

**Pathogens and Microbes Associated with Housefly (*Musca domestica* L.) from a dumpsite  
in a Major Trade Market in Egor Local Government Area of Edo State.**

**BY**

**AMBALI NENE MOROMOKE (MISS)**

**LSC1806082**

**UNIVERSITY OF BENIN**

**BENIN CITY,**

**NIGERIA**

**JULY, 2023**

**Pathogens and Microbes Associated with Housefly (*Musca domestica* L.) from a dumpsite  
in a Major Trade Market in Egor Local Government Area of Edo State.**

**BY**

**AMBALI NENE (MISS)**

**LSC1806082**

**A DISSERTATION SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE B.SC. (HONS) IN ANIMAL AND ENVIRONMENTAL BIOLOGY, UNIVERSITY OF BENIN, BENIN CITY NIGERIA.**

**JULY, 2023**

**CERTIFICATION**

**I certify that this project work was done by MISS AMBALI NENE MOROMOKE (MISS) with matriculation number LSC1806082 in the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria.**

\_\_\_\_\_  
**DR. ANTHONY OMOREGIE**  
**(Project Supervisor)**

-----  
**Date**

\_\_\_\_\_  
**PROF. MYKE OMOIGBERALE**  
**(Head of Department)**

-----  
**Date**

\_\_\_\_\_  
**EXTERNAL EXAMINER**

-----  
**Date**

## **DEDICATION**

**This work is dedicated foremost to God Almighty for his grace and to my mother, whose unwavering love and support have been my guiding light throughout this project.**

## **ACKNOWLEDGEMENTS**

**I owe my profound gratitude to my project supervisor, Dr Anthony Omoregie for his support, guidance throughout the course of this project**

**My warmest gratitude goes to the Head of Department, Prof. Myke Omoigberale and Course adviser , Dr O.C. Asemota for their encouragement.**

**My priceless appreciation goes to my mother, Mrs Ann for her prayers, support and counsel which never cease.**

**I wish to express my genuine and sincere appreciation to my friends, Okpor Esther, Akinduro ifeoluwa, Mr Speaker, Ogunjobi ifakorede for their constant assistant to all my calls.**

**To everyone who contributed in one way or the other , most especially financially, to make this project work a success, may God bless you immensely.**

## Table of Contents

COVER PAGE .....	i
TITLE PAGE .....	ii
CERTIFICATION .....	iii
DEDICATION .....	iv
ACKNOWLEDGEMENTS .....	v
LIST OF FIGURES .....	viii
LIST OF PLATES .....	ix
LIST OF TABLES .....	x
ABSTRACT .....	xi
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.0 Background of Study .....	1
1.2 Statement of Problem .....	8
1.3 Justification of Study .....	9
1.4 Aims and Objectives .....	9
CHAPTER TWO .....	11
LITERATURE REVIEW .....	11
2.1 Houseflies ( <i>Musca domestica</i> ) .....	11
2.2. Pathogens .....	11
2.2.1 Bacterial Pathogens .....	14
2.2.3 Fungal and Protozoan Pathogens .....	18
CHAPTER THREE .....	24
MATERIALS AND METHODS .....	24
3.1 STUDY AREA .....	24
3.2 COLLECTION OF HOUSEFLY ( <i>M. domestica</i> ) .....	25
3.3. STERILIZATION OF MATERIALS .....	25
3.4 PROCEDURES FOR ZOOLOGICAL COLLECTION .....	25
3.5 PROCEDURE FOR MICROBIOLOGICAL ANALYSIS .....	27
3.6. ENUMERATION AND ISOLATION OF BACTERIAL AND FUNGAL ISOLATES .....	27
3.7 PHENOTYPIC IDENTIFICATION OF BACTERIAL FROM SAMPLES .....	28
3.8 Gram Staining Test .....	<b>Error! Bookmark not defined.</b>
3.8.1 Potassium Hydroxide (KOH) test .....	29

3.8.2	Biochemical Test .....	29
3.8.2.1	Indole Test .....	29
3.8.2.2	Oxidase Test .....	30
3.8.2.3	Catalase Test .....	30
3.8.2.4	Citrate Utilization Test .....	31
3.8.2.5	Urease test .....	31
3.8.2.6	Mannitol Test .....	31
3.8.2.7	Triple Sugar Iron (TSI) test .....	32
3.9	GROWTH ON DIFFERENTIAL MEDIA .....	32
3.9.1	<i>Bacillus cereus</i> Agar Base .....	33
3.9.2	Mannitol Salt Agar (MSA) .....	33
3.9.3	<i>Pseudomonas</i> Agar .....	33
3.9.4	Eosin Methylene Blue (EMB) agar .....	33
3.10	Rhizobacterial Potential of Bacterial and Fungal Isolates .....	34
	CHAPTER FOUR .....	35
	RESULTS .....	35
	CHAPTER FIVE .....	42
	DISCUSSION .....	42
	CONCLUSION .....	44
	REFERENCE .....	46

## LIST OF FIGURES

Figure 1 (A): Adult house fly ( <i>Musca domestica</i> ) showing paired claws, paired large pulvilli and a single central bristle-like empodium,.....	4
Figure 1 (B): Wing venation and antennae contrasting the distinguishing characteristic of the genera <i>Musca</i> , <i>Muscina</i> , <i>Fannia</i> and <i>Stomoxys</i> . Note endings of veins 3 and 4 ,.....	4
Figure 2: Life cycle of housefly ( <i>Musca domestica</i> ).....	6
Figure 4.1 showing bar charts of heterotrophic bacterial count.....	47
Figure 4.2 showing bar charts of coliform bacterial count .....	48

## LIST OF PLATES

Plate 1: An open refuse dumpsite situated in Uselu Market.....	28
--	----

## LIST OF TABLES

Table 1: Cultural, microscopic observations and biochemical characteristics of the isolated pathogens.....	44
Table 2: The antibiotic sensitivity profile of bacteria isolates isolated from <i>Musca domestica</i> .....	45
Table 3: Phenotypic virulence determinants of bacterial isolates .....	46

## ABSTRACT

Houseflies, also known as *Musca domestica* L. , are a type of insect in the Muscidae family. Certain types of these bugs are capable of transmitting illnesses. They are called dirty flies because they like to hang around things like garbage and poop. This study found the germs and harmful bacteria that are linked to house flies and the potential danger in the main market in Egor Local Government Area of Edo state. We used a net to collect things from two parts of the dumpsites. Testing was done on housefly samples to find out what kind of bacteria were in them. This involved looking at the bacteria under a microscope, identifying their physical traits, and seeing how they grew on special substances. The study found certain types of bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Proteus vulgaris*. The study found that the amounts of certain types of bacteria ranged from  $1.4 \times 10^6$  to  $1.6 \times 10^6$  for coliform bacteria and from  $6.7 \times 10^6$  to  $7.4 \times 10^6$  for other bacteria.

The bacteria *Escherichia coli*, *Serratia*, *Pseudomonas*, and *Proteus* were separated. We all react strongly to Gentamycin and ciprofloxacin. *S. marcescens* is a type of bacteria. *E. coli* and *Serratia* bacteria are easily affected by chitosan. *P. aeruginosa* is a type of bacteria. The tests showed that most bacteria were sensitive to carbenicillin, but *Proteus sp* was resistant to it. The MAR index ranged from 0.333 to 0.444. It was found that houseflies in Uselu market from Egor Local Government Area of Edo state carry harmful parasites and bacteria on their bodies and inside them. So there is need to control the number of flies to prevent diseases caused by flies now and in the future..

## CHAPTER ONE

### INTRODUCTION

#### 1.0 Background of Study

Houseflies are a prevalent type of insect that can be found in nearly every part of the world, from rural to urban areas with moderate and warm climates. They make up around 90 percent of all the flies in neighborhoods and other natural areas (Nmorsi et al. , 2006). Houseflies are a type of insect that can spread disease. They are dirty because they like to be around garbage and animal waste.

Houseflies have been around since humans first showed up on Earth. They are really good at living with people, so they are called synanthropic animals. (WHO, 2008) The housefly can make people and animals sick because it carries germs and diseases from its dirty habits and the way it eats. More than 100 germs like bacteria, viruses, fungi, and parasites have been linked to houseflies.

Animals and humans are always in contact with tiny organisms that can make them sick. Luckily, animals have a natural defense system that usually keeps them safe from getting sick from exposure to harmful things. The human body has a lot of tiny organisms called normal flora. They usually stay in certain parts of the body. If there are too many of them in one part, it can cause symptoms in the body.

Musca has around 70 kinds of flies. Domestica is very common in Africa. The different species that make up the genus look very much alike. Houseflies are small insects with a hard outer covering that are about 6-9 mm long. They can be different colors, like shiny green or light grey with darker spots. Each antenna has two parts. The distal part is the biggest and looks like a tube

with hairs around it called arista. They have a special mouthpart called a proboscis that they use to suck up liquids or soft foods. It only comes out when they need to use it, and then it goes back inside their head when they're done. It has a tunnel in the middle called pseudotracheae. This is where liquids that are sucked in go into the digestive system. Houseflies can eat lots of different things like dead animals, poop, and other organic stuff, including the same food people eat. The type of food determines how houseflies eat. For thin liquids like beer, milk and fruit juice, the flies use their mouthparts to touch the liquid and then suck it up through small tubes. When eating soft foods, the labella are not used. Instead, the food is sucked directly into the food channel. The small teeth around the food tube scrape food that has been moistened by spit.

The wings of houseflies are important for telling them apart from other kinds of flies. Houseflies are different from other types of flies because they have a vein on their wings that is sharply bent to join another vein. This vein is called vein number 4 and it connects to a thick vein along the front edge of the wing called the costa, near vein 3. The housefly has sticky hairs on its legs that help it to easily stick to smooth surfaces. These hairs also pick up germs when the fly lands on poop or infected wounds.

