,22,23, 24,25,28,29,30,31,33,36,39,40)	Red to pink-white colonies surrounded by brilliant red zones	Grey color colony	Colonies with black centers
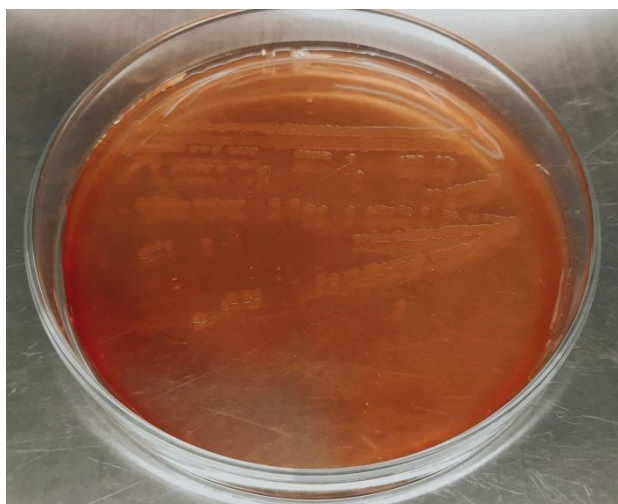
Figure 4: *Salmonella* spp. in MacConkey agar media
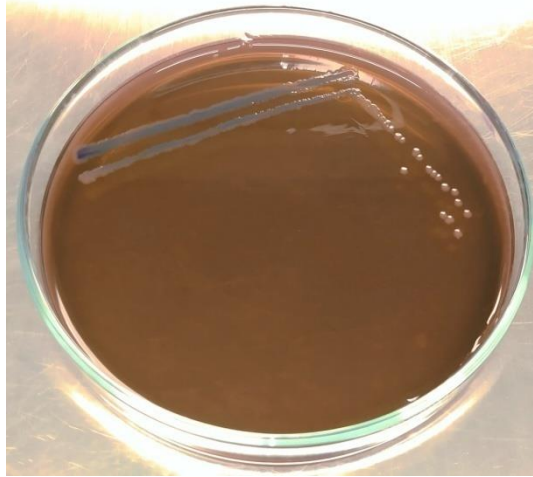
Figure 5: *Salmonella* spp. in EMB agar media
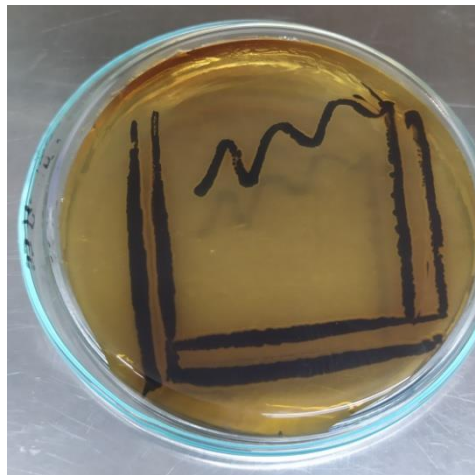
Figure 6: *Salmonella* spp. in SS agar media

4.5.3.5 Results of Gram's staining technique

Light microscopic examination after Gram's staining revealed Gram-negative, pink-colored, rod-shaped organisms arranged as single or in pairs.

4.5.3.6 Results of the biochemical tests

Tentatively confirmed *Salmonella* spp. isolates by colony characteristics, morphology and staining characteristics by motility were subjected to different biochemical tests for identification.

Table 9. Demonstration of the biochemical reactivity pattern of *Salmonella* spp. isolated from cats.

Fermentation properties of five basic sugars					MR test	V-P test	Indole production test	Citrate test
DX	ML	L	S	MN				
AG	AG	NF	NF	A↓G	+	−	−	+

Legends: DX= Dextrose; ML= Maltose; L= Lactose; S= Sucrose; MN=Mannitol; A= Acid production; G= Gas production; A↓= Less acid production; NF=No Fermentation, G= Gas production; (+) = Positive reaction; (-) = Negative reaction.

