CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Isolation and Identification of Microbial Pathogens Associated with *Musca domestica* L. (Diptera: Muscidae) Important for Human Health

by

Syed Nouman Hassan Shah

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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I dedicate my dissertation work to my family, teachers and friends. A special feeling of gratitude is for my loving parents for their love, endless support and encouragement.

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CERTIFICATE OF APPROVAL

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Abstract

House flies *(Musca domestica)*, feed and breed in disintegrating natural waste and are always in contact with various types of microorganisms. Since house fly lives in close association with people all over the world, they represent a risk of transmitting pathogenic microorganisms from infected sources to sterile places. The habitual movement of house fly from dirty materials such as human faeces, animal excreta, garbage, etc. makes them ideal candidate for disease transmission such as fever, dysentery, conjunctivitis, poliomyelitis, cholera, shigellosis, salmonellosis, typhoid, paratyphoid and others when settling on food. To explain pathogen vector capability of house flies by examining flies from various sites such as fruit and vegetable markets, garbage, slaughter houses in Rawalpindi and Islamabad Pakistan. The flies were captured by using nylon net. One hundred and fifty flies were collected on each location. Bacteria from fly samples were isolated using the different isolation techniques. *Escherichia spp, Enterobacter spp, Proteus spp, Pseudomonas spp, Klebsiella spp, Bacillus spp*, were isolated from external surfaces and internal organs of house fly. The emergence of antibiotic resistant pathogens is a growing public health concern. The *M.domestica* have been identified as potential carriers of antibiotic resistant pathogenic bacteria, *Neisseria gonorrhoeae, Staphylococcus aureus (MRSA), Klebsiella pneumonia, E.coli, Mycobacterium tuberculosis, Clostridium difficile*. It is suggested that these insects may serve as an important vectors of spreading disease-causing agents to the environment and hosts.

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Abbreviations

Acronym	**W**hat (it) **S**tands **F**or
M.domestica	*Musca domestica*
E.coli	*Escherichia coli*
S.aureus	*Staphylococcus aureus*
ART pathogens	Antibiotic resistant pathogens
P.aeruginosa	*Pseudomonas aeruginosa*
FDA	Food and drug Administration
MRSA	Methicillin-resistant *Staphylococcus aureus*
V.cholera	*Vibrio cholera*
H.pylori	*Helicobacter pylori*
SAR	Sulfonamide resistant
VRE	Vancomycin-Resistant *Enterococci*
FQRP	Fluoroquinolone-resistant *Pesudomonas aeruginosa*
PBPs	Penicillin-binding proteins
Macc Agar	MacConkey Agar
NUT Agar	Nutrient Agar
EMB	Eosin methylene blue media
MSA	Mannitol salt agar
API	Analytical Profile Index
K.pneumonia	*Klebsiella pneumonia*
ANOVA	Analysis of variance

Chapter 1

Introduction

The common housefly (*Mussca domestica*) that belongs to the family of Muscidae and it is considered that first fly of the species that intact properly with the extents were described by the Linnaeus in the year of 1758 that exhibited in the trends of over 400 described species of Muscidae. The *M. domestica* has been considered the most of the necessary insect pest with the intact behavioral incidences about human and domestic animals that may exhibit as the infection causing agent [1]. It lives with the closed interacting environment of people around the world and it has been associated that foods of humans and waste materials are in contact with it [2]. These food materials are responsible for the cause of disease and in the climates of warmer regions it is included with the subject of causing eye infections as well. These flies are responsible for the diseases as they are freely available to feed on the food of humans. It contains the pathogenic agents with their feeding and crawling extents. *M. domestica* is a standout amongst the most bottomless creepy crawly species and is nearly connected with people (synanthropic). They are plenteous in conditions, for example, open markets, fairs, eateries, reject dumps, creature pens, and restricted creature sustaining tasks and in homes. Because of their plenitude, relationship with people and fascination in both rottenness and human nourishment their part as sickness vectors is upgraded [3].

The *M.dometica* is necessary in the public health consideration to act in the extent of potential carrier with number of different pathogenic bacteria. House fly contain the ability that carry with number of germ particles and cause the typhoid, paratyphoid fevers, cholera and dysentery with poliomyelitis [4]. The mechanism of feeding and breeding established with the behavioral incidences of housefly and the synanthropic insects as house flies and develop the efficient vectors in the transmitting to human enteric parasites. The waste material products and debris are included with the regurgitate ingestion [5].

It has been reflected that about 100 of the pathogens are associated with the house fly that cause the diseases in humans and animals with including traits of typhoid, bacillary dysentery, cholera, anthrax and the tuberculosis with the biologic habits of feeding ophthalmia and the extent of infantile diarrhea as well as the parasitic protozoa and worms. The *M. domestica* is able to carry the pathogens of humans as bacteria from the sources of broiler farms, public parks, hospitals wastes, garbage, the slaughter house and the residential/domestic habitation [6]. Houseflies can transfer the pathogens such as *E.coli*, *S.aureus*, fungi and the *Helminthes* [7] and the nosocomial infections causing species *Klebsiella* from place to place [8].There four different well established and well recognized ways are used by house flies to transmit the infections as hairs and the surface of the body, the glandular hairs on the feet, regurgitation of the vomitus and the passage of alimentary tract.

The *Staphylococcal* food poisoning is caused by the enterotoxin that produced by the *S.aureus* is the important factor of food born disease across the world. The factors as dust, soil excretory or waste products of the human body as well as the animals are the major sources of staphylococcal contamination established by house flies [9].

Number of species that are included with the pathogenic bacteria have been isolated with the houseflies to collect from the multiple locations as restaurants [10], hospitals[11], city streets [12], poultry farms and the slaughter houses. It is considered that most of the extents are subjects with the diarrheal diseases including *Vibrio cholera* [13], *Shigella spp.* and the *Solmonella enterica* [14].

The bacteria that are attached on the external surface of fly are able to exist for few hours and those that are intact with internal side of the body as gut of the fly are able to survive for number of days [15]. In the studies it was examined that bacteria that are able to stay inside of the gut. The pathogenicity has been remained with the intact to surface of the body in the duration of 6 hours till thirty-five days. The transmitted pathogens use the media of body of flies. The bacteria that are highly common with transmitting body to include the causes of illness and poisoning of food in humans. The microbial pathogens that used to reservoirs with the extent of minimal hazard and people are intended towards the more risk in food items. The intact pathogens that are sticky with the abdomen of flies [16].

It is established that small number of bacteria that are isolated from the flies are intended with the conditional hygiene of prevailed items. The large number of bacteria are located on the legs get infection from the house fly and about twenty percent of bacteria recovered with the feet and ventral surface of the whole body. It is included with the seventy percent of visceral surface of body. The potential role of findings is exhibited with the dissemination [17].

The antibiotics that are defined with the substances or compounds in producing the certain microorganisms that may established in killing to inhibit the growth of other microorganisms. Currently, there number of distinguished synthetic compounds are designed to act like in same pattern as antibiotics as β-lactams, cephalosporins and carbapenems. In the initial stages these antibiotics frameworks were used in the humans and the veterinary medicines as well as in the agricultural production of food processing through the essential behavioral concerns of protecting the human and animal health against the pathogens. In the studies it has been exhibited that about 5000 different antibiotics have been discovered and about 100 of the actively uses in the treatment of human as well in the treatment of animal infections [18].

The derived applications of antibiotics are considered with the factors of pervasive antibiotic resistance problem and it is subjected with the short incidence that the

first application of antibiotics was initiated in the 1930s at time of isolation of *Staphylococcus aureus* in the hospitals [19]. In the recent years it is subjected that emerging trend of antibiotic is growing with the antibiotic resistant (ART) pathogens with the developing opportunity as MRSA *Clostridium difficile*, and the *Pseudomonas aeruginosa* that is becoming the major public health threat.

The main objective of research was to isolate and identified bacteria on the external body parts of Housefly (*M. domestica*) and also from gut. These flies were collected from different fruit and vegetable markets, garbage and slaughter houses within Rawalpindi and Islamabad, Pakistan.

1.1 Aims and Objectives

The general aim of this study is to assess the housefly skill as vector of various bacterial pathogens from garbage reservoirs, fruit and vegetable markets and slaughter house. And to evaluate the susceptibility of isolated bacteria. Multidrug resistance has turned into a significant issue in clinical medication. The principle concern is the expansion of obstruction improvement in destructive bacterial strains and its relationship to the substantial utilization of anti-infection agents. The safe microscopic organisms are chosen for by the specific weight and by dispersing and sharing the resistance qualities. House flies are a notable causative specialist of bacterial ailments.

The objectives of this research are:

1. Isolation and identification of pathogens (bacteria) associated with *M. domestica*
2. To determine the antibiotic resistance in bacteria isolated from housefly against the most common antibiotic drugs.

Accomplishing these objectives will enable us to decide with greater clearness the particular collaborations amongst vector and pathogen and particularly illustrate

the natural or mechanical capability of housefly in the transmission of detached bacterial pathogens.

1.2 Hypothesis

House flies spread bacterial pathogens from various territories of the sustenance provender zones to the encompassing condition.

Chapter 2

Literature Review

2.1 THE HOUSE FLY *(Musca domestica)*

2.1.1 Life cycle, Habitat and Behavior

The *M.domestica* is recognized as fly that belongs to the suborder of Cyclorrhapa. It is considered as most common in all categories of domestic flies and as per the estimate about 91 percent of the flies that are adopted as in the habitat of humans. It is also known as the insect that is most widely distributed in the world [20]. The report of FDA exhibited that house fly is the pest that is responsible to carry and transmit serious pathogens and these pathogens are able to cause multiple diseases in humans and animals.

FIGURE 2.1: Body structure of male and female house fly *(M.domestica)*

House fly (additionally housefly, house-fly or regular housefly), began on the steppes of focal Asia, however now happens on every possessed mainland, in all atmospheres from tropical to mild, and in an assortment of conditions from provincial to urban. They have an entire transformation with particular egg, hatchling or slimy parasite, pupa and grown-up stages. Every female fly can lay roughly 500 eggs in five to six clusters of around 75 to 150 [21] in warm, clammy material that will supply suitable sustenance for the larval formative. The Eggs are white and around 1.2 mm long; for the most part bring forth in 12-24 hours. Hatchlings (slimy parasites) brought forth from the eggs, live and feed on natural material (dead and rotting, for example, refuse or excrement. They are pale-whitish, $3 - 9$ mm long, more slender at the mouth end, and have no legs. They inhabit minimum multi week. Hatchlings have permeable pharyngeal edges in their cephalopharyngeal skeleton that is utilized to channel basic sustenance particles and microorganisms from the fluid substrates. House flies recreate and create as hatchlings in rotting natural issue, for example, creature compost, human would not, open privies, ruined creature bedding, litter and waste around sustenance and vegetable preparing plants, which all are regions abounding with differing and dynamic microbial networks [16]. Every single formative phase of house flies (e.g. hatchlings, pupae,

grown-ups) are normally debased with different microorganisms. House flies require a functioning microbial network for larval advancement[2]. Toward the finish of their third instar, the slimy parasites creep to a dry cool place and change into ≈8 mm long pupae, hued rosy or dark colored. Amid the pupation procedure, the third larval integument turns out to be hard and shapes the puparium. The grown-up flies rise up out of the pupae and live from two weeks to multi month. The grown-ups are normally dim, under 1/4-inch long with four dark stripes on the thorax and entire body is secured with hair-like projections. The grown-up house fly, is an extremely normal nuisance of extraordinary financial significance and a non-gnawing creepy crawly found in close relationship with people all through the world. Houseflies feed on fluid, semi-fluid substances (wealthy in starches), strong material (dung, open bruises, sputum), and clammy rotting (ruined nourishment, eggs foods grown from the ground) natural issue. They release spit or regurgitation on strong sustenances so as to diminish or to predigest it. They likewise disgorge somewhat processed issue and pass it again to the stomach area.