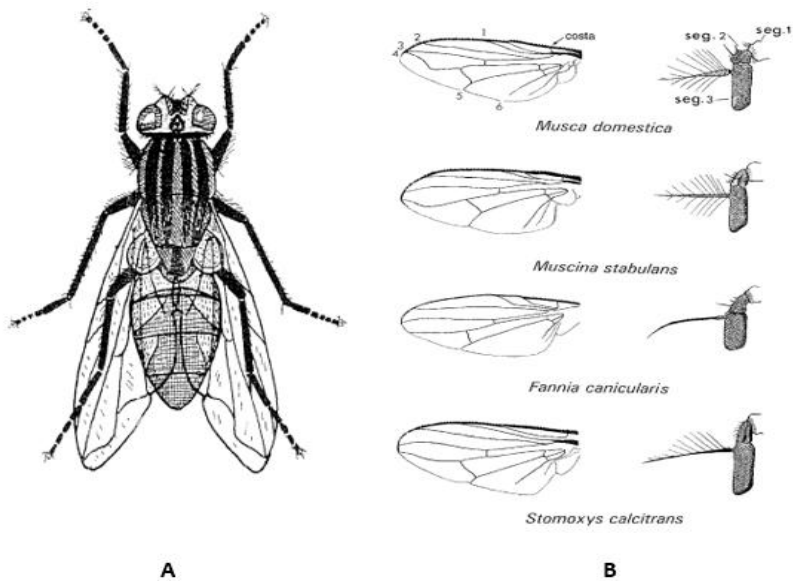


Figure 1 (A): Adult house fly (*Musca domestica*) showing paired claws, paired large pulvilli and a single central bristle-like empodium

Figure 1 (B): Wing venation and antennae contrasting the distinguishing characteristic of the genera *Musca*, *Muscina*, *Fannia* and *Stomoxys*. Note endings of veins 3 and 4

(Mike, 2010)

Houseflies, like all living things, rely on reproduction for their continuation as a species. Domestic flies lay their eggs on decaying things like animal poop, bird poop, trash, leftover food, and wet surfaces. (Tatfeng et al. , 2005; Adeleke et al. , 2017) They lay 75-100 eggs all at once or in separate groups at the same time. House flies usually lay eggs 5-10 times in their life, which adds up to 1,000 eggs. The eggs hatch 10-16 hours after they are laid. The eggs cannot survive very hot or very cold temperatures. They dry out and die when it's colder than 15°C or hotter than 40°C. When the right conditions are there, the egg changes into a larvae.

Larvae are often called maggots. They change into pupae in 3-5 days in a process called pupation. This was found by Kettle in 1995. During warm weather, the puparial stage lasts about 3-5 days, but it can last 7-14 days when it's cooler. After that, it becomes an adult.

The grown-up fly gets out of its puparia shell by pushing from the front and crawling out. Then it flies away after a little while. Adult house flies usually stay away from the sun and like to hide in buildings where people or their animals live. House flies, related flies, and calliphorid flies are often called domestic or synanthropic flies because they are usually found close to humans and their homes. House flies poop and throw up their food anywhere, which leaves gross spots.

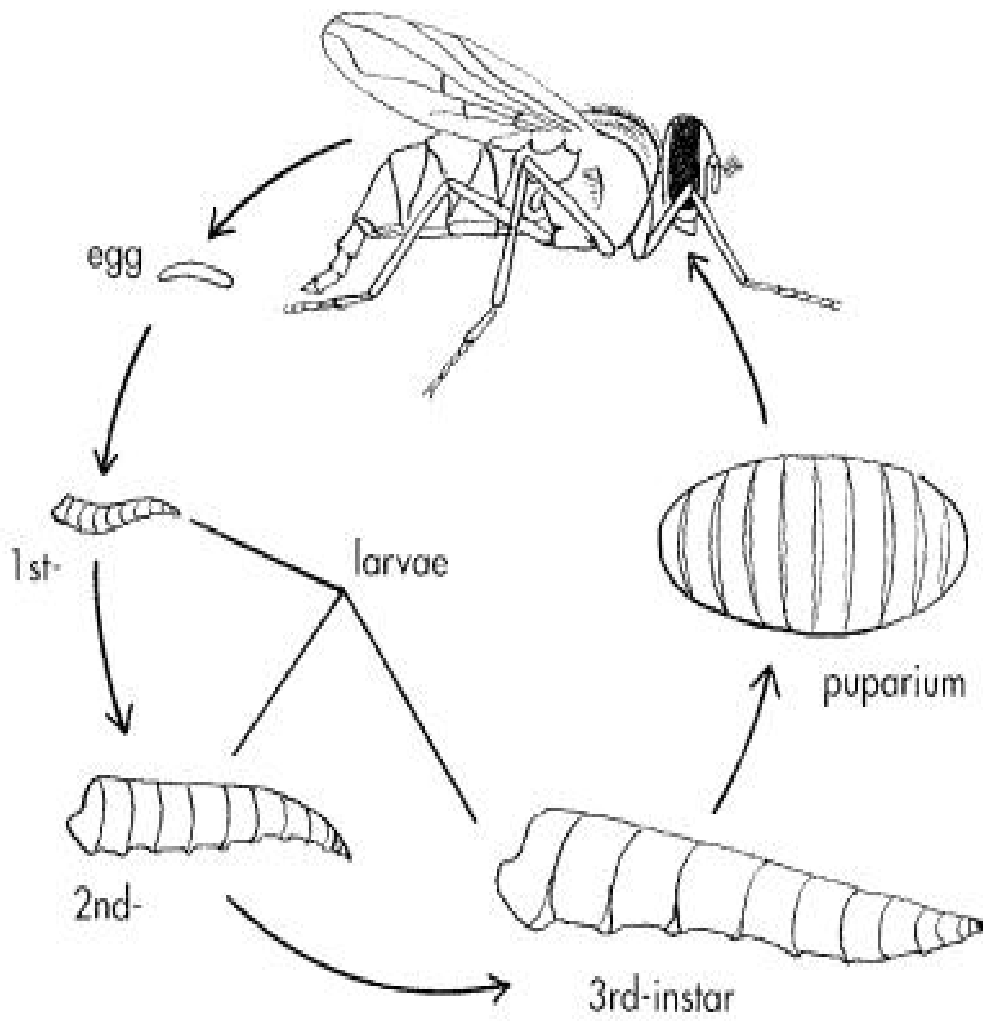


Figure 2: Life cycle of housefly (*Musca domestica*)

Houseflies are a species of fly that do not inflict bites. They leave tiny dots of poop around the place where they live. This was found by Graczyk et al. Diseases that cause diarrhea from contaminated food are more common in cities and countryside when there are more houseflies. This happens in warm places where there is not much clean water or proper sanitation. (Onwugamba, 2018; Gehad and Elshirbini, 2010).

Flies transfer germs from poop to food and can make people sick with parasites like Giardia and worms. They do this by moving between dirty things and food.

The markets in Egor Local Government Area have a lot of houseflies because of the things the traders do there. The dump is a place where houseflies breed. These flies are not taken seriously, but they can contaminate food and make people sick. This can also cause food to go bad and waste resources. Another problem is that some ready-to-eat foods like fruits, snacks, and groceries are exposed to houseflies. When people buy these products that houseflies have touched, they might not wash the food well before they eat it. This study will look at the germs that are connected to houseflies at a big trash site in Uselu market in Egor Area of Edo State.

The housefly is very important because it can spread diseases that affect both agriculture and public health around the world. The number of flies may go up as the Earth gets warmer. The too many flies around cause trouble for farmers and people who live nearby. What

Rephrase

is most important is that people have come to realize that adult flies can spread diseases by pooping and throwing up on animal and human food, leading to illness in people and animals.

Lately, people have been worried about getting sick from the food they eat. Scientists are studying how flies can spread germs that make people sick, like E. coli, Shigella, and Salmonella.

(Nayduch and Stuzenberger, 2001; Ahmad and colleagues, 2007; Macovei and others, 2008; Holt and colleagues, 2007). The germs carried by houseflies can spread to milk, meat, and potato salad. Flies that carry germs are usually found near trash and places where people and animals go to the bathroom. They then spread these germs to places where people live and do things. Greenberg (1971) found over 330 ways that houseflies can spread diseases (Greenberg, 1971). Keiding (1985) made a list of important diseases that can be spread by *M. Domestica*. Domestic animals can get sick with bacterial infections like shigellosis, salmonellosis, cholera, and *Campylobacter* under certain conditions.

Furthermore, house flies can carry the eggs and cysts of many types of intestinal worms. These worms include *Ascaris* spp. , hookworms, and tapeworms. The housefly can spread trachoma, skin infections, and wounds.

Houseflies are a problem for farm animals like cows, camels, sheep, and chickens. They can make the animals sick and even cause them to die, which costs farmers money. Houseflies make cows tired and less productive because they have to keep shooing them away. This makes it harder for farm workers to do their job. Houseflies also spread diseases to animals, which can cost farmers more money for medicine and vet services. They can also spread diseases to humans.

Houseflies have become resistant to most insecticides that are used to kill them. This has led to ongoing research on other ways to get rid of the flies that are causing food to be contaminated and reproduce too much. (Tarelli and colleagues, 2009; Huang and colleagues, 2009).

It is important to keep people safe from houseflies so that we don't lose money and other things because of them. Researchers have found ways to get rid of houseflies and how they affect people.

- Cultural control

It means using traditions and customs to manage something. Cultural control means using specific methods to make it difficult for insects to survive and reproduce, which helps decrease their numbers. Cultural control is a good way to stop houseflies from breeding. It doesn't harm the environment and includes things like keeping things clean, making sure things are dry, using baits and pesticides, managing garbage, and using ultraviolet lights.