4.5.3.7 Sugar fermentation test

The result carbohydrate fermentation test of *Salmonella* spp. was performed by inoculating a loop full of thick bacterial culture into the tubes containing five basic sugars (dextrose, maltose, sucrose, lactose, and mannitol) and incubated at 37°C for 24 hours. Acid production was indicated by the change of media from pink to yellow color and gas production was indicated by the appearance of gas bubbles in the inverted Durham's fermentation tubes (Table 9, Figure 7).
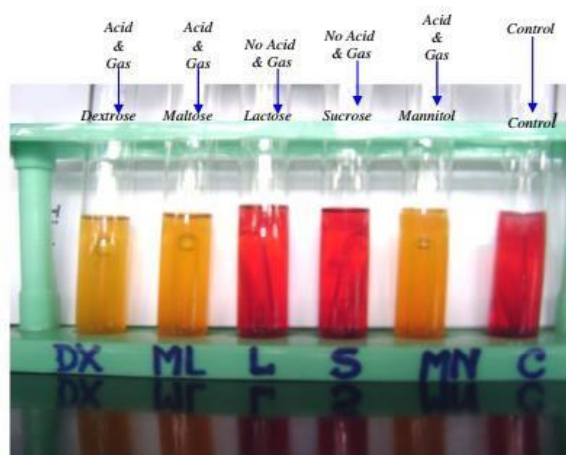
Figure 7: Sugar fermentation test of *Salmonella* spp.

4.5.3.8 Result of others biochemical test

All the isolates were Indole test positive (Table 9, Figure 8), Citrate test (Table 9, Figure 9), Methyl red (MR) test positive (Table 9, Figure 10).The above mentioned patterns of biochemical reactions were considered as *Salmonella* spp.

Figure 8: Indole test positive for *Salmonella* spp.

Figure 9: Citrate test positive (On left) for *Salmonella* spp.

Figure 10: Methyl Red positive for *Salmonella* spp.

4.6 Prevalence of *Salmonella* spp. in fecal samples of cat

A total of 40 cat fecal samples were collected for isolation and identification of *Salmonella* spp. Whereas 21 samples were isolated as *Salmonella* positives isolates. Fecal samples from three different breeds as Persian, Mixed and Local (12, 15, 13) were collected from different areas of Dhaka city where *Salmonella* positive isolates were 7, 5, 8 respectively (Table 10). The prevalence of *Salmonella* positives isolates among Persian, Mixed and Local breeds were 58%, 38% and 53% respectively. 12 *Salmonella* isolates from 23 male fecal samples and 9 isolates from 17 female fecal samples were found in this study. The prevalence of *Salmonella* isolates in male and female were 52% & 53%. The most prevalence was found between 1-3 years of cats (75%) followed by bellow 6 months (53%), 6 month to 1 year (50%), 3-5 years (50%) each and above 5 years (43%). Equal 6 positive isolates were identified in Agargoan and Mohammadpur area from 10 fecal samples of each. The highest prevalence was found in the Agargaon (60%) and Mohammadpur area (60%) followed by Mirpur (50%) and Dhanmondi (40%) (Table 10, Figure 11).

Table 10. Prevalence of *Salmonella* isolates among 40 fecal samples of cat

Variable		Frequency	Salmonella positive isolates	Percentage
Breed	Persian	12	7	58%
	Mixed	13	5	38%
	Local	15	8	53%
Sex	Male	23	12	52%
	Female	17	9	53%
Age	< 6 months	15	8	53%
	6 months-1yr	8	4	50%
	1-3 yrs.	4	3	75%
	3-5 yrs.	6	3	50%
	>5 yrs.	7	3	43%
Location	Agargaon	10	6	60%
	Dhanmondi	10	4	40%
	Mohammadpur	10	6	60%
	Mirpur	10	5	50%