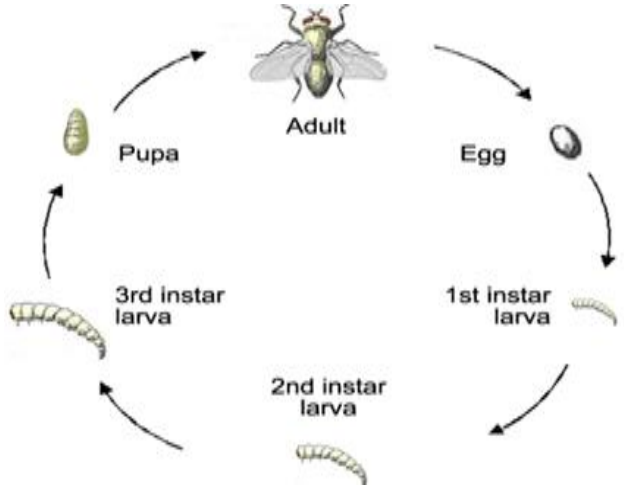

FIGURE 2.2: Housefly lifecycle: complete metamorphosis *(Lysyk,et al., 1991)*

2.1.2 Medical Importance of *M.domestica*

The nearby relationship of the housefly and microorganisms, and its part in transmission of pathogens, makes it a perfect model creature to examine the significance and variety of the microbiota of vector species. Barely any investigations have

tended to the variety in the microbiota of foulness flies under normal conditions albeit such variety is probably going to influence the phenotype of the fly. House flies (*Musca domestica L.; Diptera: Muscidae*) have for some time been thought about vectors or transporters of pathogenic microorganisms. House flies breed in creature squanders and rotting natural material, amid which their outside surfaces and nutritious water way wind up defiled by various microorganisms. Flies move from these septic substrates to local situations where they land and feed on human or creature sustenance. In view of this unpredictable and synanthropic encouraging conduct, flies exchange contaminants from rotted and sick sources to different situations [10]. This exchange might be exclusively because of dislodgement of microorganisms from their outside body parts [22]. In any case, organisms are additionally exchanged when flies spew septic harvest substance to condense dry nourishment substrates and encourage ingestion, or amid poo, which now and again happens simultaneously with sustaining [23]. Because of their capability to harbor and scatter pathogenic microbes, house flies fill in as mechanical and natural vectors of microorganisms that represent a peril to human and creature wellbeing.

Various types of pathogenic microorganisms have been disengaged from house flies gathered from various settings, for example, eateries [10], doctor's facilities [11], city boulevards [12], poultry ranches [24] and butcher houses [25]. House flies can convey microorganisms that reason diarrheal illnesses including Vibrio cholera [13], *Shigella spp.* [19] and *Salmonella enterica* [14]. A pestilence of typhoid fever episodes among military camps amid the Spanish-American war was credited to house flies that were transmitting *Salmonella typhi.* House flies additionally were embroiled as the reason for a 12 episode of colitis at a nursery school in country Japan in 1999, where flies conveyed and transmitted the *Escherichia coli* O157:H7 from a close-by creature holding office. Further, trial prove has shown that house flies can vector microscopic organisms, for example, *E. coli* O157:H7 [26], and *Yersinia enterocolitica.* A portion of the organisms that house flies convey inside their bodies are pathogenic, however the house flies themselves evidently don't

wind up sick. Assurance from microbial attack might be because of physical hindrances, for example, the fingernail skin of the exoskeleton and, in the midgut, the twofold layered Type II peritrophic grid (PM), which lines and ensures the epithelium. Past examinations have demonstrated that microbes don't escape entanglement inside the house fly PM [27]. Also, the midgut secretes stomach related chemicals including amylases, lipases, proteinases, and lysozyme that capacity to breakdown sustenance and hydrolyze ingested microbes [28].

It has been considered that bacteria are also able to isolate from the external surfaces of flies as *vibrio cholera* bacteria are recognized on the location of abdominal intersegmental membranes in the exoskeleton [29]. It is evident that some of the bacteria have been used to exist on the fly wings as *M. domestica* wings are not able to play significant role in the transmission of *V.cholerae* [22].

The typical examples of these bacteria are *Salmonella spp, Escherichia coli, Staphylococcus spp, Methicillin Resistant Staphylococcus* aureus, *Vibrio cholerae, Listeria spp, Shigella spp, Bacillus spp, Helicobacter pylori, Klebsiella spp, Serratia spp, Enterobacter spp,* and most of them are addressed in the reviews as [30] and [16]. These are exhibited with the summaries are given in below table 2.1.

TABLE 2.1: Bacterial associations with *M. domestica*

S.No	Fly Specie	Bacteria Isolated	References
1	*Musca domestica*	*Haemolytic streptococci, Coagulase positive staphylococci, Coliform bacilli, Proteus spp*	Shooter and Waterworth,1944
2	*Musca domestic*	*Helicobacter pylori*	(Grubel *et al.*, 1997)
3	*Musca domestica*	*Aeromonas hydrophila Citrobacter freundii Enterobacter agglomerans*	(Sulaiman *et al.*, 2000)

		Klebsiella oxytoca *Proteus mirabilis* *Proteus vulgaris* *Pseudomonas* *aeruginosa* *Pseudomonas* *fluorescens* *Burkholderia* *pseudomallei*	
4	*Musca domestica*	*Vibrio cholerae*	(Fotedar *et al.*, 2001)
5	*Musca domestica*	*Serratia marcescens*	(Cooke *et al.*, 2003)
6	*Musca domestica*	*Escherichia coli*	(Alam and Zurek ,2004)
7	*Musca domestica*	*Methicillin resistant* *Staphylococcus* *aureus* (MRSA)	(Boulesteix *et al.*, 2005)
8	*Musca domestica*	*Bacillus atrophaeus*	(Torres et al., 2006)
9	*Musca domestica*	*Bacillus sp* *Coccobacillus sp* *Staphylococcus sp* *Micrococcus sp* *Streptococcus sp* *Acinetobacter sp* *Enterobacter sp* *Proteus sp* *Escherichia sp* *Klebsiella sp*	(Nazni *et al.*, 2005)
10	*Musca domestica*	*E. coli* *Klebsiella spp* *Aeromonas spp* *Pseudomonas spp* *Staphylococcus spp*	(Rahuma *et al.*, 2005)

		Streptococcus spp	
11	*Musca domestica*	*Shigella spp* *Salmonella spp*	(Ugbogu *et al.*, 2006)
12	*Musca domestica*	*Coagulase-negative staphylococci* *Escherichia coli* *Klebsiella pneumoniae* *Viridans streptococci* *Morganella morganii* *Enterobacter cloacae* *Providencia stuartii* *Enterococcus spp* *Providencia alcalifaciens* *Providencia rettgeri* *Citrobacter freundii* *Enterobacter agglomerans* *Bacillus spp* *Proteus mirabilis* *Mixed Gram negative bacilli* *Citrobacter amalonaticus* *Enterococcus faecalis* *Enterobacter aerogenes* *Proteus penneri*	(Sukontason *et al.*,2007)

		Pseudomonas spp *Micrococcus spp* *Staphylococci spp* *Staphylococcus aureus*	
14	*Musca domestica*	*Salmonella enterica*	(Holt *et al.*, 2007)
15	*Musca domestica*	*Acinetobacter Baumanni* *Bacillus cereus* *Bacillus pumilus* *Bacillus thuringiensis* *Cronobacter sakazakii* *Escherichia coli 0157:H7* *Methylobacterium persicinum* *Shigella dysenteriae* *Staphylococcus saprophyticus* *sciuri* *Staphylococcus xylosus*	(Butler *et al.*, 2010)
16	*Musca domestica*	*Enterococcus faecalis* *E. hirae* *E. faecium* *E. casseliflavus*	(Ahmad *et al.*, 2011)

2.1.3 Types of Diseases Transmitted by Housefly

House flies for the most part spread irresistible ailments and these are caused by infections, microscopic organisms, protozoa and even nematodes (worms like the roundworm or threadworm). There are more than 100 pathogens (ailment causing creatures) that are related with house flies. Dissimilar to different bugs, for example, mosquitoes or ticks, these pathogens don't particularly require a creepy crawly vector. The house fly assumes no particular part in the existence cycle of these pathogens, however the fly is just a bearer in a few cases. Diarrheal sicknesses are a portion of the more typical ailments spread by house flies. This incorporates microorganisms, for example *Shigella, Enterococcus* and related microbes which usually cause diarrheal sicknesses and are found in the stool of inhabited with these diseases [31]. A portion of the maladies spread by house flies incorporate:

1. Mechanical transmission of living beings on its hairs, mouthparts, vomitus and dung: Parasitic illnesses: growths of protozoa e.g., *Entamoeba histolytica, Giardia lamblia*, and eggs of helminths, e.g., *Ascaris lumbricoides, Trichuri strichiura, Hymenolepis nana* and *Enterobiusvermicularis.*

2. Bacterial infections: typhoid, cholera, loose bowels, pyogenic cocci, and so forth. House flies have been shown to be vectors of *Campylobacter* and *E. coli* O157:H7 utilizing PCR. House flies can be observed for bacterial pathogens utilizing channel paper spot cards and PCR.

3. Infections: entero viruses, poliomyelitis, viral hepatitis (A and E) and so forth. It is apparent that flies can spread numerous irresistible ailments aimlessly, yet luckily, these infections are not much of the time spread by flies. Different courses are normally more typical and powerful for their transmission and the house fly can spread sickness through a few courses. It doesn't chomp like the pony fly or tsetse fly to infuse the pathogen into a man. Rather malady causing specialists are spread on its body, in its mouth parts or through its vomitus and excrement. House flies feed aimlessly on an extensive variety of natural issue, from dung to nourishment (organic

products, vegetables and meat). It is through this contact with the thing to that it is sustaining upon and even by coordinate contact with individuals that malady causing specialists are gained and passed on. The infective measurements for every pathogen shifts incredibly and here and there only a couple of microorganisms are required to cause genuine sickness. The defiled issue containing these microorganisms and even only the organisms itself, that are procured from one source may hold fast to the fly or be passed out in its vomitus and defecation. The tainted issue and organisms are then passed onto nourishment once the fly terrains as well as feeds on it. The circumstance is additionally exacerbated if the nourishment isn't refrigerated permitting the vaccination measurements of organisms to duplicate before the sustenance is eaten.

2.2 Breeding Sites

In spite of the fact that sewage isn't an issue for urban occupants in created countries, however creature defecation (manure) can at present be an issue, particularly with domesticated animals. Pet waste that isn't legitimately discarded can fill in as reproducing destinations and draw in flies. It may not generally be conceivable to wipe out all remainders of pet dung, particularly in a covered home yet normal washing of the floor coverings and utilization of local bug sprays over the zone can help essentially. The same applies for natural issue that isn't disposed of by the correct channels. Trash transfer units have assumed a critical part in averting of natural product, vegetable and sustenance remains from representing a danger in general garbage containers. At the point when not accessible, natural issue ought to be fixed firmly in junk packs before arranging. Fertilizer stores in the garden, particularly where excrement is utilized, can likewise fill in as another fascination source and reproducing site for flies [32].

2.3 Contact between Flies and Pathogens

A housefly just needs a couple of moments to reach a wellspring of pathogens keeping in mind the end goal to transport it somewhere else. Defecation are one of the substances that are loaded down with an extensive variety of organisms, particularly on the off chance that it is passed from a man who is sick. Current toilets have disposed of this hazard to a vast degree in created countries. Be that as it may, dirtied child diapers are as yet a hazard if not disposed of appropriately. More established individuals, who are crippled, similar to the sick, may likewise be a source and parental figures need to guarantee that any excreta are cleaned as quickly as time permits. Grown-up diapers might be valuable in such manner yet it must be discarded in like manner. Open injuries and bruises and tainted eyes can likewise fill in as another source. Creature butcher may represent another issue, especially in regions where chasing is a typical practice. Brisk butchering and disposing of the leftovers suitably, such as covering insides, can decrease this hazard [32].

2.4 Contact with People, Food and Eating Utensils

House flies can't be totally annihilated and even the best endeavors in the home can decrease fly populaces yet it can rapidly return. Keeping in mind the end goal to anticipate maladies, the fly's contact with individuals, sustenance and eating utensils ought to along these lines be avoided or intruded. Self-shutting entryways and nets/screens over entryways and windows are extremely powerful in keeping of flies from entering the home. Indeed, even electric fans blowing air over an entryway can block flies from entering the home. At the point when these measures can't stop flies altogether, at that point airborne splashes and fly traps might be [32].