- Chemical control

It means using chemicals to manage or eliminate pests or unwanted plants. There are different ways to control houseflies with chemicals. One inexpensive way is by using a chemical called dieldrin. (Hertz et al. ) In 2011, dieldrin comes from plants and is not very harmful to other living things. Fly attractant (z-9 tricosene) and imidacloprid are good at killing flies. But a study by Fouda et al. , 2022 suggested that using ozone is better for getting rid of houseflies because it turns into oxygen quickly and doesn't stay in the air or on surfaces. Plant chemicals are good for the environment and can help get rid of pests. Pangnakorn and other researchers. In the year 2011, they used Wood Vinegar to control flies. Basil oil is an effective and affordable way to control house flies. (Ojianwuna et al. , 2011)

- Biological Control

It is the use of living organisms to control pests and diseases. Biological control is a good way to get rid of houseflies and other pests by using living things like other bugs and plants. We use different bugs and other natural things to control house flies. These

control agents can appear as predators. Bio-pesticides are a good choice for getting rid of houseflies..

## **1.2 Statement of Problem**

The transmission of human parasites by houseflies mainly occurs through the transportation of parasites on their bodies and subsequent dispersion through their droppings and regurgitation. Throwing up (Grazyk et al 2005). Houseflies can transport germs from dirty sources like excrement and wound discharge to new locations when they land. Their consumption of unsanitary substances puts them at risk for carrying potentially harmful bacteria on their bodies. Due to their habit of consuming unclean items, houseflies are able to transmit germs from one place to another

(World Health Organization 1990, Manzon and Sanchoz 1997). The focus of this study is to investigate the connection between houseflies and the spread of germs and bacteria.

## **1.3 Justification of Study**

Insufficient knowledge exists about the pathogens that houseflies harbor and transmit, despite the fact that they can cause illness in humans and animals and can also spoil food. The objective of this research was to determine the specific types of germs and small organisms carried by houseflies. It will show how some of these germs and tiny organisms can make people sick. This research will be helpful for other scientists, especially those who study the spread of diseases.

## **1.4 Aims and Objectives**

This study aimed to evaluate the various pathogens and microbes associated with *Musca domestica*.

The specific objectives of the study were to;

1. Identification of microbes
2. Evaluate the phenotypic virulence properties of the microbes
3. Anti-microbial susceptibility

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1 Houseflies (*Musca domestica*)**

The Muscidae family of insects, which encompasses 4200 different types of flies in 190 groups, includes houseflies. Houseflies are the most common and important type of fly. There are other types of flies like the greater housefly and the stable fly, but they are not as common as the housefly. Houseflies carry germs that can make people and animals sick. They are found in places where people and animals live. (Akpan et al. , 2018) Human waste like poop, spit, and animal poop is a good place for houseflies to have babies because they have the food they need to grow and lay their eggs. (Barreiro et al. , 2013).

#### **2.2. Pathogens**

Microbes are tiny living things that are too small to see without a microscope.

Microorganisms are minuscule living organisms that are impossible to observe without the aid of a microscope.

Microscopic organisms are microscopic life forms that cannot be visible to the naked eye.

Tiny living creatures called microbes are only visible under a microscope.

They can be made up of many cells or just one cell.

Pathogenic microorganisms are tiny living things that can spread and make a living thing sick.

They can cause sickness in plants and animals by changing how the cells work. We can

understand why germs would grow inside a body, but it's not clear why they make us sick. One reason could be that sometimes,

Rephrase

the ways microorganisms affect the body help them to spread more and survive better, which is good for them.

In 2019, Neupane and his team studied how likely it is for cows to carry and spread disease-causing bacteria that cause bovine respiratory disease. Respiratory disease is a big problem for cattle on feedlots in the USA. It can cause cattle to get sick and cost a lot of money. This disease is usually caused by things like stress and changes in what the cattle eat. The researchers collected houseflies from a pen with sick cattle. They put the flies in a clean plastic bag and kept them cold while they brought them to the lab. The crushed up fly was put onto a special dish that helps things grow, called blood agar. We then confirmed what kind of species it was using a method called Polymerase chain reaction (PCR) and sequencing. The tiny living things we saw were mostly *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. This study suggests that female flies who eat food like manure, animal secretions, and animal feed in a feedlot can get sick from pathogens released by infected animals. Yes, *M P haemolytica Multocida* and *H. Bacteria* are often found in the tonsils and lungs of sick cows and can also be found in their nose secretions. Flies can pick up these bacteria when they eat the cow's nose secretions or contaminated food and water. When flies get sick from bacteria, they can spread the bacteria to different places, even where there are healthy cows. But because we mixed all the flies together, we couldn't tell where the BRD bacteria came from - whether it was from inside the flies' stomachs, on their outer bodies, or both.

Scientists discovered that insects have many different types of tiny organisms living on them. The common fly can carry germs that make people and animals very sick. More than 100 germs like bacteria, viruses, fungi, and parasites have been found in insects. Germs like bacteria, viruses, and other tiny things can make you sick. All types of germs need ways to get into their host and avoid being destroyed by the host's immune system right away. Pathogens are spread to their host mainly through contact with a carrier or through contact with dirty surfaces. Houseflies can carry and spread these pathogens to people. (Sarwar, 2015; Khamesipour et al. , 2018)

Williams and Kelechi, in 2015, studied bacteria found in soil from four different places in Port Harcourt. They collected ten samples from dump sites to see how harmful the bacteria were. The samples were tested to see how hot or cold they were, how acid or basic they were, and how many bacteria and fungi were in them. The average soil temperature was between 27 and 28 degrees Celsius, and the average pH was between 6.5 and 7.8. The number of bacteria ranged from 24 million to 120 million per gram of soil, and the number of fungi ranged from 4.8 million to 23 million per gram of soil. The germs found in the trash were bacteria like *Bacillus*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus*. It also had fungi like *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Saccharomyces*. The study found that the pH levels at locations R and U were different at a confidence level of 0.05. The temperature, bacteria, and fungi levels at sites R and U were about the same and didn't vary much. In the dump sites R and U, the number of bacteria and fungi found were about the same in both sites.

### 2.2.1 Bacterial Pathogens

Bacteria are small organisms that are capable of independent survival and can perform most of their functions autonomously, although they depend on a host for nourishment. Out of all the bacteria we come across, only a few are harmful germs. These harmful bacteria are bigger and more complicated than viruses. Most bacteria are not harmful. Harmful germs have certain genes that help them interact with the body and make it easier for them to grow and spread.

In a research study done by Adeleke and colleagues in 2017, they looked at the parasites and harmful bacteria found on houseflies in Osogbo, Southwestern Nigeria. They collected houseflies from places like slaughterhouses, dumping areas, and places where palm wine is made, and tested them for parasites and bacteria. We used regular methods to separate the bacteria and looked at the parasites through a microscope. The bacteria were tested to see which antibiotics could kill them. All 70 samples had harmful bacteria in them. The bacteria found are Salmonella, Klebsiella, Proteus, Escherichia coli, and Providencia. Some bacteria names are Enterobacter aerogenes, Pseudomonas aeruginosa, Morganella morganii, Vibrio cholera, Shigella species and Serratia marcescens. The types of bacteria were different in each place we studied, but the difference was not very important ( $p > 0.05$ ). Few houseflies were found to have the cyst of *Entamoeba histolytica* and the eggs of *Ascaris lumbricoides*.

Many of the symptoms and signs of a disease caused by harmful bacteria are how the body's immune system reacts to fight the infection. Bacterial infections in people and animals can cause redness and swelling where the infection is. Pus, which is made up of dead white blood cells, is also made as the body tries to fight off the bacteria. A fever is also a way for your body to protect itself. When your body gets hotter, it can stop some germs from growing (WHO, 2008).

Deakpe and his team did a study in Makurdi, Nigeria to find out which harmful parasites and bacteria are found on houseflies in that area. 210 flies were found in 7 different places in Makurdi from October 2015 to March 2016. These places are Wurukum Market, High-level Market, Kwararafa Quarters, Wadata Market, Railway Market, Modern Market and North Bank Market. We checked the samples for harmful parasites and bacteria using regular lab tests. The study found that 15 people (25. 42%) had *Ascaris lumbricoides*, 11 people (18. 64%) had *Entamoeba histolytica*, 9 people (15. 25%) had *Taenia saginata*, 7 people (11. 86%) had *Schistosoma mansoni*, 5 people (8. 47%) had *Enterobius vermicularis*, 5 people (8. 47%) had *Hymenolepis nana*, 3 people (5. 08%) had *Giardia lamblia*, 2 people (3. 39%) had *Schistosoma haematobium*, and 1 person (1. 69%) had *Trichostrongylus* sp. House flies were found to have a small amount of *Ascaris lumbricoides* and *Trichuris trichiura*. "Unhealthy bacteria found in house flies are *Staphylococcus* sp. " 31 out of 100 (or 38. 27%) of a type of bacteria called *Bacillus*. 16 out of 81 (19. 75%) of the bacteria were *Klebsiella* species, and 16 out of 81 (19. 75%) were *Escherichia* species. 6 out of every 100 *Proteus* sp. represent 41% 6 out of 100 (7. 41%) samples tested positive for *Salmonella* bacteria. 4 per cent (4. 94%) and a type of bacteria called *Moraxella* sp. 2 out of every 100 is equal to 2. 47%The results showed big differences in the number of bacteria and how often they showed up. It has been found that houseflies in Makurdi carry harmful parasites and bacteria on their bodies and inside them.

Each germ has its own way of making someone sick, which makes it hard to understand how infections work. However, when thinking about how germs affect our bodies, we use the term "pathogenicity" which means the ability of a germ to make a person sick. Bacterial pathogens use their shapes to infect their hosts. Bacteria are sorted into groups based on their shapes: (A)

spheres (cocci), (B) rods (bacilli), and (C) spiral cells (spirochetes). They are also categorized as either Gram-positive or Gram-negative (Prescotts, 2011).

Only a small number of bacteria can make people sick.

- Few bacteria have the ability to cause illness in humans.
- The number of bacteria capable of making people sick is limited.
- Only a small percentage of bacteria are able to cause sickness in humans.

Some germs that make you sick can only grow inside your body's cells. They are called obligate pathogens. Some germs can live in water or soil and only make people sick if they come into contact with someone who can get sick; these germs are called facultative pathogens. Many bacteria start off harmless but can cause illness in a person with a weakened immune system or an injury. These bacteria are called opportunistic pathogens.