Figure 11: Prevalence of *Salmonella* isolates of fecal samples of cat collected from different locations of Dhaka city.
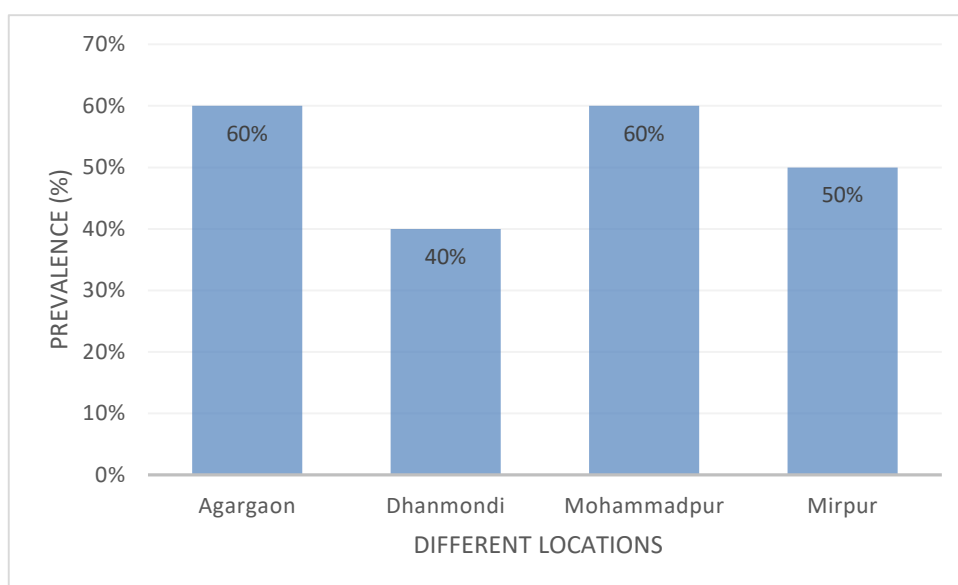
4.7 Results of drug sensitivity and resistance pattern of *Salmonella* spp. isolated from fecal samples of cat

A total of 21 different *Salmonella* isolates collected from 40 different samples from cats were used for drug sensitivity testing. Ten different drugs were used for the disc diffusion method test.

A large number of *Salmonella* isolates from fecal samples of cats were found sensitive to CTR (77%) and CIP (72%). Others antibiotics as CFM (67%), AMC (67%), COT (53%), CXM (52%) were found to sensitive followed by GEN (48%), S (47%) and AZM (43%). A little number was sensitive to AMX (19%). No drugs were found 100% resistance in this study. The highest resistance was against AMX (57%) & CFM (43%). None of the isolates showed Intermediate sensitivity & complete sensitivity. They showed comparatively lower resistance against AZM (29%), S (29%), GEN (19%), CXM (19%) followed by CTR, AMC, COT and CIP 9% of each. Most of the isolates showed intermediate sensitivity against COT (38%), CXM (33%) GEN (33%) and AZM (28%). Equal 24% of the isolates were found intermediate sensitive against CFM, AMC, AMX and S each. Among 10 drugs, the lowest intermediate sensitivity was found against CTR (14%) (Table 11, Figure 12, 13).

Table 11. Demonstration of the sensitivity and resistance pattern of different *Salmonella* isolates to different drugs in percentage