2.5 Antibiotics

Antibiotics are such type of compounds which are produced by certain microorganisms that could kill or inhibit the growth of other microorganisms. Nowadays, in antibiotics also include many synthetic compounds with similar functions such as β-lactams, cephalosporins and carbapenems. After the initial discovery, the antibiotics have been broadly used in human, as a veterinary medicine, and have been essential for protecting human and animal health against pathogens. Number of times bacterial infections have been effectively treated by antibiotics, it is greatly enhanced the life probability and quality of lives of human beings and other animals globally. It has been estimated that, more than 5000 antibiotics have been discovered and about 100 are actively used to treat human and animal infections [18]. There are wide range of applications of antibiotics, which started shortly after the first applications of antibiotics in the 1930s when sulfonamideresistant *Streptococcus pyogenes*, methicillin- resistant *Staphylococcus aureus* (MRSA) and *streptomycin−resistant Mycobacterium tuberculosis* were first isolated in hospitals [19]. In recent years, the rapid emergence of hospital-acquired infections by antibiotic resistant (ART) pathogens and opportunistic pathogens, such as MRSA, *Clostridium difficile*, *vancomycin-resistant Enterococci* (VRE) and fluoroquinolone-resistant *Pesudomonas aeruginosa* (FQRP) have become a major public health threat [20].

2.5.1 Examples of antibiotics used in treatment of infections caused by bacteria

2.5.1.1 Amikacin

It is broad-range spectrum antibiotic and use against the different bacterial infections in humans. It includes joint infections, intra-abdominal infections, meningitis, pneumonia, sepsis, and urinary tract infections. The side effects of Amikacin includes hearing loss, balance problems, kidney problems and can cause paralysis.

Amikacin is mostly used against the multi-drug-resistant gram negative bacteria especially *Pseudomonas spp*, *Klebsiella spp*, *Enterobacter spp* [33].

2.5.1.2 Ceftazidime

Ceftazidime is a semisynthetic, broad-spectrum, beta-lactam antibacterial drug. This medication belongs to a class of drugs known as cephalosporin antibiotics. It works by stopping the growth of bacteria. Ceftazidime is a bactericidal agent that acts by inhibition of bacterial cell wall synthesis. Ceftazidime has activity in the presence of some β-lactamases, both penicillinases and cephalosporinases, of Gram-negative and Gram-positive bacteria.It is used for the bacteria that cause the joint infections, meningitis, pneumonia, urinary tract infection [34].

2.5.1.3 Cefixime

Cefixime is used to treat a wide variety of bacterial infection. This medication is known as a cephalosporin antibiotic. Cefixime is used to treat certain infections caused by bacteria such as bronchitis (infection of the airway tubes leading to the lungs); gonorrhea (a sexually transmitted disease); and infections of the ears, throat, tonsils, and urinary tract [35].

2.5.1.4 Ceftriaxone

Ceftriaxone was introduced in 1980s.It is broad-range antibiotic and use against the different bacterial infections in humans these include meningitis in adults and infants, gonorrhea, acute pyelonephritis and spontaneous bacterial peritonitis. Ceftriaxone used extensively to treat bacterial infections due to its stability against P-lactamases, produced by members of *Enterobacter spp* [36].

2.5.1.5 Imipenem

Imipenem is a broad-spectrum antibiotic is given in figure. It is recommended in initial therapies and is used for the treatment of severe bacterial infections including nosocomial infections, febrile neutropenia, ventilator associated pneumonia(VAP),hospital acquired pneumonia(HAP) [37].Imipenem the IUPAC name (N-formimodoyl-thienamycin) is not used individually because it rapidly degraded by enzyme dehydropeptidase which produced by the human kidney and has toxic effects on kidney.Therefore it is used with cilastatin in the ratio of 1:1.It act as an inhibitor of the dehydropeptidase enzyme and neutralize the toxic effect of the antibiotic [38]. Transpeptidases enzymes also called penicillin binding proteins (PBPs) cross link the peptidoglycan and provides the rigidity to bacterial cell wall. These PBPs are the main targets for imipenem. Imipenem inactivates the transpeptidases moreover it inhibits the Dalanine carboxypeptidase in *E.coli* [39].

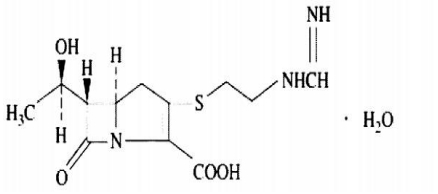

FIGURE 2.3: N-formimodoyl-thienamycin (Rodloff *et al.*, 2006)

2.5.2 Classes of Antibiotics

Antibiotics can be classified by their mode of action. The antibiotics may be bactericidal or bacteriostatic, the spectrum of activity of antibiotics are two types (broad spectrum or narrow spectrum), and routes of administration are (oral or injection).The most well-known class among the mostly used antibiotics in clinical therapy is the $\beta-$lactam antibiotics. These antibiotics usually kills the bacteria (bactericidal) works against the Gram positive and Gram negative bacteria, these antibiotics interfere with the chemical composition of the peptidoglycan layer of bacterial cell walls by irreversibly blocking penicillin-binding proteins (PBPs), including carboxypeptidases, endopeptidases and transpeptidases.PBPs are a group

of proteins that facilitate crosslinking of newly synthesized peptidoglycan to the existing cell wall structure [24]. Once treated with β-lactam antibiotics, susceptible bacterial cells develop a weak cell wall and are eventually subject to cell lysis. There are four major classes of antibiotics and their derivatives belong to the β-lactam antibiotics category, including penicillin, cephalosporins, carbapenems and monobactams. Based on their antimicrobial activities and antibacterial spectrum, they can be further divided into different generations and groups. For example, now there are presently five generations of cephalosporins with differing antibacterial spectrums and activities in each generation. Improved activity against Gram-positive bacteria than Gram-negative bacteria was found in first generation cephalosporins, while the next generation of cephalosporins has significantly greater activity against the Gram-positive bacteria than Gram-negative. In this way, each subsequent generation of cephalosporins has significantly greater activity than previous ones. Gram-negative antimicrobial has better properties than the preceding generation, but with reduced activity against Gram-positive organisms, with the exception of fourth-generation cephalosporins which have true broad-spectrum activity [40].

Cephalosporin exist in broad spectrum category due to their broad spectrum of activity, cephalosporins are one of the most widely prescribed classes of antimicrobials. The earlier generations of cephalosporins are commonly used for community-acquired infections, while the later generation antibiotics, with their better spectrum of activity against Gram-negative bacteria make them useful for hospital acquired infections. [41].

Cephalosporins are also commonly used in veterinary medicine. For example, first and second generation of cephalosporins are approved worldwide for the treatment of mastitis infections in dairy cattle [39]. While another third generation cephalosporin, ceftiofur, has worldwide approved for the treatment of respiratory disease in swine, ruminants (cattle, sheep and goats) and horses and has also been approved for foot rot and metritis infections in cattle.Ceftiofur has also been approved in various countries for early mortality infections in day-old chicks [39].

Aminoglycosides are another example of bactericidal antibiotics. Their key structure includes an aminocyclitol ring in the molecule, with different glycosidic linkages and side chains among members in this family [41]. Aminoglycosides affect bacterial cells synthesis by displacing Magnesium and calcium ions such as Mg^{2+} and Ca^{2+} on the outer bacterial membrane, disrupting normal permeability. Moreover, aminoglycosides can also be bacteriostatic, as they impair the growth of bacterial cells by binding to the 30S ribosomal smaller subunit of the bacterial ribosome. In this way it inhibits the protein synthesis. The bacteria which are susceptible to aminoglycosides are primarily aerobic Gram-negative bacteria, such as *Klebsiella spp*, *Enterobacter spp* and *Pseudomonas aeruginosa* [42]. The most common members of aminoglycosides which are present in market including gentamicin, kanamycin, neomycin and streptomycin. The members of Aminoglycosides are primarily used when treating infections on the surface of the skin and in the respiratory system.

Glycopeptides are another type of bactericidal antibiotics. Similar to β-lactam antibiotics, glycopeptides affect the bacterial cells by inhibiting the cell wall peptidoglycan synthesis. However, in contrast to $\beta-$lactam antibiotics, glycopeptides mode of action includes the interaction with a substrate of the enzyme which catalyzes transglycosylase reaction, and apparently shields it from the active site of the enzyme. One of the well-known glycopeptides is vancomycin, has been given the most attention because of its performance in treating MRSA infections [43].

In recent years, however, vancomycin resistant organisms are becoming more common. Because resistance may be due to the widely use of glycopeptides as growth promoters in food animal production. For example, until 2000, avoparcin, chemically similar to vancomycin, has been widely used around the world (except North America) as a growth promoter [44].

TABLE 2.2: Common Antibiotic Classes (Wang et al., 2013)

S.No	**Name of Drugs**	**Class of drug**	**Mode of action**
1	Ciprofloxacin	Fluoroquinolone	Broad-spectrum, antibiotic

			inhibit cell division
2	Levofloxacin	Fluoroquinolone	Broad-spectrum, antibiotic acts as a bactericide
3	Imipenem	Carbapenem	Inhibition of cell wall, synthesis of various gram-positive and gram-negative bacteria
4	Aztreonam	β-lactam	Inhibits mucopeptide synthesis in bacterial cell wall, thereby blocking peptidoglycan crosslinking
5	Meropenem	Carbapenem	Inhibition of cell wall synthesis
6	Amikacin	Aminoglycoside	Disrupts bacterial protein synthesis by binding to the 30S ribosome of susceptible organism
7	Gentamicin	Aminoglycoside	Interrupting protein synthesis
8	Tobramycin	Aminoglycoside	Tobramycin binds irreversibly to a specific aminoglycoside receptor on bacterial 30S ribosomal subunit and interferes with initiation complex between messenger RNA and 30S subunit thereby inhibiting initiation of protein synthesis
9	Tazobactam	β-lactamase	Tazobactam is a penicillanic acid sulfone derivative and $\beta-$lactamase inhibitor with antibacterial activity
10	Cefoperazone	Cephalosporins	Inhibits bacterial cell wall synthesis

11	Cefixime	Cephalosporin	Inhibits bacterial cell wall synthesis
12	Cefuroxime	Cephalosporin	Inhibits bacterial cell wall synthesis
13	Amoxycillin -clavulanic acid	Penicillin-like antibiotics	Stopping growth of bacteria synthesis
14	Ceftriaxone	Cephalosporin	Inhibits bacterial cell wall synthesis
15	Cefoxitin	Cephalosporin	Inhibits bacterial cell wall synthesis
16	Doxycycline	Tetracyclines	Inhibits bacterial cell wall synthesis
17	Trimethoprim-sulphamethoxazole	Sulfonamides	Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by comparing with para-aminobenzoic acid (PABA). Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting the required enzyme, dihydrofolate reductase
18	Tigecycline	Glycylcyclines	Inhibits protein synthesis
19	Piperacillin	penicillin	Inhibits bacterial cell wall synthesis
20	Sulbactam	β-lactamase	β-Lactamase Inhibitor

Chapter 3

MATERIAL AND METHODS

3.1 Required Materials

Table 3.1: Materials Required

S.No	Materials	Chemical Composition
1	House fly	Adult ,wild sized
2	0.7% saline solution	0.7 gm Nacl +100ml dH_2O
3	Nutrient Agar	Peptic digest of animal tissue 5gm/L Beef extract 3 gm/L, Agar-agar 15 gm/L
4	Mac Conkey agar	Peptone 17gm/L, Peptocomplex 3gm/L, Lactose 10 gm/L, Bile Salts 5 gm/L NaCl 5 gm/L, Neutral Red 0.05 gm/L, Agar 15 gm/L
5	Eosin methylene blue	Peptone 10 gm/L, Lactose 10 gm/L, Dipotassium hydrogen phosphate 2 gm/L Eosin yellow 0.4 gm/L, Methylene blue 0.065 gm/L, Agar No.2 15 gm/L
6	Mannitol salt agar	Agar 15 gm/L, Phenol red 0.025 gm, D-mannitol 10 gm, NaCl 75 gm Peptones 10 gm, Meat extract 1 gm