In 2016, Imarhiagbe and others studied how well different antibiotics worked against germs found in smoked and frozen fish sold in Benin and Warri. Using standard methods for studying microorganisms, the number of bacteria and fungi ranged from 5.4 million to 25.1 million at Ekpan market and from 110,000 to 290,000 at Effurun market and Ekpan and New Benin market. Six bacterial species namely; *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Pseudomonas* sp., *Corynebacterium* sp., *Micrococcus* sp. and four fungal species namely; *Aspergillus* spp., *Fusarium* sp., *Mucor* sp. and *Penicillium* spp. were identified. Majority of the bacteria were isolated from fish samples from Benin City Markets (51.1%, n = 23) while (48.9%, n = 22) were obtained from Warri Markets. *Bacillus* spp. were the most predominant (35.6 %) and

*Micrococcus* sp. was the least predominant among the bacterial isolates (2.2%). *Aspergillus* sp. was recorded the most predominant (37.1%) while *Penicillium* sp. (14.3%) was the least predominant among the isolated fungi. The isolated bacteria (*Escherichia coli*, *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Corynebacterium* sp. and *Micrococcus* sp.) were sensitive to ciprofloxacin and showed resistance to zinnacef and rocephin. These findings show that fish samples from different markets harbour varied levels of potentially pathogenic organisms which could pose health threat to consumers. Proper handling, processing and storage methods should be put in place to reduce potential health risk.

Some bacterial pathogens are fastidious in their choice of host and will only infect a single species or a group of related species, whereas others are generalists. *Shigella flexneri*, for example, which causes epidemic dysentery (bloody diarrhea) in areas of the world lacking a clean water supply (Hrudey *et al.*, 2006), will only infect humans and other primates. By contrast, the closely related bacterium *Salmonella enterica*, which is a common cause of food poisoning in humans, can also infect many other vertebrates, including chickens and turtles.

The opportunistic pathogen *Pseudomonas aeruginosa* is capable of causing disease in plants as well as animals. Other pathogenic bacteria use several independent mechanisms to cause toxicity to the cells of their host. *Anthrax*, for example, is an acute infectious disease of sheep, cattle, other herbivores, and occasionally humans. It is usually caused by contact with spores of the Gram-positive bacterium, *Bacillus anthracis*. Unlike cholera, anthrax has never been observed to spread directly from one infected person to another. Dormant spores can survive in soil for long periods of time and are highly resistant to adverse environmental conditions, including heat, ultraviolet and ionizing radiation, pressure, and chemical agents. After the spores has been picked up by houseflies and placed on a host, when the spores are inhaled, ingested, or rubbed

into breaks in the skin, the spores germinate, and the bacteria begin to replicate. Growing bacteria secrete two toxins, called lethal toxin and edema toxin.

In another study carried out by (Philip *et al.*, 2011) to ascertain the pathogens associated with houseflies (*Musca domestica*). The test insects was collected near the rear entrances and dumpsters of 4 restaurants in north central Florida. Live house flies were collected and individually transferred to blood agar plates for 1 hour. After removing the flies, the plates were incubated overnight at 37°C. Bacterial colonies that were morphologically distinct were isolated from other colonies by streaking onto new plates. The bacteria were identified by fatty acid analysis and sequence of their 16S rRNA gene. The bacterial isolates included 5 new bacterial records for house flies: *Acinetobacter baumannii*, *Bacillus pumilus*, *Cronobacter sakazakii*, *Methylobacterium persicinum*, and *Staphylococcus sciuri*. Other bacteria identified have been associated previously with houseflies, including *Bacillus cereus*, *B. thuringiensis*, *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus*. Most of the organisms recovered from the house fly are serious pathogens, known to produce diseases such as meningitis, food poisoning, diarrhea, abscesses, bloodstream infections, and hemorrhagic colitis. The possible exception is *Bacillus thuringiensis*, a known pathogen for insects that only occasionally produces allergic reactions in humans. If these organisms are not prevented from entering the food preparation and consumption areas, they could become a serious risk in the transmission of diseases.

A study conducted by Uredo-ojo and his colleagues explored the potential for houseflies to harbor drug-resistant bacteria. They caught houseflies in certain places in Port Harcourt, Rivers State, Nigeria, like poultry farms and residential areas. They looked at the flies with a microscope and put some of the bacteria they found on a special plate to see what kinds of

bacteria were there. They found *Salmonella* spp. bacteria on 80 flies. 'were the most common, along with *Escherichia coli*, *Proteus* spp. ' can be simplified to 'were the most usual,' along with 'Escherichia coli, *Proteus* spp. ' *Klebsiella* spp accounted for 81. 4% of all the bacteria. The majority of the bacteria were not killed by amoxicillin (82. 9%) and co-trimoxazole (81. 4%), but only a few were resistant to ciprofloxacin (6. 2%) A total of 58 different antibiograms were found, but almost half of them (29 out of 59) were seen in only one isolate. This research found that a lot of bacteria (74. 4%) were resistant to multiple drugs. This study shows that houseflies can hold dangerous bacteria and also resistant bacteria. The different types of bacteria found suggest that there could be a big problem for public health because it might be hard to stop flies from spreading the bacteria. Efforts to control the flies may need to focus on keeping them away from people's food and water.

Rahuma, et al. , 2005, did a study about how houseflies in Libya carry bacteria that can make people sick. They used standard lab techniques and collected 150 houseflies. They found bacteria that can make people sick on the flies collected from the streets and the abattoir, but not on the flies collected in the hospital. The bacteria they found were *Salmonella*, *Yersinia enterocolitica*, and *Edwardsiella tarda*. *Shigella sonnei* was found in only one fly, which was caught in the slaughterhouse. Flies from the hospital, streets, and slaughterhouse were tested and some of them had bacteria on them. 42% of hospital flies had *Escherichia coli*, 70% had *Klebsiella* spp. , 2% had *Aeromonas* spp. , 96% had *Pseudomonas* spp. , 20% had *Staphylococcus* spp. , and 24% had *Streptococcus* spp. Meanwhile, 42% of street flies had *Escherichia coli*, 50% had *Klebsiella* spp. , 20% had *Aeromonas* spp. , 36% had *Pseudomonas* spp. , 12% had *Staphylococcus* spp. , and 22% had *Streptococcus* spp. Lastly, 32% of slaughterhouse flies had *Escherichia coli*, 62% had *Klebsiella* spp. , 10% had *Aeromonas* spp. , 34% had *Pseudomonas* spp. , 16% had

Staphylococcus spp. , and 18% had Streptococcus spp. When the researchers looked at which antibiotics would work on the bacteria found in flies, they found that the bacteria from hospital flies were resistant to more antibiotics than the bacteria from flies caught on the streets or at the slaughterhouse. In many houseflies, no matter where they were caught, there is a type of bacteria called Pseudomonas. The Pseudomonas bacteria that were found with these flies were often resistant to many different antibiotics. More than half of them were resistant to at least 10 different antimicrobial medicines. This study shows that houseflies in the hospital can carry bacteria that are resistant to many antibiotics, including one called methicillin-resistant Staphylococcus aureus. Because houseflies can move around easily, it's possible that they spread germs from hospitals to nearby areas, and back again.

### **2.2.3 Fungal and Protozoan Pathogens**

Pathogenic fungi and protozoan are eukaryote, they are usually saprophytic, but have the capacity to cause diseases. It is therefore more difficult to find drugs that will kill them without killing the host. Consequently, antifungal and anti-parasitic drugs are often less effective and more toxic than antibiotics. A second characteristic of fungal and parasitic infections that makes them difficult to treat is the tendency of the infecting organisms to switch among several different forms during their life cycles. A drug that is effective at killing one form is often ineffective at killing another form, which therefore survives the treatment (Kohler, *et al.*, 2014).

Protozoa is another infectious agents in the eukaryotic group of pathogens. They are single-celled even though they possess animal like characteristics such as predation, motility and lack of cell wall. One of the most common infectious diseases on the planet, shared by about a billion people at present, is an infestation in the gut by *Ascaris lumbricoides*. This nematode closely resembles its cousin *Caenorhabditis elegans*, *C. elegans*, however, is only about 1 mm in length,

whereas *Ascaris* can reach 30 cm. The bulk of protozoan pathogens is the major cause of most human and animal disease such as; amoebiasis, malaria, babesiosis, toxoplasmosis, chagas disease and African trypanosomiasis. Evidence supporting the role of the house fly in transmission of these diseases are inconclusive, with the strongest evidence pointing to the correlation between the rise in incidence of most of these diseases mentioned and an increase in the insect population (Farag *et al.*, 2013; AHA *et al.*, 2014).

Philips *et al.*, 2011 carried out a study titled “Indicator microbes correlate with pathogenic bacteria, yeasts and helminthes in sand at a subtropical recreational beach site” with an objective to evaluate distribution of various pathogens associated with yeasts (fungi), samples used in this study was gotten from beach sides, then the samples was subjected to organic analysis, the microbial analysis was done using three different methods, the pathogens identified were protozoa [*Cryptosporidium* spp. and *Giardia* spp., the fungi colonies that were subsequently identified in this study to determine the presence of some specific potential human pathogens *Candida tropicalis*, *Candida albicans*, *Candida guilliermondii*, *Candida glabrata* and *Candida parapsilosis*.

Elkanah, *et al.*, (2020) carried out a study titled “Houseflies (*Musca domestica*) as potential carriers of human intestinal parasites in jalingo metropolis” in this particular study 200 flies were collected from four different locations (Abattoir, Jalingo Main Market, Kasuwan Bera and Mile-Six Market) within Jalingo metropolis. Three species of flies were identified during the study namely; *Musca* spp, *Sacophage* spp and *Stomoxys* spp. The house flies were analyzed for the presence pathogenic parasites using standard laboratory techniques. The results showed that the parasites *E. histolytica* 25(28.74 %), *Taenia* spp 25(28.74 %), *A. lumbricoides* 20(22.99 %), *Giardia lamblia* 8(9.20 %) and *Trichuris trichiura* 9(10.34 %) are associated with the house flies.