Sample / Drug	S-03	S-06	S-07	S-09	S-11	S-13	S-16	S-17	S-19	S-22	S-23	S-24	S-25	S-28	S-29	S-30	S-31	S-33	S-36	S-39	S-40	R	IN	S
GEN	R	S	S	IN	IN	S	R	S	IN	IN	IN	S	S	R	S	S	IN	IN	R	S	S	19	33	48
AZM	S	S	R	R	IN	R	S	S	IN	S	IN	IN	S	IN	S	R	R	R	S	IN	S	29	28	43
CFM	R	R	IN	S	S	IN	R	R	S	R	S	S	R	R	IN	S	IN	S	R	R	IN	43	24	67
CXM	S	IN	R	S	S	S	IN	IN	R	S	R	IN	S	S	S	IN	S	IN	S	R	IN	19	33	52
CTR	S	S	S	IN	S	S	R	IN	S	S	S	S	S	IN	S	S	S	S	S	S	R	9	14	77
AMX	R	R	R	R	IN	R	S	S	R	IN	R	R	R	IN	R	S	R	IN	IN	S	R	57	24	19
AMC	S	S	S	S	S	S	S	IN	R	S	S	S	IN	R	S	S	IN	IN	IN	S	S	9	24	67
COT	IN	IN	S	S	IN	S	R	S	IN	S	IN	S	S	IN	S	S	R	S	S	IN	IN	9	38	53
CIP	S	S	S	S	IN	S	IN	R	S	S	S	S	R	S	S	IN	S	IN	S	S	S	9	19	72
S	IN	IN	S	IN	R	S	S	S	R	IN	R	R	S	S	R	S	S	R	IN	S	S	29	24	47

Legends: GEN = Gentamicin; AZM = Azithromycin; CFM = Cefuroxime; CXM = Cefixime; CTR = Ceftriaxone; AMX =Amoxicillin; AMC = Amoxicillin + Clavulanic Acid; COT = Co-Trimoxazole; CIP = Ciprofloxacin; S= Streptomycin; S = sensitive; IN = intermediate; R = resistant;S-03, S-06, S-07, S-09, S-11 S-13, S-16, S-17, S-19, S-22, S-23, S-24, S-25, S-28, S-29, S-30, S-31, S-33, S-36, S-39, S-40 = Fecal samples of cat
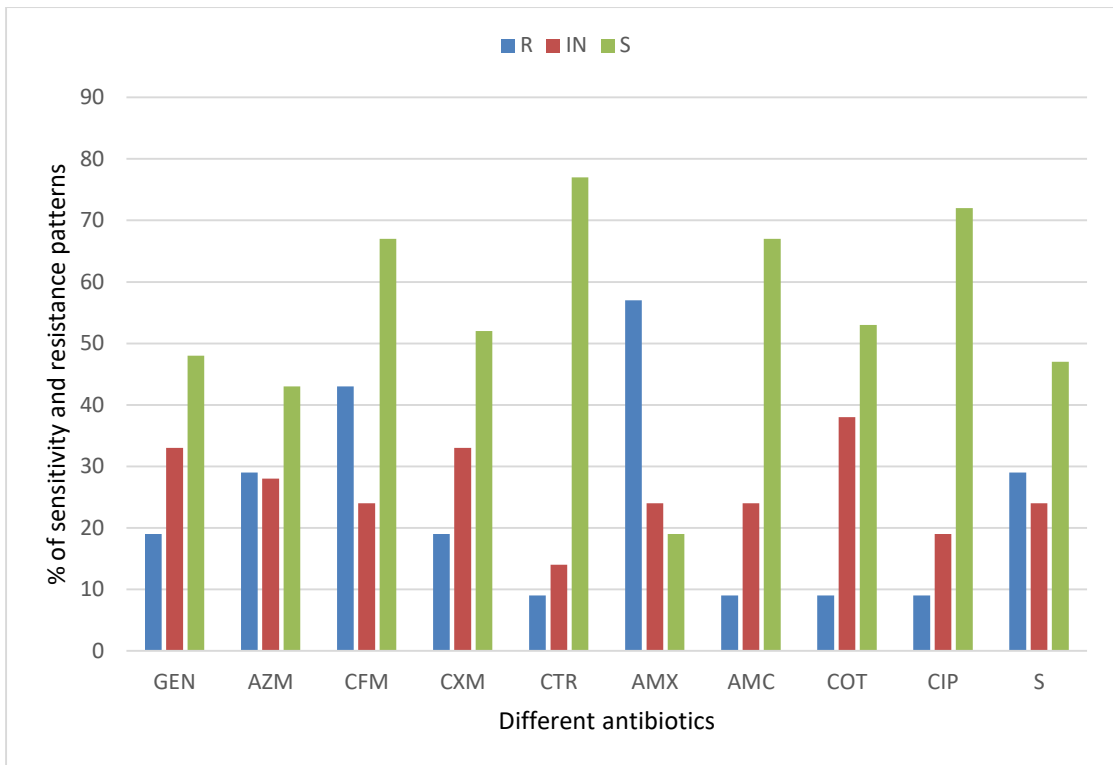
Legends: R= Resistance, IN= Intermediate, S= Sensitive