7	Crystal violet solution	5 gm crystal violet in 25 ml ethanol, 0.25 gm ammonium oxalate in 25 ml dH_2O, 4.5 ml dH_2O + 0.5 ml Crystal violet solution + 25 ml Ammonium oxalate
8	Grams Iodine solution	1 g of iodine + 2 g of potassium iodide + 3 g of sodium bicarbonate in 300 ml of water
9	Ethanol	Ethanol absolute 95 ml + distilled water 5 ml
10	Safranin	0.5 gm Safranin + 100 ml ethanol
11	Catalase test(Chemicals)	H_2O_2 3%, Acetophenetidine
12	Oxidase test(Chemicals)	Formulation per 100 ml N,N,N,N-Tetramethyl-p-phenylenediamine Dihydrochloride 0.60gm, Stabilizing Agent 0.02 gm Dimethyl Sulfoxide 100 ml
13	Distilled water	dH_2O

3.2 Apparatus

Table 3.2: Apparatus

S.No	**List of Apparatus**	**Specifications**
1	Insect Nylon Net	Nylon
2	Eppendorf tubes	Plastic tubes, 1.5 ml
3	Petri dishes	Glass , 25 ml size
4	Micropipette	SOCOREX-100-1000 μl, SOCOREX-10-100μl, SOCOREX-0.1- 2 μl
5	Inoculation loop	Iron metal
6	Glass rod	Solid glass rod
7	Glass slides	Company Sigma-Aldrich 25*75mm
8	Spirit lamps	90% conc ethanol
9	Glass beakers	250 ml and 500 ml

10	Dissecting Needle	Made up of iron specific for the dissection of flies
11	Dropper	Plastic
12	Conical Flask	Glass, 250 ml
13	Glass Bottles	Jam jars
14	Magnetic stirring bar	Medium and large sized
15	Graduated cylinder or Measuring cylinder	10 ml, 250 ml, 100 ml
16	Foreceps	Iron
17	Parafilm	Bemis company
13	Aluminium foil	Company home foil
13	Spatula	Iron

3.3 Equipments

Table 3.3: Equipments

S.No	**List of Equipments**	**Specifications**
1	Autoclave	Company Wise clave, 121°C
2	Microscope	Company LABOMED, 1000X
3	Laminar flow	Company Technico Scientific,Model=PA11SV00
4	Incubator	$10 - 45\ ^0C$ temperature range, Pakistan
5	Refrigerator	Dawalance, -20°C
6	Weighing scale	Company Mettler Toledo Model=ML802E
7	Microwave oven	Haier
8	Magnetic stirrer	Company WiseStir
9	Vortex Mixer	Company Wisemix

3.4 Methodology

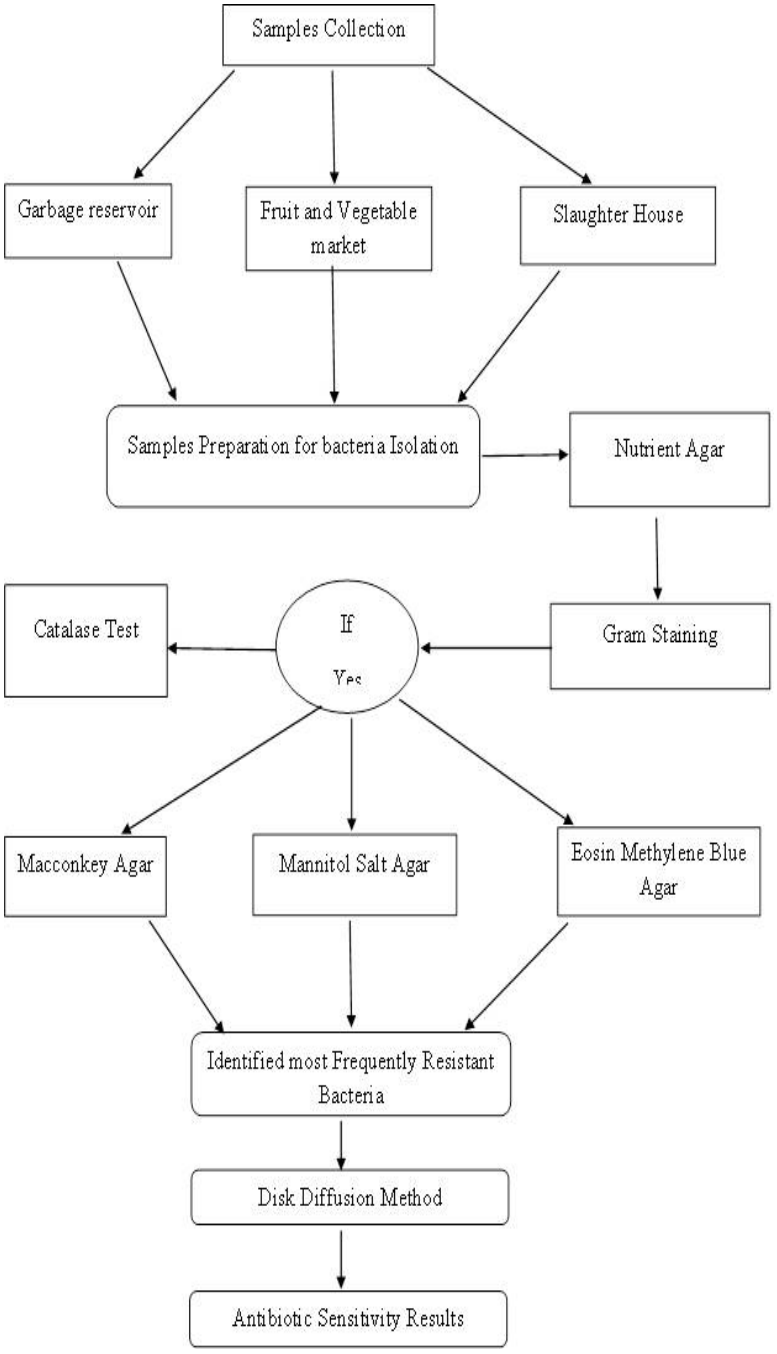

FIGURE 3.1: Research Methodology Flow Chart

3.4.1 Sampling Locations

Keeping in view the significance of locations as concluded from literature following locations were selected for sample collection.

TABLE 3.4: The locations and number of house flies collected form three different locations

S.No	Sampling Locations	Number of samples
1	Fruit and vegetable Market (Peer wadhai, Islamabad), (Khanna pull, Islamabad), (Raja Bazar, Rawalpindi)	150/per location
2	Slaughter House (Model Town Humak, Islamabad), (Kahuta, Rawalpindi), (Raja Bazar, Rawalpindi)	150/per location
3	Garbage (Khanna pull, Islamabad), (Shakrial, Rawalpindi), (Iqbal Town, Islamabad)	150/per location

3.4.2 Collection Method Using Insect-Net

Adult *M. domestica* were collected from fruit & vegetables markets, slaughter houses and garbage reservoirs by using nylon insect-Net. One hundred fifty flies were captured from each location. Then the house flies were brought to the laboratory and were kept in refrigerator at 20°C. Randomly 3-5 flies from each sample were placed individually in autoclaved eppendorf tube containing 3 mL 0.7 percent saline solution and mashed them by using sterile dissecting needle and mashed content of each individual fly were kept in eppendorf tube till further procedure. For control 3-5 houseflies were placed in eppendorf tube and were washed with the distilled water and then washed water was kept in the eppendorf tube till further processing [15].

3.4.3 Nutrient Agar Preparation

To confirm the presence or association of bacterial pathogens with *M. domestica*, the samples were first cultured on the nutrient agar [45]. Nutrient agar were prepared according to the manufacturer procedure. 0.46g of the powder was weighed using a balance and transferred into 20 ml distilled water contained in a conical flask. The media was stirring properly using magnetic stirrer and was then heated in microwave oven for 60 seconds to dissolve the media completely. This was followed by autoclaving at 121°C for 30 minutes.The prepared media of 20ml was dispersed into each petri dish and allowed to solidify at room temperature. Total 21 plates were prepared to replicate the each location including control.

3.4.4 Media Inoculation

0.5ml of the diluent from eppendorf tubes of different samples were transferred to the petri dishes using a micropipette. Each location sample including control were replicated. The inoculated petri dishes were placed upside down in an incubator at 37°C for 48hrs.

3.4.5 Gram Staining

Preparation of Crystal Violet Solution

Initially dissolve 5 gm crystal violet in 25 ml ethanol to make crystal violet solution. Similarly dissolve 0.25 gm ammonium oxalate in 25 ml sterilized water to make an oxalate solution. The working solution obtained by mixing 0.5 ml of crystal violet solution with 4.5 ml of distilled water and 25 ml of ammonium oxalate stock solution [42].

Preparation of Methylene Blue Solution

Initially dissolve 1 gm of methylene blue in 100 ml of ethanol to make methylene blue solution. Similarly add 0.03 gm of Potassium hydroxide in 300 ml of sterilized water. By mixing both solutions yields the working solution [46].

Preparation of Gram Iodine Solution

One gram of iodine crystals, Two gram of potassium iodide, and 3 gram of sodium bicarbonate dissolve in 300 ml of sterilized water. Gram iodine stock solution is prepared [42].

Preparation of Gram Safranin Solution

Initially dissolve 2.5 gram safranin in 100 ml of 95 percent concentrated ethanol to make a stock solution. The working solution is obtained by adding one part of stock solution in five parts of sterilized water [42].

Gram Staining Procedure

The gram staining method is developed by Hans Christian Gram in 1844. It is a differential staining method of differentiating bacterial species either Gram positive or Gram negative. A glass slide was cleaned with 75 percent ethyl alcohol then place a drop of sterile water on slide and a loop of sample inoculum was picked using sterilized inoculation loop and was placed on glass slide. It was allowed to air dry. The heat fixation was done. One drop of crystal violet solution was applied on the slide with the specimen and was left for 30 seconds, and it was rinsed with sterile water. After that, 3-4 drops of Grams iodine was added on the slide and was left for one minute. After one minute, the slide was rinsed with sterile water. The slide was washed with decolorizer (95 percent ethanol) and was run over the stained area until no more colour washes out, and the slide was again rinsed with sterile water. Safranin 3-4 drops were applied on the slide with the specimen for

one minute and the rinsed with sterile water .The prepared slides were observed under microscope at 100X oil emulsion objective lens. The gram negative bacteria shows pink colour while gram positive bacteria shows purple colour [42].

3.4.6 Identification of Bacterial Pathogens

3.4.6.1 Identification of Bacterial Pathogens Using Differential Media

To identify the bacteria, the specimens were further cultured on differential media MacConkey Agar (MAC), Eosin Methylene Blue Media (EMB), Manitol Salt Agar (MSA) [45].

MacConkey Agar

1.1g of the dry powder was weighed using a balance and transferred into 20ml distilled water contained in a conical flask. The media was stirred properly using magnetic stirring bar and was then heated in microwave oven for 60 seconds to dissolve the media completely. This was followed by autoclaving at 121°C for 30 minutes. The prepared media of 20 ml was dispersed into each petridish and allowed to solidify at room temperature. Total 21 plates were prepared to replicate the each location including control.

Eosin Methylene Blue Media

0.75g of the dry powder was weighed using a balance and transferred into 20ml distilled water contained in a conical flask. The media was stirred properly using magnetic strirrer and was then heated in microwave oven for 60 seconds to dissolve the media completely. This was followed by autoclaving at 121°C for 30 minutes. The prepared media of 20 ml was dispersed into each petridish and allowed to solidify at room temperature. Total 21 plates were prepared to replicate the each location including control.

Mannitol Salt Agar

2.22g of the dry powder was weighed using a balance and transferred into 20ml distilled water contained in a conical flask. The media was stirred properly using magnetic stirrer and was then heated in microwave oven for 60 seconds to dissolve the media completely. This was followed by autoclaving at 121°C for 30 mins. The prepared media of 20ml was dispersed into each petridish and allowed to solidify at room temperature. Total 21 plates were prepared to replicate the each location including control.

Media Inoculation

0.5ml of the diluent from eppendorf tubes of different samples were transferred to the petri dishes using a micropipette. Each location sample including control were replicated. The inoculated petri dishes were placed upside down in an incubator at 37°C for 48hrs.