The results showed no significant variation ( $\chi^2 = 0.61$ ,  $p > 0.05$ ) between the parasites species. From the result gotten from this it can be concluded that house flies in Jalingo harbor pathogenic parasites on their bodies. The fact that houseflies have been incriminated to be mechanical transmitters of pathogenic diseases to man because of their anthropogenic lifestyles, measures must be taken to control fly population in order to avert both the present and future outbreak of disease conditions emanating from the flies activities.

In 2018, Deakpe and his team investigated the types of germs and parasites present in house flies in Makurdi, Nigeria. 210 flies were found at 7 places in Makurdi from October 2015 to March 2016. These places are Wurukum Market, High-level Market, Kwararafa Quarters, Wadata Market, Railway Market, Modern Market, and North Bank Market. The samples were checked for harmful parasites and bacteria using regular lab methods. The study found that 25.42% of people had *Ascaris lumbricoides*, 18.64% had *Entamoeba histolytica*, 15.25% had *Taenia saginata*, 11.86% had *Schistosoma mansoni*, 8.47% had *Enterobius vermicularis*, 8.47% had *Hymenolepis nana*, 5.08% had *Giardia lamblia*, 3.39% had *Schistosoma haematobium*, and 3.39% had *Trichostrongylus* sp. House flies were connected to two types of worms, which were each found in 1.69% of the cases. Harmful bacteria found in house flies are *Staphylococcus* sp. 31 out of 100 samples (38.27%) contain *Bacillus* bacteria. 16 out of every 19.75% of the bacteria are *Klebsiella* species, and the rest are *Escherichia* species. *Proteus* sp accounts for 6 out of every 100 individuals (7.41%) *Salmonella* bacteria make up 6 out of every 100 germs in the sample. It is a type of bacteria called *Moraxella* sp. that makes up 4.94% of the sample. 2 out of every 100, or 2.47% The study found that there were big differences in the number of parasite species and houseflies in different places, but there were no big differences in the number of bacteria. In Makurdi, it was found that houseflies carry harmful parasites and bacteria on their

bodies and insides. So, flies can spread these harmful germs to people because they live close to humans and can carry the germs on their bodies. We need to take steps to control the number of flies to stop them from causing diseases now and in the future. Words: Housefly; disease-causing bacteria; harmful organisms; Makurdi.

Alaku and his team studied the germs and parasites carried by houseflies in Lafia, Nigeria. They collected 215 houseflies from five different places in Lafia between September 2019 and March 2020. The scientists studied the flies to see if they had any harmful parasites using regular lab techniques. The study found that the parasite *Entamoeba histolytica* was the most common, with 22.11% of people infected. *Ascaris lumbricoides* was next most common at 15.05%, followed by *Taenia* species and *Schistosoma mansoni* at 14.70% each. Hookworm and *Trichuris trichura* were the least common, with only 1.12% of people infected with each. The amount of parasites and houseflies varied a lot in different places. It can be said that houseflies in Lafia carry germs and bacteria on their bodies inside and out. The common housefly can carry and spread disease to humans. We need to control the number of flies to prevent diseases now and in the future.

House flies spread diseases by carrying germs from one place to another. This is a well-known way that germs can be passed on (Fisher et al., 2017). This takes place when germs are transmitted from one animal to another without proliferating or evolving in the intermediary carrier.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 STUDY AREA**

The research was conducted in a major landfill in Uselu market, situated in the Egor Local Government Area in Edo state, Nigeria. Uselu is the main town in this area. Egor Local Government Area is in a place with a warm climate and it covers an area of about 93 square kilometers. The Area has two main seasons, rainy and dry. The average temperature is 28°C and humidity is 68%. The Uselu market is located at 6 degrees 22 minutes 25. 9 seconds North latitude and 5 degrees 36 minutes 45. 5 seconds East longitude of the equator. Traders sell different things like food, clothes, and drinks at the market.

### **3.2 COLLECTION OF HOUSEFLY (*M. domestica*)**

Houseflies were collected on August 4th, 2023, from the dumpsite adjacent to Uselu market. The net is 30cm deep, with a 12 inch ring and a 15 inch wooden handle. Gloves were worn to make sure that no additional germs were transferred to the housefly. Samples were put into a jar with a special liquid to kill them. The liquid is called ethyl acetate. These samples were collected from two separate parts of the dumpsite that were 12 feet (3.65 meters) away from each other. The food vendor is 20 feet (6 meters) away from the dumpsite. We used a measuring tape to take all the measurements..

### **3.3. STERILIZATION OF MATERIALS**

All these materials used were submerged in a clean bucket with 1% of alcohol (Active compound: Sodium hypochlorite). After sterilization, the materials were sundried for about 30-40mins.

### **3.4 PROCEDURES FOR ZOOLOGICAL COLLECTION**

In order to effectively capture bugs, we employed small, see-through bottles with cotton inside, as well as a small quantity of ethyl acetate and a cut piece of paper.

After gathering, all samples were brought to the lab to be organized. A clean white cloth was put on a table. Then, all the flies from a jar were emptied onto it. They were separated into different dishes using a clean spoon instead of tweezers. This was done for all the jars used to kill insects.

10 housefly samples were collected from different parts of A and B at the dumpsite. These samples were taken for microbial analysis.



**Plate 1: An open refuse dumpsite situated in Uselu Market.**

### **3.5 PROCEDURE FOR MICROBIOLOGICAL ANALYSIS**

The Petri dishes, pipettes, glass containers, and bottles were all cleaned and left to dry." They were covered with aluminum foil and cleaned in a hot air oven at 160 degrees Celsius for one hour. They were able to cool down after being cleaned before they could be used. Using a Bunsen burner to eradicate germs and wiping down surfaces with alcohol, we established a hygienic work environment.

We used media from Oxoid and followed the instructions from the manufacturer to prepare it. The study used different types of agar such as Plate count agar, Bacillus cereus agar (BCA), Eosin methylene blue agar (EMB), Mannitol salt agar (MSA), Pseudomonas cetrinide agar (PCA), Triple sugar iron agar (TSI), Simmons citrate agar (SCA) and Mueller Hinton agar (MHA) (Willey et al. , 2008).

### **3.6. ENUMERATION AND ISOLATION OF BACTERIAL AND FUNGAL ISOLATES**

We diluted a small quantity of flies by mixing it with water. Then we put a little bit of this mixture onto different plates with food for bacteria to see how many bacteria grow. We also added a medicine to some of the plates. We made copies of samples for bacterial and fungal plates using the pour plate method. The equation used to figure out dilution factor is shown in equation (1) (Ogofure and Igbinosa, 2021). The bacteria and fungi were counted using the formula (Willey et al., 2008).

After growing bacteria from the samples, individual colonies were put on tryptone soya agar and kept in a warm place for 24 hours. at room temperature (approximately 20-30 degrees Celsius). The colonies were looked at under a microscope and identified using specific methods as described in the Oxoid manual. We used different types of special media to grow and isolate

bacteria from samples. These included Chromogenic Bacillus cereus agar, Sorbitol MacConkey agar, Eosin methylene blue agar, Pseudomonas cetrimide agar, Salmonella Shigella agar, Mannitol salt agar, and triple sugar iron agar slants. The bacteria were identified by doing some tests like citrate, indole, oxidase, urease, sugar fermentation, catalase, 3% KOH, gas formation, and H<sub>2</sub>S formation. By observing the appearance of the fungi and using a microscope, we were able to make an educated guess about their type. Lactophenol cotton blue and wet mount staining was used to look at the fungi we found. Certain fungal characteristics like spores, hyphae, and conidia were used to identify them, following guidelines from Barnet and Hunter (1996)..

### **3.7 PHENOTYPIC IDENTIFICATION OF BACTERIAL FROM SAMPLES**

Special techniques were used to obtain individual bacteria from a single colony. We examined their physical characteristics and actions as part of our study. Many tests were done to identify bacteria, like the Gram reaction, catalase, urease, indole, oxidase, and citrate utilization tests. The bacteria's responses to the triple sugar iron agar were also noted. The purpose of this was to obtain an initial identification of the bacterial isolates.

### **3.8 Gram Staining Test**

A The Gram staining test was performed to determine the existence of Gram-positive or Gram-negative bacteria. Clean and dry microscope slides with labels were used to make a sample. The sample was dried and fixed over a flame. The stain was covered in purple dye, left for a minute, and then rinsed with water. Lugol's iodine was added to the fabric, left for one minute, and then washed off with distilled water. The stain was removed with 95% ethyl alcohol, left for 30 seconds, and then washed off with distilled water. After that, the streak was colored again using safranin solution for one minute and washed with clean water. Finally, the smear was left to dry

in the air. Then we added immersion oil to see the smear under a microscope with a special lens. Different colors, shapes, and how things were arranged were used to figure out what the organisms were. Gram-positive bacteria kept the crystal's purple color, while Gram-negative bacteria stayed pink from safranin.

### **3.8.1 Potassium Hydroxide (KOH) test**

-The KOH test was employed to detect a specific bacteria and to distinguish between two other types of bacteria. It was used along with another test called Gram staining. KOH breaks the walls of some bacteria but not others. Breaking the walls of bacterial cells causes them to burst and release their insides, including their genetic material. I put a small amount of 3% KOH solution on a clean microscope slide and spread a clean culture using a loop. It was mixed slowly and noticed that the liquid became thick and heavy.

A slimy or sticky string formed within 60 seconds, showing that Gram-negative isolates were present. Viscous suspensions that were not slimy still showed no positive results.

### **3.8.2 Biochemical Test**

To better characterize these isolates, biochemical tests were conducted, which included:

#### **3.8.2.1 Indole Test**

We conducted an experiment to determine if certain bacteria have the ability to metabolize tryptophane into indole.