Figure 12: Demonstration of the sensitivity and resistance pattern of different *Salmonella* isolates to different drugs in percentage

Figure 13 : Antibiotic sensitivity test for *Salmonella* isolates

4.7.1 Results of drug sensitivity and resistance pattern of *Salmonella* spp. against 10 different drugs in different areas of Dhaka city

In this study, the antibiotic sensitivity and resistance pattern of isolated *Salmonella* spp. from four different area against 10 different drugs were investigated. Among the areas, the most sensitive of *Salmonella* was found in Dhanmondi (60%) and Mirpur (56%) followed by Mohammadpur (47%) and Agargaon (43%). The drug resistance of *Salmonella* isolates among Mohammadpur, Agargaon, Mirpur and Dhanmondi, areas were 28%, 25%, 20%, 17% respectively. The highest intermediate sensitivity was found in Agargaon (32%) followed by Mohammadpur (25%), Mirpur (24%) and Dhanmondi (23%), (Table 12, Figure 14).

Table 12. Demonstration of the sensitivity and resistance pattern of *Salmonella spp.* against 10 different drugs in different areas of Dhaka city

Location	No. of Salmonella isolated sample	Percentage of sensitivity and resistance		
		R	IN	S
Dhanmondi	4	17	23	60
Mohammadpur	6	28	25	47
Agargaon	6	25	32	43
Mirpur	5	20	24	56