3.4.6.2 Identification Using Oxidase and Catalase Test

Catalase test

This test exhibit the nearness of catalase, a compound that catalyzes the arrival of oxygen from hydrogen peroxide (H_2O_2). It is utilized to separate those microscopic organisms that creates a compound catalase, for example, staphylococci, from non-catalase delivering microbes, for example, streptococci. The catalyst catalase intervenes the breakdown of hydrogen peroxide into oxygen and water. The nearness of the compound in a bacterial disconnect is obvious when a little inoculum is brought into hydrogen peroxide, and the quick elaboration of oxygen bubbles happens. The absence of catalase is clear by an absence of or feeble air pocket creation. For this reason, 24hours old culture was used [45].

$$2H_2O_2 + \text{Catalase} \rightarrow 2H_2O + O_2(\text{gas bubbles})$$

Catalase Reagent Preparation

For routine testing of aerobes, use commercially available 3% hydrogen peroxide. Store the hydrogen peroxide refrigerated in a dark bottle. For the identification of anaerobic bacteria, a 15% H_2O_2 solution is required [47]. The catalase test is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from *Bacillus* species, which are positive [48].

Test Procedure

1. Use a sterile loop or to transfer a small amount of colony growth taken from the all three locations grown on nutrient agar for 24 hrs on 37°C in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.

Immediate formation of bubbles or the absence of bubbles were used to evaluate the test as positive or negative. Catalase-positive microbes incorporate strict aerobes and facultative anaerobes, for example, *Bacillus* and *Staphylococcus*. They all can breathe utilizing oxygen as a terminal electron acceptor. Catalase-negative microscopic organisms might be anaerobes, or they might be facultative anaerobes that exclusive mature and don't breathe utilizing oxygen as a terminal electron acceptor (i.e. *Streptococci*).

3.5 Antibiotic Drugs

In this study, antibiotics were used according to the recommendations of clinical and Laboratory Standards Institute published in M100s Performance Standards for Antimicrobial Susceptibility Testing (26th edition).Isolated *E.coli* and *K.pneumoniae* were tested for their susceptibility against five antibiotics selected from major Antibiotic groups given in table 3.5

TABLE 3.5: Antibiotic drugs used in Disk Diffusion method

S.No	Class of Antibiotic	Antibiotic drugs	Disk content
1	Aminoglycosides	Amikacin	30μg
2	Cephalosporin	Ceftazidime	30μg
3	Cefems	Cefixime	5μg
4	Cefems	Ceftriaxone	30μg
5	Carbapenems	Imipenem	10μg

3.6 Kirby Bauer Method Procedure

1. Muller-Hinton agar media was set having standardized composition.
2. Muller-Hinton agar media was poured into 150 mm petri dishes at a level of 4mm deep.
3. The agar media was maintained at pH range of 7.2 to 7.4 and broth culture was used for inoculation.
4. The culture plates was made inoculated by streaking a sterile swab passed through broth culture of *E.coli* and *K.pneumoniae* separately.
5. The agar media plates inoculated with *E.coli* and *K.pneumoniae* separately was left for about five minutes to dry.
6. The antibiotics disks were transferred to the inoculated agar plates by using sterilized needles.
7. The discs were gently press by using flame-sterilized foreceps to make sure that each disc is in contact with surface of agar media properly.
8. The plates were incubated at incubation temperature of 37°C for the night.
9. The zone of inhibition was measured for each antibiotic disc by using scale or screw gauge which determined the effectiveness status of the antibiotic against *E.coli* and *K.pneumoniae* as shown in below (Figure 3.2 and 3.3).

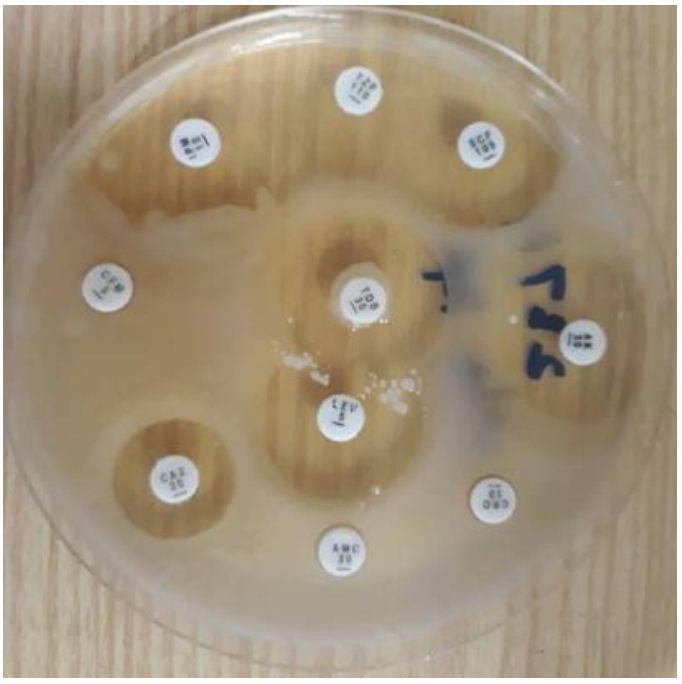

FIGURE 3.2: Zone of inhibitions for *E.coli*

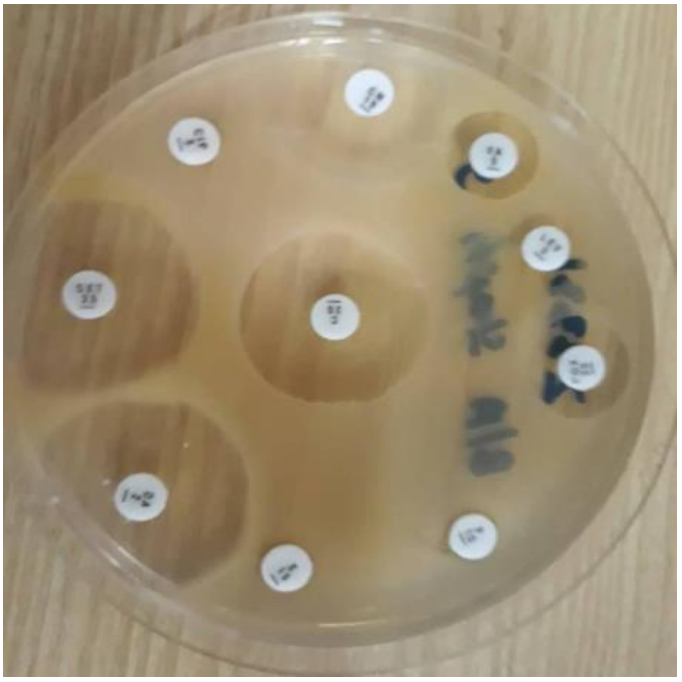

FIGURE 3.3: Zone of inhibitions for *K.pneumoniae*

Criteria for Antimicrobial susceptibility test were taken from M100s (26^{th} Edition) of CLSI published in January 2016 Table 3.6.

Table 3.6: CLSI criteria for zone of inhibition (mm) of the Antimicrobial

S.No	**Antibiotic Drugs**	**Symbol**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin	AK	>=17	15-16	<=14
2	Ceftazidime	CAZ	>=21	18-20	<=17
3	Cefixime	CF	>=19	16-18	<=15
4	Ceftriaxone	CFT	>=23	20-22	<=19
5	Imipenem	IMP	>=23	20-22	<=19

Chapter 4

RESULTS AND DISCUSSION

4.1 Growth of Bacteria on Media and Gram Staining

4.1.1 Nutrient Agar

For the culturing of non-fastidious microorganisms Nutrient agar culture medium is recommended. Nutrient agar is commonly used. The Nutrient agar is chemically composed of peptone, beef extract and agar. This type of simple formula composition provides the sufficient nutrients to bacteria which are favorable for their growth and reproduction and their genome replication [6]. Pathogens were isolated form *M. domestica* collected from slaughter house were grown on Nutrient agar. The results showed that it contains bacterial growth indicating that housefly carries the pathogens. Nutrient Agar allows the growth of gram-positive as well as gram-negative bacteria. It is clear from the growth in the petridishes that *M. domestica* collected from Slaughter house contain gram-negative and gram-positive bacteria as shown in Figure 4.1 and 4.2.

FIGURE 4.1: Bacterial growth on nutrient agar

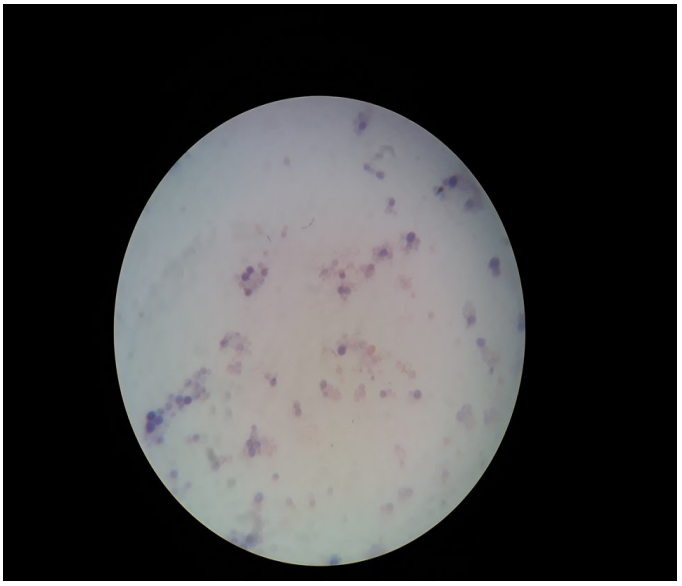

FIGURE 4.2: Gram staining

Similarly, *M. domestica* collected from Fruit and vegetable market and garbage reservoirs contains bacterial growth confirming the status of housefly as vector for bacterial pathogens. Further confirmation was done by performing gram staining technique that showed the presence of both gram positive and gram negative bacteria in the culture. Bacterial colonies isolated from *M.domestica* collected from Fruit and Vegetable Market are shown in Figures 4.3 and 4.4 while Bacterial

colonies isolated from *M.domestica* collected from garbage reservoirs are shown in Figures 4.5 and 4.6.