Rephrase

The indole production test helps to find a type of bacteria called Enterobacteriaceae that can break down the amino acid tryptophan and release indole. This test uses a special chemical called

Kovac's indole reagent on a piece of filter paper. A small amount of bacteria from a clean and separate group was placed on the wet part of the paper with a loop. It was okay to look at the color change after 2-3 minutes. In this test, when indole was mixed with the reagents on the paper, it made the bacteria turn blue or blue-green. Any bad reactions stayed colorless or light pink.

### **3.8.2.2 Oxidase Test**

The purpose of the oxidase test was to detect the presence of specific enzymes in the bacteria that are capable of transferring electrons and altering the color of a specific dye. The dye would turn dark purple if it had positive reactions.

The dye will become a deep purple if it elicits favorable responses. In the presence of positive reactions, the dye will change to a dark purple color, If there are positive responses, the dye will take on a deep purple hue.

Many different chemicals can be used for this research, but we used a substance called Kovacs oxidase reagent, which is made of 1% tetra-methyl-p-phenylenediamine dihydrochloride in water. The filter paper was soaked in a Kovacs oxidase reagent solution, and a tiny bit of the pure culture was spread on it using a platinum loop. Color development was allowed and watched for 10-60 seconds. When a deep purple-blue/blue color showed up, it means that oxidase was produced. A negative result is when there is no change in color..

### **3.8.2.3 Catalase Test**

This test aids in identifying bacteria that produce the catalase enzyme, such as Staphylococci, and those that do not produce it, such as Streptococci. Catalase helps to break down hydrogen peroxide into oxygen and water. "In the test, 2 milliliters of hydrogen peroxide solution was put

into a test tube. Then, some colonies of the test organism were picked and put into the H<sub>2</sub>O<sub>2</sub> solution using a clean glass rod. " Bacteria that have catalase enzyme make bubbles of gas, but bacteria that don't have catalase enzyme don't make bubbles.

#### **3.8.2.4 Citrate Utilization Test**

The citrate utilization test shows if organisms can use citrate as their main energy source or not. By conducting a citrate test, we aimed to distinguish the bacteria within the Enterobacteriaceae family that possess the capability to utilize citrate by means of an enzyme. Simon made a special kind of agar with citrate in it, which is an important energy source. Afterward, he placed it in Petri dishes to allow bacteria to proliferate. We used clean plates and a clean tool to put bacteria from a pure culture onto the plates. The plates were kept warm at 37 degrees Celsius for one whole day. The color changed because bacteria grew on the food and used citrate, which showed a positive test for citrate. The change in pH makes the bromothymol blue in the liquid go from green to blue, which shows a positive result. The test showed that nothing grew and the color of the medium stayed green.

#### **3.8.2.5 Urease test**

The urease test helps to find bacteria that can make the urease enzyme. Organisms that produce urease can break down urea into ammonia and carbon dioxide. This test was used to tell the difference between bacteria that have urease and other types of Enterobacteriaceae bacteria. The clean bacteria were put into a special liquid and kept at a certain temperature for a day. Cultures that are urease-positive turn pink because the indicator changes color when there is ammonia. Cultures that are urease-negative do not change color or turn yellow-orange.

#### **3.8.2.6 Mannitol Test**

The purpose of the mannitol test is to ascertain if the bacteria has the capability to ferment mannitol, as well as to screen for bacteria that can withstand high levels of salt. Fermenting mannitol makes the liquid turn from red to yellow as it becomes more acidic. Mannitol salt agar was sterilized, cooled, and then spread onto plates. Injected with pure samples and kept warm for 24 hours at 37 degrees Celsius. We observed some yellow items growing that looked promising. The bad result stayed red-pink with stuff growing on it.

### **3.8.2.7 Triple Sugar Iron (TSI) test**

The TSI test examines an organism's ability to ferment sugars and produce hydrogen sulfide (H<sub>2</sub>S) or gas (O<sub>2</sub>), or both. The test was used to tell apart different bacteria based on how they break down sugar, and to separate them from other similar bacteria. A TSI agar slant was used in a test tube at an angle. We put bacteria on a slanted surface using a needle. We poked the needle into the middle of the surface and then moved it around to spread the bacteria. After giving the vaccines, the test tubes were covered with foil paper and left at a normal temperature of 36°C to sit for 24 hours. We looked at what happened when we mixed stuff in test tubes. When sugar fermented, it made hydrogen sulfide, gas, and changed the color from red to yellow. When the test showed a red top or yellow bottom, it meant that only glucose was being broken down. When the yellow top and yellow bottom of the acid/acid slant turned up, it meant that dextrose, lactose, and/or sucrose were fermenting. The red top and red bottom in the test tube shows that there is no sugar fermentation happening. The darkening of the area in the test tube showed that H<sub>2</sub>S was made. Bubbles, cracks, or raised area at the bottom of the slanted agar showed that gas (CO<sub>2</sub> and H<sub>2</sub>) was made.

## **3.9 GROWTH ON DIFFERENTIAL MEDIA**

### **3.9.1 *Bacillus cereus* Agar Base**

*Bacillus cereus* agar was employed by us to detect and differentiate *Bacillus* and pathogenic *Staphylococci* bacteria. *Bacillus cereus* agar stops some bacteria from growing, and it also helps tell the difference between different kinds of *Bacillus* bacteria. The *Bacillus cereus* agar was heated at a very high temperature and then cooled before being put into dishes. We put pure bacteria on a dish and let it grow at a warm temperature for a day. Based on the shape, color, and spore shape, we visually inspected the plates for signs of typical bacteria growth.

### **3.9.2 Mannitol Salt Agar (MSA)**

MSA is used in differentiating and selecting mostly *Staphylococcus* species, which was prepared and autoclaved at 121°C for 15 minutes, then was allowed to cool and poured into Petri dishes. The isolated pure cultures were inoculated by streaking on the medium, and the plates were incubated at 37°C for 24 hours. Plates were examined and observed for typical growth.

### **3.9.3 *Pseudomonas* Agar**

*Pseudomonas* bacteria produce various pigments, including one known as fluorescein. *Pseudomonas* agar was used to see if *Pseudomonas* bacteria make pigment. *Pseudomonas* agar is a special kind of dish that helps to find and study *Pseudomonas* bacteria by stopping other kinds of bacteria from growing. We prepared *Pseudomonas* agar Petri dishes by heating the medium and then pouring it into plates to grow bacteria on. Dishes were filled with the pure isolated samples and kept at 37 degrees Celsius for a day. The sample was looked at to see if any microorganisms were growing. The test showed that the sample turned a light yellow color in the agar and glowed under UV light.

### **3.9.4 Eosin Methylene Blue (EMB) agar**

Eosin Methylene Blue (EMB) agar is a special kind of agar that helps to tell the difference between different types of bacteria. It shows if certain harmful bacteria are present by changing color. It stops one type of bacteria from growing, and shows if another type can ferment lactose. A clean plate was prepared with a type of bacteria stuff, then heated up in a special machine, and then cooled down. Next, it was covered with a pure sample of bacteria. We put the plates with bacteria in a warm place for a day and looked at them to see if there were any changes in how the bacteria looked. Bacteria that can ferment lactose make dark or pink colonies, and bacteria that cannot ferment lactose are colorless.

### **3.10 Rhizobacterial Potential of Bacterial and Fungal Isolates**

In order to produce Indole Acetic Acid (IAA), we examined the growth of rhizobacterial isolates in a liquid containing L-Tryptophan, a necessary component for IAA synthesis. We used a special liquid and a chemical called Salkowki's reagent to do this test. Broth with vaccine was kept warm at 30°C for 3 days in a shaking machine. After leaving it to grow, the liquid was spun around at a high speed for 15 minutes. Mix 0 ml of the liquid with 2. 0 ml of Salkowski reagent, and leave it at room temperature for 25 minutes. Pink color appeared after leaving it at room temperature, which shows that IAA was produced (Patten and Glick in 2002, Kumar et al. in 2012, and Ngoma et al..)

$$\frac{\text{colony diameter} + \text{halozone diameter}}{\text{colony diameter}}$$

## CHAPTER FOUR

### RESULTS

The data in Table 1 provides details of the testing performed on the identified microbes. We found four types of bacteria in the samples from houseflies. We did more tests to confirm that they were bacteria, and they grew on the growth medium we used to find them. Four types of bacteria were found. They are called *Proteus vulgaris*, *Pseudomonas aeruginosa*, *E. coli* and *Serratia marcescens*.

The number of bacteria in the microbial isolates is displayed in table 2. The amount of coliform bacteria ranged from 1.4 million to 1.6 million in sample M1 to M3, and the amount of other bacteria ranged from 6.7 million to 7.4 million in sample M1 to M3.

The bacteria's response to antibiotics was shown in Table 2. The bacteria *Escherichia coli*, *Serratia*, *Pseudomonas*, and *Proteus* were separated. We are all allergic to Gentamycin and ciprofloxacin. The bacteria called *Pseudomonas*, *E. coli* and *Serratia* bacteria were easily affected by Chitosan. The test showed that most bacteria were sensitive to carbenicillin, but *Proteus sp.* was resistant to it. The MAR index was between 0.333 and 0.444, which means these bacteria are a public health concern.

Table 3 displays the traits that make the bacteria from houseflies harmful. *E. coli* and *Pseudomonas* bacteria both tested positive for the hemolysin test.

Figure 4.1 shows the total amount of bacteria that can't make their own food, and figure 4.2 shows the amount of coliform bacteria. M3 had the most counts in the two places where they

took samples. There wasn't much of a difference in the number of bacteria in flies from two different areas in the dump. The number of bacteria was about the same in both places.