Legends: R= Resistance, IN= Intermediate, S= Sensitive

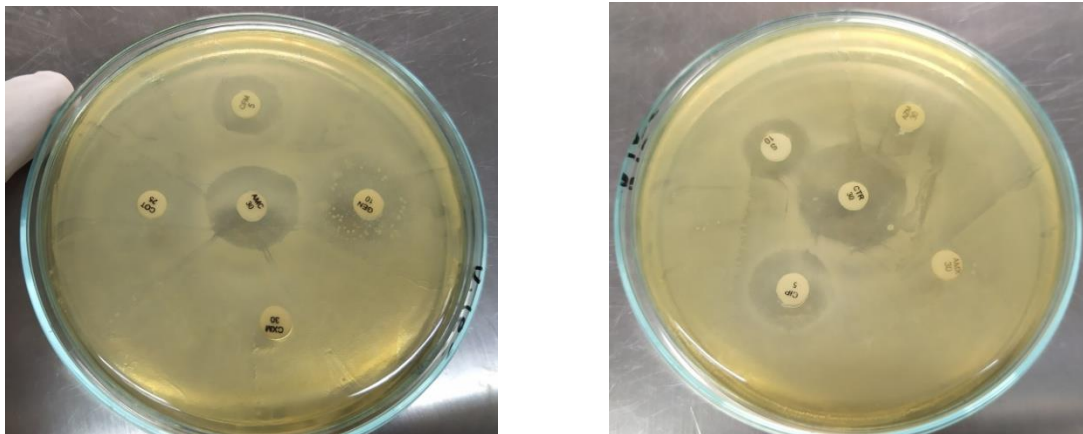**Figure 14: Demonstration of the sensitivity and resistance pattern of *Salmonella* isolates to different drugs in different areas of Dhaka city**
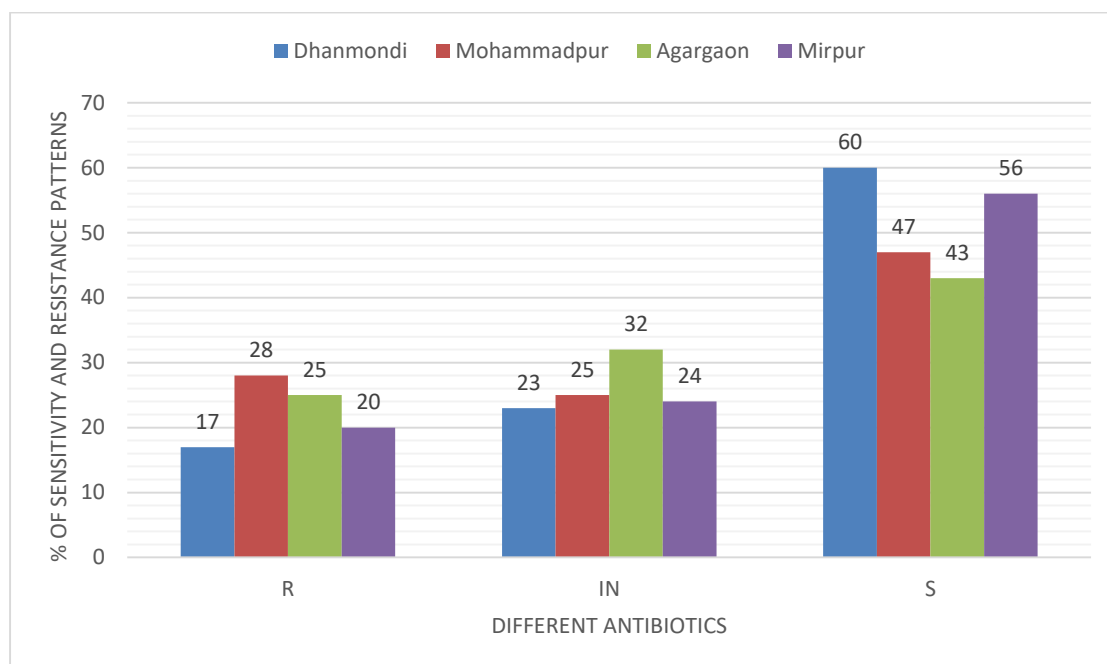
Legends: R= Resistance, IN= Intermediate, S= Sensitive

4.8 Discussion

The present study was conducted primarily for the isolation and identification of the *Salmonella* spp. isolated from fecal samples of cats in four different areas of Dhaka city and also to determine the current status of drug sensitivity and resistance pattern of the isolates to determine the drug of choice for therapeutic use against infection caused by these organisms.

Isolation and identification results of the study indicated that the selected samples contained Gram-negative and motile organisms (*Salmonella* spp.). All the *Salmonella* isolates were able to produce Red to pink-white colonies surrounded by brilliant red zones in MacConkey agar media, grey color in EMB agar & Colonies with black centers in SS agar. In Gram's staining, the morphology of the isolated bacteria exhibited pink, small rod-shaped Gram-negative bacilli. These findings were supported by several authors such as Buxton and Fraser, (1977), Freeman, (1985) and Jones *et. al.,* (1987). Another fundamental basis for the identification of the *Salmonella* spp. organism was determining the ability or inability of fermentation of five basic sugars with acid and gas production. However, species identification and

differentiation by fermentation reaction was difficult (Freeman, 1985) and showed similar reactions indifferent sugars (OIE Manual, 2000). Differentiation of *Salmonella* into species level was difficult based on their sugar fermentation pattern. All the isolates of this study fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which satisfied the statement of (Buxton and Fraser, 1977), (Hossain, 2002) and (Han *et. al.* 2011).

The result of Indole test for *Salmonella* was negative (formation of yellow ring), MR positive & result of V-P test was negative which satisfy the statement of (Buxton and Fraser, 1977).