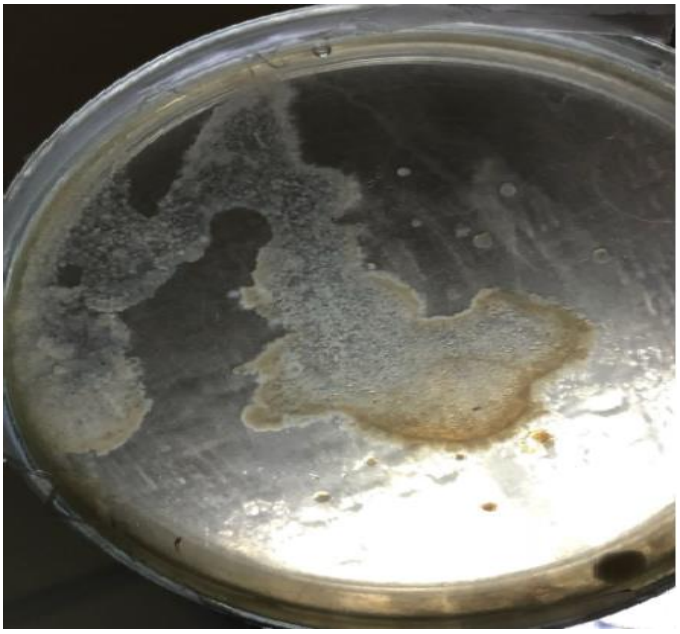

FIGURE 4.3: Bacterial Growth on nutrient agar

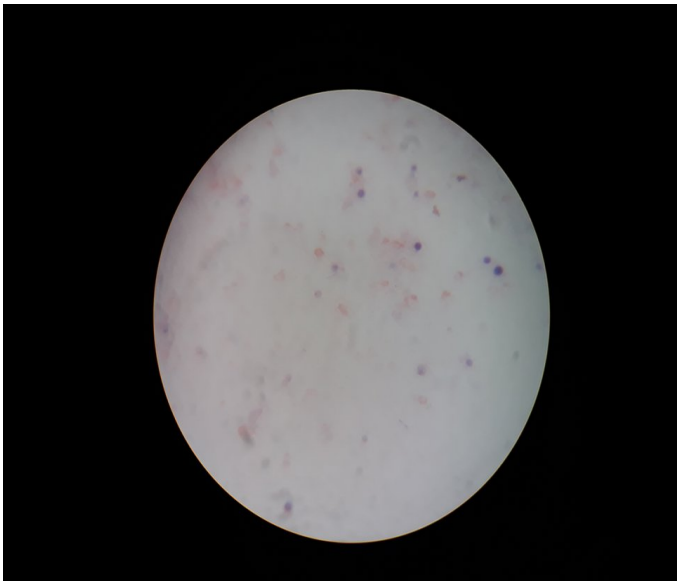

FIGURE 4.4: Gram staining

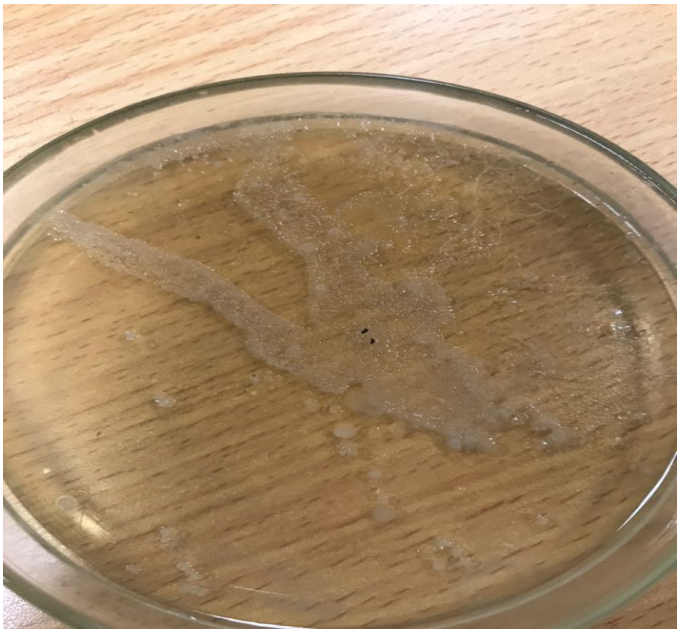

FIGURE 4.5: Bacterial Growth on nutrient agar

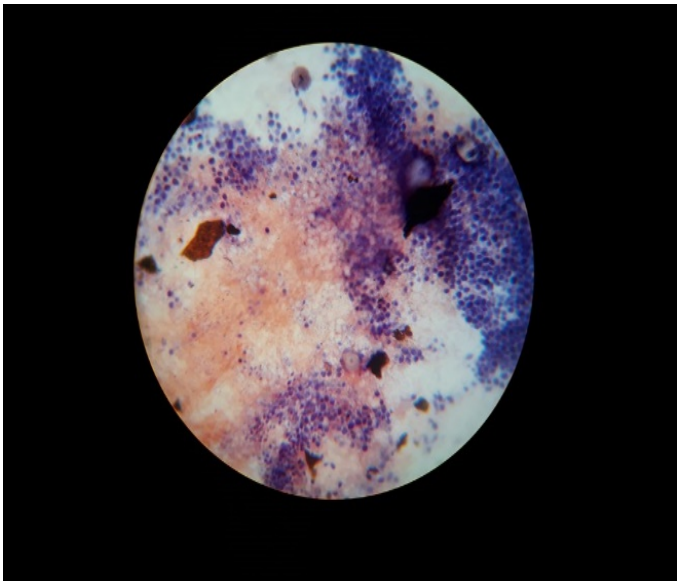

FIGURE 4.6: Gram staining

4.1.2 MacConkey Agar

MacConkey medium is commercially available in dehydrated form. The dehydrated form of this medium consists of 20.0 g peptones, 10.0 g lactose, 1.5 g bile salts, 5.0 g sodium chloride, 13.5 g agar, 0.03 g neutral red, and 0.001 g crystal violet. Final pH 7.1 $\pm$ 0.2. The characteristic of MAcConkey agar for not allowing

growth of gram-positive bacteria and can be used for identification of organism. The purpose of MacConkey agar used is to isolate the gram negative bacteria extracted from the gut of *M.domestica* and also used to differentiate between lactose fermenting gram negative bacteria from lactose non-fermenting gram negative bacteria. MacConkey agar is chemically composed of gelatin and peptones which extracted from meat and casein. These different chemicals provides the necessary nutrients and vitamins which helps in the growth of microorganisms. These Organism includes *E. coli*, *Enterococcus*, *Aerobacter pseudomonas*. McConkey media only allows the growth of gram-negative bacteria hence it inhibits the growth of gram-positive bacteria (Himedia: Technical Data).Pathogens were isolated by maceration method from adult houseflies. *M. domestica* collected from slaughter house, fruit and vegetable market and garbage reservoirs locations and were grown on MacConkey agar. MacConkey agar inhibits the growth of gram-positive bacteria. The results showed that all the three locations specimens showed the bacterial growth indicating the presence of gram negative bacteria. MacConkey agar contain bile salts which prevent most of gram-positive organisms to grow. Neutral red and crystal violet present in this medium are very lethal to bacteria. Gram-negative bacteria are more resistant to the dyes present in this medium than gram-positive bacteria. Moreover, bile salts reduces toxicity for gram-negative bacteria and increase toxicity for gram-positive bacteria. Gram negative bacteria usually shows more significant growth on medium and these bacteria can differentiate due to their lactose fermenting ability. The lactose fermenting bacterial strains shows red or pink coloured colonies and which may be surrounded by a zone of acid precipitated bile. The red colored pattern is just due to the releasing of acid from lactose, when pH of medium drops below 6.8 in the result.

Absorption of neutral red starts and lateral change in colour of the dye occurs. While lactose non-fermenting bacterial strains like *salmonella* and *shigella* shows transparent and colourless appearance which normally do not change the medium appearance (Himedia: Technical Data).

The samples were collected from Slaughter house and Fruit and vegetable market, cultured samples showed a shiny pink color colony indicating the presence of *E.*

coli. *E. coli* colony grown on MacConkey media isolated from *M.domestica* shown in figures 4.7 and 4.8 (Himedia: Technical Data).

FIGURE 4.7: Slaughter House

FIGURE 4.8: Fruit and Vegetable Market

The result of pathogens isolated from garbage samples also showed gram negative bacterial growth. The colorless colonies indicate the probability of presence of *Salmonella spp* and *Proteus vulgaris* as shown in Figure 4.9 (Himedia: Technical

Data). To further confirm the presence of gram negative bacteria, gram staining procedure was carried out.

FIGURE 4.9: *Proteus spp* grown on MAC isolated from *M.domestica* collected from garbage reservoirs

4.1.3 Eosin Methylene Blue Media (EMB)

In dehydrated premixed form, EMB is available for commercial use. The commercial powder produces a medium comprising the following components when it is rehydrated (g/L): peptone (Bacto-peptone or Gelysate) 10.0, lactose 5.0, sucrose 5.0, dipotassium phosphate 2.0, agar 13.5, eosin 0.4, and methylene blue 0.065. Final pH is 7.2 ± 0.2. EMB contains the dyes methylene blue and eosin which inhibit Gram positive bacteria, thus favoring growth of Gram Negative. Eosin methylene blue media helps in the identification of *E.coli*, from nonpathogenic lactose-fermenting gram negative rod shaped bacteria [49].

All samples were collected from Salughter house, Fruit and vegetable market and Garbage reservoirs isolated from *M. domestica* were grown on EMB media. *M. domestica* sample collected from slaughter house showed the presence of *E.coli* with the green metallic sheen colour and *Klebsiella pneumonia* with pink mucoid colour.

Bacterial growth on EMB isolated from *M. domestica* collected from slaughter house are shown in Figures 4.10 and 4.11 (Highmedia: Technical Data).

FIGURE 4.10: *E. coli*

FIGURE 4.11: *Klebsiella pneumonia*

Fruit and Vegetable market samples cultured showed the luxuriant growth of *E.coli* with characteristic green colour on media and *Klebsiella pneumonia* glossy pink

colour. *E. coli* and *Klebsiella pneumoniae* growth on EMB isolated from *M. domestica* collected from Fruit and vegetable market are shown in Figure 4.12.

FIGURE 4.12: *E. coli* and *Klebsiella pneumoniae* growth on EMB isolated from *M. domestica* collected from fruit and vegetable market

E. coli and *Klebsiella pneumoniae* growth on EMB isolated from *M. domestica* collected from Fruit and vegetable market

The *Enterobacter* species having colour pattern pink without sheen was present in samples.These samples were collected from different garbage reservoirs as shown in Figure 4.13. Dyes with reversible oxidation-reduction potentials, such as methylene blue, are toxic to bacteria. EMB media inhibits the growth of gram-positive bacteria so the samples collected cultured on EMB showed the presence of gram negative bacteria. Dyes with reversible oxidation-reduction potentials, such as methylene blue, are toxic to bacteria [3]. Gram staining of the culture grown in EMB showed the presence of gram negative bacteria.

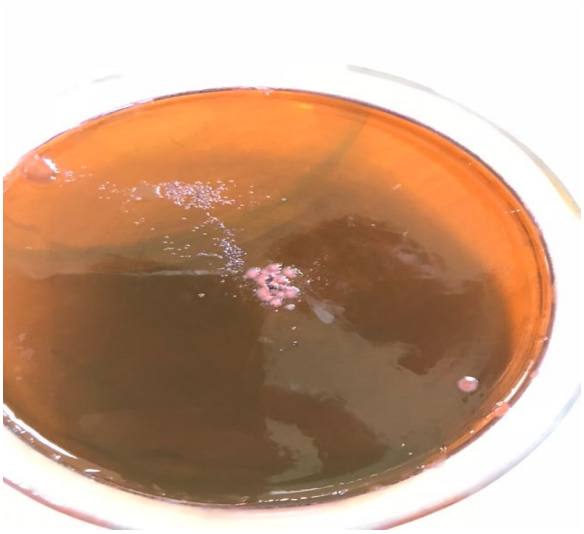

FIGURE 4.13: *Enterobacter sp* growth on EMB isolated from *M.domestica* collected from fruit and vegetable market

4.1.4 Mannitol Salt Agar (MSA)

For the isolation of *Staphylococci*, Mannitol salt agar (MSA) is used that is both selective as well as differential medium. This medium consists of 7.5% sodium chloride, thats because it is chosen for those bacteria which can bear high salt concentrations. The only carbohydrate in the MSA is sugar mannitol which is used to distinguish bacteria on the basis of fermentation. Mannitol fermentation is demonstrated by changing of media colour, not only by colony colour. This process is predominantly significant as several micrococci are pigmented. The plates that are inoculated, refrigerated over the time may show colour loss. Re-incubation can bring back some colour is observed by some scientists on the other hand some people have found that driving plates thicker reduces the colour loss [28].

All the samples collected from three locations showed no growth ruling out the probability of presence *Staphylococci* in the isolates. The absence of any bacterial growth also indicates or confirm the results of MAC and EMB results as MSA inhabits the growth of *E.coli*, *Klebsiella*, *Enterobacter* and *Proteus spp.* MSA agar paltes showing no growth of the samples isolated from *M. domestica* collected from

slaughter house, fruit and vegetable market and garbage reservoirs are shown in (figures 4.14, 4.15 and 4.16).