**Table 1:** Cultural, microscopic observations and biochemical characteristics of the isolated pathogens

<b>Morphological</b>	<b>Isolate 1</b>	<b>Isolate 2</b>	<b>Isolate 3</b>	<b>Isolate 4</b>
Elevation	Flat	Raised	Flat	Raised
Margin	Entire	Entire	Undulate	Entire
Color	Cream	lemon	Cream	Cream
Shape	Circular	Circular	Irregular	Circular
Size	Medium	Medium	Large	Medium
Gr. diff. agar	SSA	PCA	EMB	EMB
Colour	black	green	green	opaque
<b>Staining</b>				
Gram stain	-	-	-	-
cell type	rod	rod	Rod	rod
Arrangement	disperse	disperse	disperse	disperse
Color	pink	pink	pink	red
Spore staining	-	-	-	-
<b>Biochemical</b>				
KOH String Test	+	+	+	+
Catalase	+	+	+	+
Indole	+	-	+	-
Citrate	+	+	-	+
Oxidase	-	+	-	-
Motility	+	+	+	+
Urease	+	+	-	-
Glucose	+	-	+	+
Sucrose	-	-	-	+
Lactose	-	-	+	+
Mannitol	-	-	-	+
Gas formation	-	-	+	-
H <sub>2</sub> S formation	+	-	-	-
TSI (Slant/Butt) reaction	K/A H <sub>2</sub> S	K/K	A/AG	K/A (*A/A)
Esculin Hydrolysis	+	-	-	-
Identity	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Serratia marcescens</i>

**Table 2:** The antibiotic sensitivity profile of bacteria isolates isolated from *Musca domestica*

<b>Isolates</b>	<b>GEN</b>	<b>CS</b>	<b>CB</b>	<b>M</b>	<b>AG</b>	<b>E</b>	<b>CIP</b>	<b>TE</b>	<b>CD</b>	<b>MAR index</b>
<i>Escherichia coli</i>	S	S	S	R	S	R	S	S	R	0.333
<i>Serratia sp.</i>	S	R	S	R	R	S	S	R	S	0.444
<i>Pseudomonas</i>	S	S	S	R	R	R	S	S	S	0.333
<i>Proteus</i>	S	R	R	S	S	R	S	R	S	0.444

**KEY:** GEN = Gentamicin, E= Erythromycin, CIP= Ciprofloxacin, AG= Augmentin,

Table 3: Phenotypic virulence determinants of bacterial isolates

<b>Isolates</b>	<b>Hemolysin</b>	<b>DNase</b>	<b>Gelatinase</b>	<b>Lipase</b>
<i>Escherichia coli</i>	$\beta$	-	-	+
<i>Pseudomonas</i> sp.	$\Gamma$	+	+	-
<i>Proteus</i>	$\alpha$	+	+	-
<i>Serratia</i>	$\beta$	+	+	-

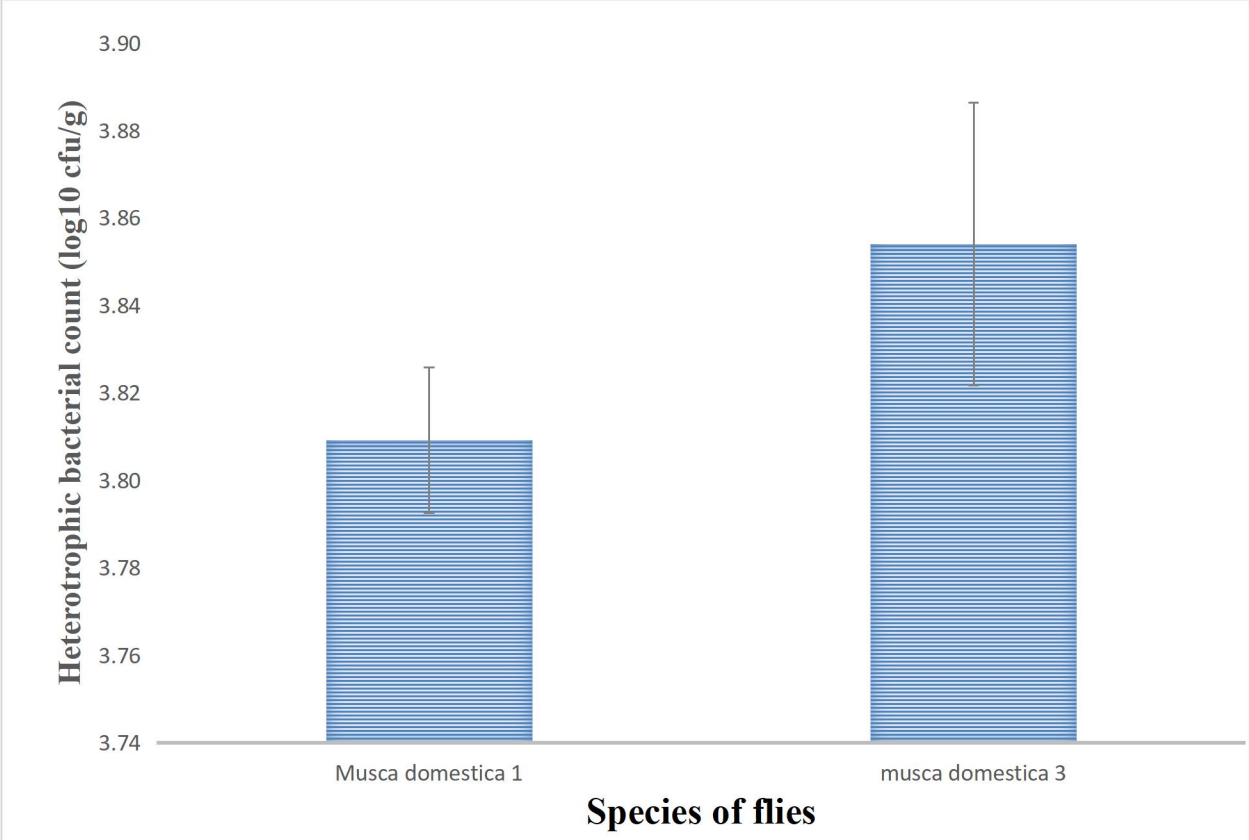


Figure 4.1 showing bar charts of heterotrophic bacterial count

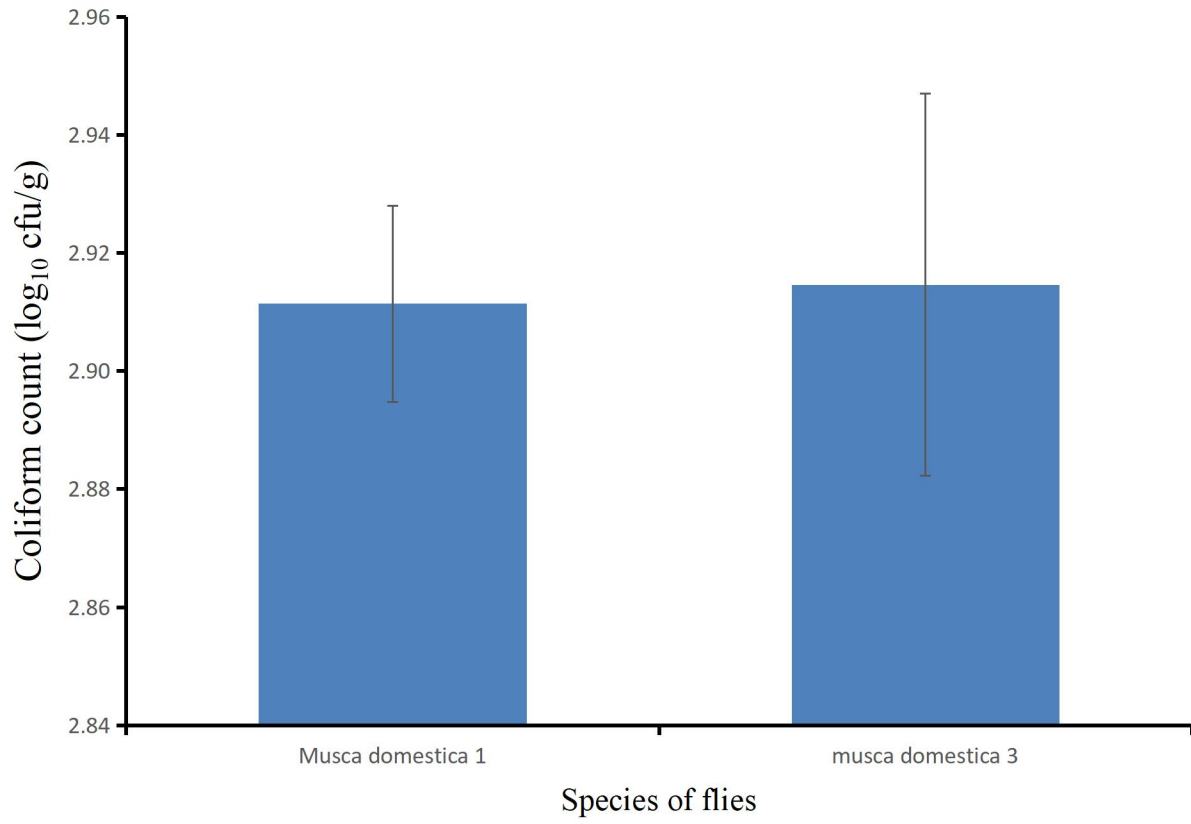


Figure 4.2 showing bar charts of coliform bacterial count

## CHAPTER FIVE

### DISCUSSION

The study revealed that *E. coli* bacteria were present in houseflies. Bacteria called *coli*, *Serratia*, *Proteus* and *Pseudomonas*. The bacteria found on houseflies in the garbage area are a big source of poop and other dirty things. This is similar to a study in 2004 that found germs in the water from the River Nile. The Kobayashi et al. , 1999 study found *E. coli* in the isolates they tested. *Coli O157:H7* is common in the intestines of *M. House flies* make food dirty because they regurgitate while eating, so any germs in their stomachs can get into the food and make it contaminated. *coli O157:H7* is one of the most common causes of foodborne illness. This type of bacteria can be found in contaminated food and can cause severe sickness when ingested. *Coli* bacteria can make some people sick, depending on how old they are, how strong their immune system is, and how strong the bacteria are.