In the present study, a total of 40 cat fecal samples were collected for isolation and identification of *Salmonella* spp. Whereas 21 samples were isolated as *Salmonella* positives isolates. Fecal samples from three different breeds as Persian, Mixed and Local (12, 13, 15) were collected from four different areas of Dhaka city where *Salmonella* positive isolates were 7, 5 and 8 respectively. The prevalence of *Salmonella* positives isolates among Persian, Mixed and Local breeds were 58%, 38% and 53% respectively. 12 *Salmonella* isolates from 23 male fecal samples and 9 isolates from 17 female fecal samples were found in this study. Gelaw *et. al.* (2018), conducted a study in South Africa reported a prevalence of *Salmonella* 0.5% (six cats) while a study of 542 cats in the United States revealed that 0.6% (three cats) were positive for *Salmonella* (Reimschuessel *et. al.* 2014). In the current study, the prevalence of *Salmonella* in cats was at variance with that of other countries, probably because of differences in sample size, pet feeding habits, pet sanitary practices, sampling strategies, and pet owner's awareness of zoonosis.

In the present study, the prevalence of *Salmonella* isolates in males and females were 52% and 53%, respectively. The most prevalence was found between 1-3 years of cats (75%) followed by bellow 6 months (53%), 6 month to 1 year (50%), 3-5 years (50%) each and above 5 years (43%). %). Equal 6 positive isolates were identified in Agargoan and Mohammadpur area from 10 fecal samples of each. The highest prevalence was found in the Agargaon (60%) and Mohammadpur area (60%) followed by Mirpur (50%) and Dhanmondi (40%). In a previous study, Degi, *et. al.* (2021) found the prevalence of *Salmonella spp.*18.82% according to conventional and molecular testing methods. In addition, seasonal, geographical, and regional

differences among studies probably led to some disparities (Seepersadsingh, *et al.* 2010).

Bacteria can counteract the effects of antimicrobial medications by creating enzymes and metabolites that either break down the antimicrobial agents or aid the bacteria's survival in different ways. As a result, the present status of the *Salmonella* isolates' sensitivity and resistance patterns to various medications should be established in order to select the optimal antibiotic for treatment reasons. To perform this study a total of 21 different *Salmonella* isolates collected from 40 different fecal samples from cats were used for drug sensitivity testing where 10 different drugs were used. The sensitivity test revealed that *Salmonella* isolates showed the highest sensitivity against CTR (77%) and CIP (72%) followed by CFM (67%), AMC (67%), COT (53%), CXM (52%), GEN (48%), S (47%) and AZM (43%). In a previous study, Hosain *et al.,* (2012) revealed that 80% of the *Salmonella* isolates were sensitive to ciprofloxacin followed by sulphamethoxazole (70%),chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%).In this present study, most of the isolates showed 38 % intermediate sensitivity against COT followed by CXM (33%) GEN (33%) and AZM (28%). Equal 24% of the isolates were found intermediate sensitive against CFM, AMC, AMX and S each. The sensitivity test also revealed that most of the *Salmonella* isolates were resistant to at least two drugs among tested dugs. No isolates showed complete resistance against tested drugs. Drug resistance of *Salmonella* is becoming increasingly serious, although the susceptibility of *Salmonella* to antimicrobial drugs varies among countries and regions. In Thailand, among 86 strains of *Salmonella* from pigs, the resistance rates to tetracycline and ampicillin were more than 81% (30). Degi *et. al.* (2021) conducted a study in Romania where the resistancy was observed to trimethoprim/sulfamethoxazole (68.8%), ampicillin (62.5%), ampicillin/sulbactam (56.3%), gentamicin (56.3%), nitrofurantoin (50.0%), and amikacin (31.3%). They found no resistance against ciprofloxacin, ertapenem, imipenem, levofloxacin, piperacillin/tazobactam, and tobramycin. Karim, (2019) conducted a study in Dhaka, Bangladesh, found that *Salmonella* isolates collected from pigeon oral and cloacal swab showed the highest rate of resistance to tetracycline (100%) followed by nalidixic acid (82%) and erythromycin (46%), amoxicillin (36%).Tsai, et al. (2007) reported that in Taiwan,

Salmonella strains from dogs showed resistance rates to sulfamethoxazole/trimethoprim (37.5%) and tetracycline (77.5%).