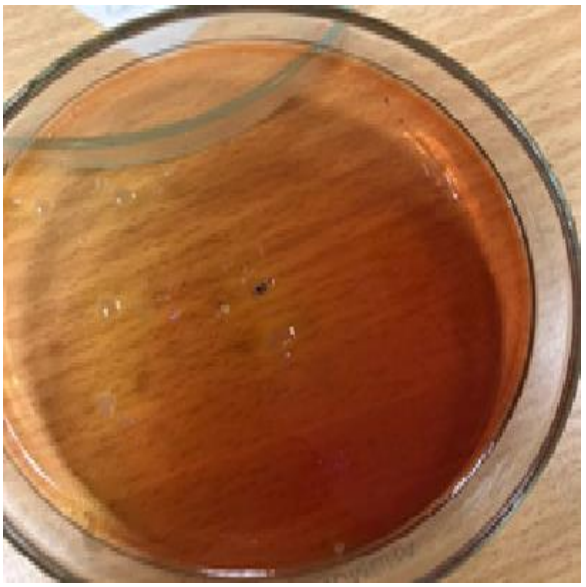

FIGURE 4.14: Slaughter House

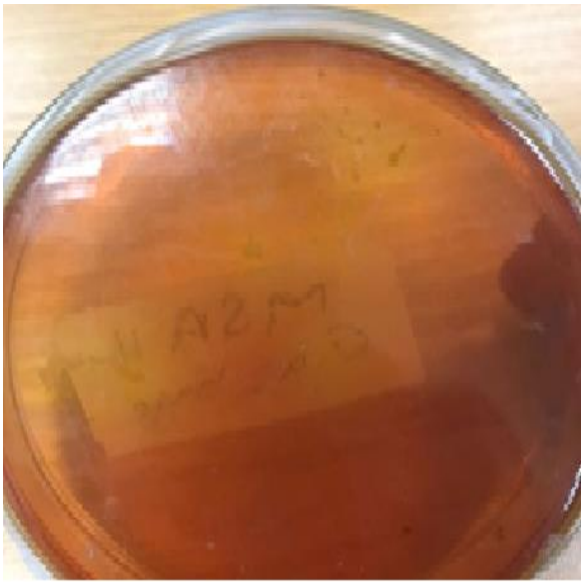

FIGURE 4.15: Fruit and vegetable market

FIGURE 4.16: Garbage Reservoirs

4.1.5 Catalase Test

Catalase test is primarily performed to differentiate between Gram-positive cocci shaped bacteria which are the members of genus *Staphylococcus* which are catalase positive while the members of genus *streptococcus* and *Enterococcus* are catalase negative. The use of catalase test is to differentiate between gram positive and gram negative bacteria like aerotolerant strains of *Clostridium* which are basicaly catalase nagative from *Bacillus spp* which is basicaly gram positive specie. Another type semi quantitative catalase test is applied for the identification of *Mycobacterium tuberculosis* bacteria which cause Tuberculosis in humans.The catalse test is also used for the identification of *Enterobacteriaceae*.The different members of *Enterobacteriaceae* family are basicaly catalase positive.

Based upon the results of MSA that showed the absence of *Staphylococci*, catalase test with15% H_2O_2 solution was performed to differentiate the aerotolerant strains of *Clostridium* from *Bacillus* species. It was observed that instantly the process of bubble formation starts.The bubble formation process indicates the presence of *Bacillus* species in all the three samples which were isolate from *M.domestica* and

which were collected from location Slaughter house hence proving the ability of housefly act as a mechanical carrier of *Bacillus* species.

In few years of last decade much attention has been given to *M.domestica* because these flies have potential in the transmission of bacterial pathogens. Different studies have shown the symbiotic relationship of bacterial pathogens with *M.domestica*. The most common examples of bacteria which can be isolate from the body surfaces of *M.domestica* are *E.coli*, *salmonellaspp*, *Staphylococcus spp*, Methicillin Resistant *Staphylococcusaureus* (MRSA), *Vibrio cholerae*, *Listeria spp*, *Shigellaspp*, *Bacillus spp*, *Helicobacter pylori*, *Klebsiellaspp*, *Serratiaspp*, *Enterobacter spp* many of these species have been discussed in recent researches [16]. The proboscis of flies contains large number of fine hairs, when the flies sits on the surface of garbage or filthy places they collect harmful bacteria from environmental detritus instantly. It has been demonstrated that when the flies lands on wounds of guinea pigs they carries *Anthrax bacilli*.Number of flies collected from different food processing units and factories and their microbiological analysis of vomitus and faeces showed the presence of *Bacillus spp* [15].

House flies and bluebottles landed on the surface of food the *Bacillus atrophaeus* spores drop off in food [37].It has been found that the flying insects have potential to transmit the gram positive rod shaped, spore forming bacteria so the mechanical transmission of *Bacillus spp* especially spore forming *Bacillus cereus* is possible through flying insects [50]. In another research [15] observed flies from different breeding areas like food halls, food processing units and poultry farms. *Bacillus spp*, *Coccobacillusspp*, *Staphylococcus spp*, *Microccus spp*, *Streptococcus spp*, *Acinetobacter spp*, *Enterobacterspp*, *Proteus spp*, *Escherichia spp*, *Klebsiellaspp*, isolated from the excretory products of house flies. (Sulaiman *et al.*, (1988)) in Malysia had isolated different bacterial species from the body of *M. domestica* and *Chrysomya megacephala* from hospitals. Some of the bacterial pathogens isolated are the *Acinetobacter spp*, *Bacillus spp*, *Enterobacter spp*, *Proteus spp*, *Escherichia spp* and *Klebsiella spp*. Eighteen bacterial species were found to be associated on the body surface of *M. domestica*, by [51] including *Burkholderia pseudomalle*

igram negative bacteria that caused the disease melioidosis, But, [52] *B. pseudomallei* was not found in any of the samples captured from different locations. It is also mentioned in different past research works that *E.coli* lives in the mouth part the crop of house flies for minimum four days.

[53] made a comparison between the transmitted bacteria through houseflies and American cockroach. They isolated *E. coli*, *Staphylococcus spp*, *Streptococcus spp*, *Shigella spp*, *Salmonella spp*, *Proteus spp*, *Klebsiella spp*, *Enterobacter spp*, *Serratia spp* from the external body surface of both insects. House flies are responsible for the transmission of multiple pathogenic organisms and the anatomy of these flies exhibit the sites of contamination. The three most common means are established through which houseflies are able to transmit the pathogens. The mechanism of transmission is based on the anatomy and as well as on the behaviour of fly and their habit of association with the waste products as animal manures and execretion of the humans [36]. The *E.coli* are present on the alimentary canal and on the mouthparts of *M.domestica* [26].The number of bacteria have been identified which have been isolated from the surfaces as gut of flies particularly *Salmonella enterica* [14].

Vibrio cholera bacteria are recognized on the location of abdominal inter segmental membranes in the exoskeleton [29]. It is evident that some of the bacteria have been used to exist on the wings of flies as *M. domestica* wings as Vibrio cholera [22]. *Klebsiella spp* are present in the respiratory tract of human and causes pneumonia and also causes others eye infections. It also have ability to produce urinary tract infection. *Klebsiella spp* also causes nosocomial infections which are associated with the inflammation of upper respiratory tract. *Enterobacter spp* are also known to cause urinary tract infection [54].

Staphylococcus spp, and *Bacillus spp*, are causative agents for diarrhea and is common in Pakistan. It is a potential in houseflies to carry different pathogenic bacteria that are resistant to multiple antibiotics have also been reported in different research works show that many of the *Enterococcus spp* isolates from house flies were resistant to tetracycline and erythromycin. Data from the past studies

shows that house flies which grows on animal manure and decaying organic material can play important role in development and dissemination of these antibiotic resistance commensal bacteria in environment. In conclusion, the current study shows that houseflies collected from different locations are all capable of carrying ART bacteria. The free exposure of houseflies to animal farms, poultry farms, slaughter houses facilities resulted in greater prevalence of antibiotic resistance bacteria and there is a great capability of house flies to carry multi-drug resistant bacteria. To control the production of house flies is still an important public health concern in the 21^{st} century especially in developing countries.The conclusion is it is proved that the flying insects act as a mechanical vectors and responsible for the spreading of diseases. The possible way is to eliminate or reduced the breeding places of flies. To eliminate the breeding sites it is necessary to improve the sanitary conditions and hygienic conditions.

4.1.6 Antibiotic Sensitivity Test

The antibiotic sensitivity test was performed only for the samples which were collected from slaughter house (Model town Humak, Islamabad), (Raja bazaar, Rawalpindi) and (Kahuta, Rawalpindi).The isolates of *E.coli* and *k.penumonia* for the five antibiotics. For this purpose disk diffusion method was used. [55]. The disk diffusion susceptibility method is simple and practical and standardized method which is mostly used in clinical labs. In this test the bacterial inoculums introduced to the surface of large (150 mm diameter) Muller-Hinton agar culture plate. The disc diffusion method is mostly prefer due to their simplicity because the test does not require any specialized equipment and the final results can easily interpreted by clinicians. The drug resistance status of all of the antibiotics in the form of zone of inhibition against *k.pnumonia* is given in the appendex 1 and 2. The percentage ratio of sensitivity was 100% in amikacine and imipenem with 100%. Whereas Ceftazidime high resistance ratio i.e. 78.33, intermediate with 15% and susceptibility 6.67% as shown in table 4.1.

TABLE 4.1: Percentage ratios of Antibiotic drugs against *K.pneumonia*

	Amikacin	**Ceftazidime**	**Cefixime**	**Ceftriaxone**	**Imipenem**
Resistant	0	47	15	44	0
Intermediate	0	9	10	5	0
Sensitivity	60	4	35	11	60
R%	0	78.33	25.00	73.33	0
I%	0	15.00	16.67	8.33	0
S%	100	6.67	58.33	18.33	100

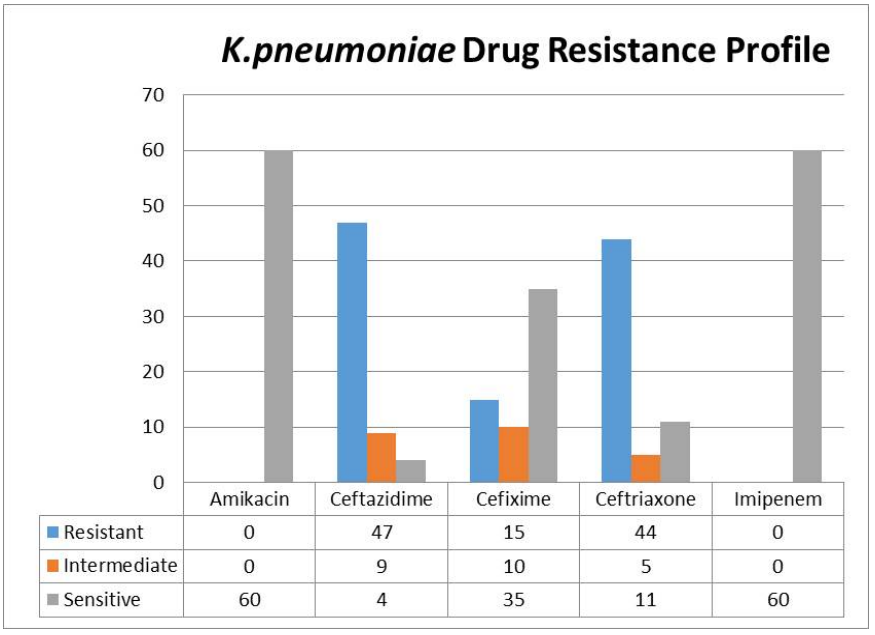

FIGURE 4.17: *K.pneumonia* Drug Resistance Profile

ANOVA (Analysis of variance) is a statistical method which is used to find and analyze the differences among group mean values.It provides the statistical analysis test which describes the means of several groups either they are equal or not.In this method the fishers exact test is applied which is statistically most significant test that helps to calculate exactly the deviatron from null hypothesis instead of approximation. SPSS 17.0 was used for analysis of variance of antibiotics used in this study the degree of freedom for *k.pnumonia* for between group was 4 and within group was 295. Table 4.2

The F value calculated is 93.26 which is beyond the F (critical) equals to 2.40 that indicates that results are significantly different. It means that the drug tested showed variation in antibiotic sensitivity of resistance. Table 4.2

TABLE 4.2: Analysis of variance of Antibiotics

Source of Variation	**SS**	**df**	**MS**	**F**	**alpha**	**F crit**
Between Groups	9097.22	4	2274.305	93.266	0.05	2.402
Within Groups	7193.566	295	24.384			
Total	16290.786	299				

The similar trends of drug sensitivity were observed in *E.coli* with the sensitivity ratio of 100% in amikacin and imipenem. High the resistance ratio was found in Ceftriaxone with value of 81.67% and sensitivity ratio of 13.33% as given in Table 4.3.