In this present study, the antibiotic sensitivity and resistance pattern of isolated *Salmonella* spp. from four different areas against 10 different drugs were investigated. Among these areas, the most sensitive of *Salmonella* was found in Dhanmondi (60%) and Mirpur (56%) followed by Mohammadpur (47%) and Agargaon (43%). The drug resistance of *Salmonella* isolates among Mohammadpur, Agargaon, Mirpur and Dhanmondi, areas were 28%, 25%, 20%, 17% respectively. The highest intermediate sensitivity was found in Agargaon (32%) followed by Mohammadpur (25%), Mirpur (24%) and Dhanmondi (23%). Wei *et. al.* (2020) revealed that the differences in the resistance to various antimicrobial agents may have been observed because of differences in serotypes and the use of antibiotics in humans, livestock, and pets in different countries and regions. They observed Macrolides, Quinolones and cephalosporins have become common in clinical use drugs for the treatment of *Salmonella* and their frequent use is an important cause of the high resistance to azithromycin, ciprofloxacin, cefixime and ceftriaxone.

Salmonella carriage was shown to be substantially greater in cats in this investigation. Furthermore, the majority of the *Salmonella* isolates were highly resistant to antimicrobial agents used in the treatment of bacterial infections in pets, animals, and humans, raising the risk of humans becoming infected with multi-drug resistant *Salmonella* through close contact with pets, particularly in children and the elderly. As a result, it is essential to improve public knowledge of zoonotic infections and encourage appropriate hygiene behaviors. Pet owners must also pay attention to the food their pets eat on a daily basis, ideally providing prepared food or pet food, to limit the risk of *Salmonella* infection and transmission to people. Routine laboratory isolation and drug sensitivity testing of the microbes, on the other hand, is impractical. As a result, frequent testing of the medication sensitivity and resistance pattern of organisms is more necessary in order to identify the best medicine of choice for the treatment of contagious diseases.

CHAPTER V

SUMMARY AND CONCLUSION

The present study was conducted for isolation and identification of *Salmonella* microorganisms from cats in four different areas of Dhaka city and also to perform a comparative study to determine the sensitivity and resistance pattern of the isolates to different antimicrobial agents.

After collection, the samples were subjected to various tests and experiments for isolation and identification of *Salmonella* spp. in cats. 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 SS agar for the determination of their colony characteristics. A total of 40 fecal samples were collected from the cats of four different areas of Dhaka city. Total Viable Count (TVC), Total Coliform Count (TCC) and Total Salmonella Count (TSC) were done by 10 fold dilution method. 21 isolates were found as *Salmonella* and the rest 19 couldn't be identified in this study. They were identified on the basis of colony morphology. Gram's staining technique were 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 also by Catalase test, MR test, V-P test and Indole production test.

The study was conducted according to breed, sex, age and area basis where the most prevalence of *Salmonella* were found between 1 to 3 years of age and the Agargaon and Mohammadpur area were subjected to most *Salmonella* infected area.

The study was also extended to investigate in vitro sensitivity and resistance pattern of the *Salmonella* 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 *Salmonella* were good sensitivity against CTR, CIP, AMC & CFM followed by COT, CXM and GEN while highest resistance were found to AMX & CFM.

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

From the present study it may be concluded that

(a) Fecal samples collected from the cats from four different areas of Dhaka city are infected with *Salmonella* spp.

(b) *Salmonella* infections of different animals and birds and also of human being may be treated effectively with CTR & CIP followed by AMC, CFM, COT, CXM and GEN.

Indiscriminate use of antimicrobial agents should be avoided in order to eliminate health hazards in man and animals caused by *Salmonella* organisms through preventing the development of multi-drug resistant mutants in nature.

CHAPTER VI

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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 extract	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 extract	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. Salmonella-Shigella agar

Proteose 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 |

6. 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

7. Methyl Red Indicator

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

8. 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

9. 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