TABLE 4.3: Percentage ratios of Antibiotic drugs against *E.coli*

	Amikacin	**Ceftazidime**	**Cefixime**	**Ceftriaxone**	**Imipenem**
Resistant	0	20	26	49	0
Intermediate	0	3	12	3	0
Sensitivity	60	37	22	8	60
R%	0	33.33	43.33	81.67	0
I%	0	5.00	20.00	5.00	0
S%	100	61.67	36.67	13.33	100

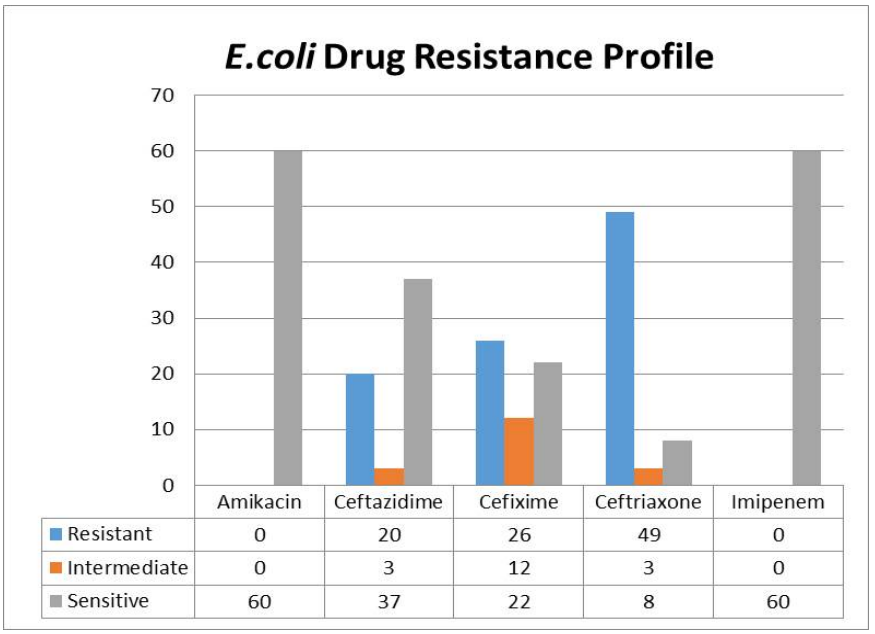

FIGURE 4.18: *E. coli* Drug Resistance Profile

ANOVA (Analysis of variance) is a statistical method which is used to find and analyze the differences among group means values.It provides the statistical analysis test which describes the means of several groups either they are equal or not.In this method the fishers exact test is applied which is statistical most significant test that helps to calculate exactly the deviatron from null hypothesis instead of approximation [56]. SPSS 17.0 was used for analysis of variance of antibiotics used in thus study the degree of freedom for *E.coli* for between group was 4 and within group was 295. Table 4.4

The F value calculated is 51.29 which are beyond the F (critical) equals to 2.40 that indicate that results are significantly different. It means that the drug tested showed variation in antibiotic sensitivity of resistance. Table 4.4

TABLE 4.4: Analysis of Variance of Antibiotics

Source of Variation	**SS**	**df**	**MS**	**F**	**alpha**	**F crit**
Between Groups	4892.013	4	1223.003	51.295	0.05	2.402
Within Groups	7033.516	295	23.842			
Total	11925.53	299				

It has been accepted that the number of bacteria has create or creating the resistance against the antibiotic drugs. It is very serious issue in worldwide. The extensive use of antibiotics in the field of agriculture and medicine producing resistance in different gram negative bacteria against the antibiotic drugs. There are number of bacteria which are present on different food reservoirs these bacteria have create resistance against the number of drugs. It has been reported in different studies the *E.coli* which have create resistance against many antibiotic drugs is found in vegetables, poultry, egg, milk and raw meat [57].In another research it is reported that the *E.coli* that has been create a highest percentage of resistance against the drugs were isolate from chicken (23.3%), vegetable salad (20%), raw meat (13.3%), raw egg-surface (10%) and unpasteurized milk (6.7%).

The *E.coli* is the gram negative bacteria and most common pathogen in humans. So now a days antibiotic resistance in *E.coli* is the main concern because *E.coli* is responsible for number of infectious diseases like it is the main cause of UTI (Urinary tract infection), common cause of hospital-acquired infections and also a cause of diarrhea [58].The resistant *E.coli* bacterial strains transmit the antibiotic resistance determinants to other strains of *E.coli* and it is reported in different studies that the resistant *E.coli* have ability to transmit the antibiotic resistant determinants in other bacterial strains within the gastrointestinal tract [30].

It has been observed that the vegetables and meat contains large number of *E. coli* and *klebsiella spp.* These bacterial strains extracted from the patients who consumed contaminated fruits and vegetables [44].In contrast the persons who consumed sterile diet have lower number of *E.coli* in their clinical tests reports of feces. The bacteria which passed alive through digestive tract to colon are often transient. The resident flora having a protective effect against intruders. The bacteria which are responsible for the transmission of antibiotic drug resistance is still possible, so if our consumed food contains resistant bacteria it could be an important source of creating resistance in gastrointestinal tract.

It is suggested that it is possible the bacterial populations spreading the resistance from one ecosystem to other [2].The spreading of antimicrobial resistance among

different bacterial species is a major problem in worldwide and this problem is increasing day by day.

The antibiotic drugs are mostly used for the treatment of infected persons against different infections.The number of findings recommend that poor selection of antibiotics may lead to create resistance in various bacteria and in the result the treatment against the bacterial infections become more difficult [26].

The resistance against antibiotics in *E.coli* is reported in worldwide. In present the infections which were caused by *E.coli* has been increasingly problematical due to the production of resistance in bacteria [59]. From last few decades resistance against the cephalosporins is increasing in the members of enterobacteriaceae. The main cause is spreading of Extended-spectrum β-Lactamases (ESBL) [9].

Hence the aim of this research was to find the antimicrobial sensitivity pattern of *E.coli* and *K.pneumoniae* that was isolated from the *M.domestica* which were collected from slaughter houses, garbage reservoirs, fruit and vegetable markets in the areas of Rawalpindi and Islamabad Pakistan. The results of this study demonstrate the findings of susceptibility and resistance against the five different drugs.

Chapter 5

CONCLUSION AND RECOMMENDATION

The house flies (*M.domestica*) plays a significant role in public health. It is involved in the spreading of different food-borne diseases. These common house flies (*M.domestica*) act as a vector because when these flies sits on the garbage or other waste materials the number of bacteria attached with their body surfaces.when these flies lands on different fruits, vegetables and meat the bacteria drop off from their body surfaces, so it creates contamination. In the present research samples of *M.domestica* were found to be contaminated with pathogens included *E.coli*, *K.pneumonia*, *Proteus spp*, *Enterobacter spp*, *Bacillus spp*. Most commonly found associated pathogens with the samples of *M.domestica* from locations slaughter house, garbage reservoirs and fruit & vegetable markets were *E.coli* and *K.pneumonia*. Pathogens isolated from slaughter house samples are *E.coli* and *K.pneumonia*. These are pathogenic bacteria and responsible for different infectious diseases in humans. The results showed that the bacterial pathogens contaminate the meat, fruits and vegetables by using *M.domestica* as a vector. The present research suggested that the eating materials must be sterile. Improvement in environmental health conditions through the use of an appropriate waste disposal system. In order to attain the good hygienic practices it is necessary to prevent the contamination of fruit, vegetables and cattle products.

Now a days the bacteria creating resistance against the antibiotic drugs is a challenging problem in worldwide. Furthermore in this research the microbial activity against the antibiotic drugs were tested. The *E. coli* and *k. pneumonia* susceptibility was checked against the five antibiotics amikacin, ceftazidime, cefixime, ceftriaxone and imipenem. *E.coli* is highly resistant against ceftriaxone and highly sensitive against amikacin and Imipenem while *K.pneumonia* is highly resistant against ceftazidime and highly sensitive against amikacin and imipenem. The previous studies suggested that *E.coli* strains which are present inside the gastrointestinal tract of human creating resistance against the drugs and also transferring this ability into other bacterial species. The future goal of this research is the pathogens must be isolated from external body parts and gut intestine separately to identify the locations of pathogens being carried by *M.domestica*. Antibiotic sensitivity must be studied on molecular level from cafeterias and restaurants which are food source areas in close interaction with the human. The other goal is to analyze the genome of pathogenic bacterial species and identify the genes which are responsible of creating resistance in bacteria with the passage of time and also find the different environmental factors which effect on them.

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Appendix

Table 5.1: *E.coli* Zones of Inhibition

S.No	Amikacin	Ceftazidime	Cefixime	Ceftriaxone	Imipenem
1	21	22	17	11	25
2	23	24	9	13	26
3	18	16	16	12	24
4	23	14	9	7	24
5	18	23	13	18	31
6	22	26	24	6	25
7	25	15	12	11	26
8	18	29	22	8	27
9	19	16	19	11	24
10	21	15	12	13	30
11	23	28	19	11	34
12	28	22	18	19	32
13	23	24	14	17	29
14	22	29	22	18	24
15	31	21	12	25	28
16	19	13	11	25	27
17	29	23	24	8	24
18	20	23	16	11	28
19	30	19	18	13	33
20	21	26	10	7	24
21	25	23	14	11	24

22	27	29	11	14	28
23	23	14	13	28	25
24	22	32	11	9	27
25	28	16	19	15	29
26	19	13	22	12	32
27	20	25	18	21	33
28	17	26	13	13	24
29	32	16	12	11	27
30	21	26	21	17	27
31	21	22	14	15	26
32	19	21	9	19	23
33	21	19	18	29	25
34	22	23	18	11	23
35	19	14	29	14	25
36	19	12	28	13	31
37	25	22	22	13	24
38	17	26	20	11	27
39	19	16	11	17	26
40	18	21	19	16	25
41	24	26	21	16	26
42	22	27	23	25	27
43	22	13	27	21	31
44	22	29	11	10	25
45	18	15	7	13	24
46	22	26	9	18	25
47	29	27	9	11	24
48	20	13	12	10	26
49	22	28	18	19	23
50	27	12	17	6	25
51	21	23	12	17	28
52	21	29	20	21	26

53	18	19	21	25	26
54	22	15	13	19	29
55	28	13	16	28	32
56	24	28	28	28	24
57	21	23	27	15	26
58	17	22	19	18	25
59	19	12	12	19	26
60	22	26	17	18	28

TABLE 5.2: *K.pneumonia* zones of Inhibition

S.No	**Amikacin**	**Ceftazidime**	**Cefixime**	**Ceftriaxone**	**Imipenem**
1	18	18	8	15	33
2	19	12	8	18	31
3	21	17	20	15	27
4	19	17	22	9	29
5	19	11	24	23	24
6	21	17	21	3	27
7	19	21	12	6	25
8	23	18	22	21	26
9	19	6	18	11	29
10	22	18	23	11	33
11	27	17	27	5	31
12	22	18	24	11	32
13	19	13	11	16	24
14	24	19	16	6	24
15	21	9	23	9	32
16	21	22	29	11	35
17	27	19	22	13	35
18	22	18	30	19	37
19	22	17	21	10	33
20	19	22	12	13	26

21	21	6	21	8	26
22	24	9	19	2	26
23	26	9	13	18	27
24	21	11	27	11	25
25	19	20	22	17	29
26	22	11	22	9	26
27	23	13	21	14	24
28	21	14	22	14	32
29	19	10	14	12	29
30	22	15	18	22	26
31	21	9	11	28	26
32	21	6	17	17	28
33	19	13	19	22	25
34	22	15	18	18	28
35	26	14	30	21	32
36	21	17	23	25	29
37	25	12	24	26	27
38	22	13	14	11	31
39	19	8	21	10	29
40	19	3	26	14	25
41	24	6	24	14	33
42	26	12	26	28	33
43	23	13	17	11	32
44	26	11	22	26	29
45	21	21	24	25	34
46	22	15	8	12	27
47	19	9	18	28	27
48	20	9	24	12	26
49	19	7	18	24	26
50	19	8	16	11	25
51	24	19	23	14	26

52	26	4	9	7	28
53	19	7	13	24	33
54	28	4	17	25	31
55	22	9	9	7	29
56	24	9	20	12	29
57	22	11	22	14	26
58	22	13	8	11	29
59	21	14	26	11	23
60	25	12	11	21	23