*Pseudomonas aeruginosa* is often found in non-sterile places on people who are not sick. It is similar to the bacteria *S. Epidermidis* is normally found on a human's skin. The bacteria usually live harmlessly on the skin and in the mouth, but can cause infections in any part of the body they touch. *P* has flexible and not strict metabolic needs. *Pseudomonas aeruginosa* can live in many different places, which makes it a good example of a germ that takes advantage of opportunities to cause illness. Because the bacteria are usually not harmful, infections mainly happen in people who are already sick and in the hospital. People with weakened immune systems, such as those with AIDS, cystic fibrosis, bronchiectasis, low white blood cell count, and certain types of cancer, can get infected with *P. aeruginosa* throughout their bodies or in specific

areas. Aeruginosa Infections caused by aeruginosa bacteria. Diseases can spread when dirty things carried by infected flies touch other things (Mengal et al. , 2019)

*Serratia marcescens* was found to be a harmful germ in *Musca domestica*, which is the common house fly. *Maarcescens* is a type of germ that can make you sick. It is hard to kill because it can resist the body's natural defenses. These pathogens account for a small percentage of hospital-acquired infections and often target surgical wounds and soft tissues. These infections are spread by houseflies. Certain types of *Serratia* bacteria can make a color on wounds that goes from dark red to pink.

*Proteus* can make you sick by causing food poisoning. Different types of *Proteus* that grow in your body also make a substance that stops white blood cells from working properly. Many kinds of *Proteus* bacteria. These infections are caused by bacteria and they make pus. They also make your gums swell and can get into the holes in your teeth.

The bacteria's response to the antibacterial was shown in Table 3. The single bacteria *Escherichia coli*, *Serratia*, *Pseudomonas*, and *Proteus*. All of us react strongly to Gentamycin and ciprofloxacin. *Pseudomonas* bacteria Chitosan was able to kill *E. coli* and *Serratia* bacteria. and a type of bacteria called *Pseudomonas*. The test results showed that most bacteria were sensitive to carbenicillin, except for *Proteus* sp which was resistant. The multiple antibiotic resistance index ranged from 0. 333 to 0444 Many bacteria release Beta-lactamase, which attaches to the active sites of antibiotics and reduces their ability to stop the growth of bacteria. (Ogunshe and Olabode, 2009). *Serratia marcescens* can make a beta lactamase that helps it resist certain antibiotics.

Some types of bacteria that are resistant to many drugs. *Pseudomonas aeruginosa* can resist most antibiotics, even carbapenems. This study also shows the same results as Ogunshe and Olabode

(2009), who found that bacteria from *Clarias gariepinus* are resistant to antibiotics. The isolates were easily killed by gentamicin, ciprofloxacin, and chloramphenicol, which was similar to what was found in another study (Okpo et al. , 2016) The MAR index found that the bacteria were not resistant to some antibiotics because those antibiotics are small and can get into the bacteria where they stop them from working properly (Okpo et al. , 2016)

We checked samples for signs of harmful germs like coliform bacteria, *E. coli*, and other types of bacteria. Coli bacteria, enterococci bacteria or coliphage viruses. Rewritten: Coli bacteria, enterococci bacteria, or coliphage viruses. These indicator microbes might not be bad, but they show that there could be other harmful germs around. In this study, the amount of germs and bacteria found ranged from 1.4 million to 1.6 million in some samples, and from 6.7 million to 7.4 million in others. More bacteria were found in site M3 because there was more garbage and rotting stuff there, like old fruit and dead animals. Site M1 had less garbage so there were fewer bacteria. This is similar to what Williams and Kelechi found in 2015, that places with less trash had less bacteria and fungi. The findings of this study support the research of Deapke et al. , (2018), who said that the cleanliness of the environment can either weaken or help the harmful parasites and bacteria carried by houseflies. In a study done by Amaechi and others. In 2017, more houseflies picked up germs from trash dumps and toilets.

## **CONCLUSION**

House flies in Egor Local Government Area of Edo State are carriers of harmful germs and bacteria. *Coli*, *Serratia*, *Pseudomonas* and *Proteus* bacteria were found on their bodies and inside them. These bacteria are harmful to public health because they are strong and resist antibiotics. So, flies can pass these germs to people because they live close to humans and can move around

easily. It is necessary to take action to manage the population of flies in order to prevent the diseases they can spread presently and in the years to come.

## REFERENCE

- Adeleke, M. A., Oyewole, V. O., Olabiyi, K. O. and Oforka, L. C. (2017). Parasites and pathogenic bacteria associated with houseflies and the public health implications in Osogbo, Southwestern Nigeria. *Munis Entomology and Zoology*, **12** (1): 94-98.
- Akhtar, M., Hirt, H. and Zurek, L. (2009). Horizontal transfer of the tetracycline resistance gene tetM mediated by pCF10 among *Enterococcus faecalis* in the house fly (*Musca domestica* L.) alimentary canal. *Microbiology and Ecology*, **58**:509–518.
- Akpan, N., Anwan, E. and Antia, U. (2017). Housefly (*Musca domestica*) as a carrier of pathogenic microorganisms in Hospital Environments in Calabar, Nigeria. *Journal of Science and Technology*, **24**(2):193- 199.
- Alaku, A. I., Danladi, S. and Mohammed, A. (2021). Bacteria and parasites pathogens associated with the houseflies (*Musca domestica*) in Lafia metropolis of Nasarawa State, Nigeria: *Journal of Applied Life Sciences International*.
- Alves, T. D., Lara, R. P., Maluta, M. G., and Leite, D. D. (2018). Carrier flies of multidrug-resistant *Escherichia coli* as potential dissemination agent in dairy farm environment. *Science Total Environment*, **633**:1345– 1351.
- Amaechi, A. A., Ukaga, C. N., Iwunze, J. I., Nwachukwu, M. O. and Anumudu, B. (2017). Epidemiological implications of houseflies (*Musca domestica*) in the dissemination of diseases in Owerri, south-east Nigeria. *Nigerian Journal of Parasitology*, **38**(2): 298-301.
- Bahrndorff, S., De Jonge, N., Skovgård, H. and Nielsen, J. L. (2017). Bacterial Communities Associated with Houseflies (*Musca domestica* L.) Sampled within and between Farms. *Plos One*, **12**(1) 15-22.

- Casolari, C., Pecorari, M., Fabio, G., Cattani, S. and Vettorelli, C. (2005). A simultaneous outbreak of *Serratia marcescens* and *Klebsiella pneumoniae* in a neonatal intensive care unit. *Journal of Hospital Infection*, 61:312–20.
- Cohen, D., Green, M. and Block, C. (1991) Reduction of transmission of *shigellosis* by control of houseflies (*Musca domestica*). *Lancet*, 337: 993–7.
- Crivaro, V., Bagattini, M., Salza, M. F., Raimondi, F., Rossano, F. and Triassi, M. (2007). Risk factors for extended spectrum beta lactamase producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *Journal of Hospital Infection*, 67(2):135–41.
- Deakpe, T. E., Manyi, M. M. and Utume, L. N. (2018). Pathogenic parasites and bacteria associated with the housefly (*Musca domestica*) in Makurdi; a fly-infested area in central Nigeria. *Nigerian Journal of Parasitology*, 39(1): 111-115.
- Elkanah, S. O., Elkanah, D. S., Fumlack, L. D., Wahedi, J. A. and Kela, S. L. (2020). Houseflies (*Musca domestica*) as potential carriers of human intestinal parasites in Jalingo metropolis. *FUDMA Journal of Sciences*, 4(3):402-405.
- Emerson, P. M., Bailey, R. L., Walraven, G. E. and Lindsay, S.W. (2001) Human and other faeces as breeding media of the trachoma vector *Musca sorbens*. *Medical and Veterinary Entomology*, 15: 314–20.
- Graczyk, T. K., Knight, R., Gilman, R. H. and Cranfield, M. R. (2001). The role of non-biting flies in the epidemiology of human infectious diseases. *Microbes Infection*, 3:231–235.
- Grema, H. A., Geidam, Y. A., Suleiman, A., Gulani, I. A. and Birma, R. B. (2015). Multi-Drug resistant bacteria isolated from fish and fish handlers in Maiduguri, Nigeria. *Journal of Animal Veterinary*, 7(3): 49-54

- Hrudey, S.E., Hrudey, E. J., Pollard and Simon J.T. (2006). Risk management for assuring safe drinking. *Environmental International. Environmental Risk management-the state of the art*, **32** (8): 948-957.
- Huang, J.G., Zhou, L. J., Xu, H. H. and Li, W. O. (2009). Insecticidal and cytotoxic activities of extracts of *Cacalia tangutica* and its two active ingredients against *Musca domestica* and *Aedesal bopictus*. *Journal of Economical Entomology*, **102**(4): 1444- 1447
- Imarhiagbe, E. E., Ofuya, M. E., Osarenotor, O. and Ekhaise, F. O. (2016). Antibiotic Sensitivity Pattern of Microorganisms Isolated from Smoked and Frozen Fishes Sold in Benin and Warri Metropolis. *Nigerian Journal of Biotechnology*, 31:40– 45.
- Jalili, D., MohamadHadi, D., Abdolmajid, F, and Mahmood, A. (2021). Assessment of Airborne Bacterial and Fungal Communities in Shahrekord Hospitals. *Journal of Environmental and Public Health*, 2021: 1-7.
- Jirillo, E., Magrone, T., and Mirgliotta, G. (2014). Immune response to parasitic infections. *Immune Response to Parsitic infections*. Volume 2. Bethan Science Publishers.
- Kohler, J. R., Casadevall, A. and Perfect, J. (2014). The Spectrum of fungi that infects humans. *Cold Spring Harbor Perspectives in Medicine*. **5**(1):1-17.
- Mengal, S., Azam, S. and Kamran, M. T. (2019). *Pseudomonas aeruginosa* as a pathogenic organism. *International Journal of Biosciences*, **14**(4):286-291.
- Mian, L. S., Maag, H. and Tacal, J. V. (2002). Isolation of Salmonella from muscoid flies at commercial animal establishments in San Bernardino County, California. *Journal of Vector Ecology*, 27: 82-85.
- Ogunshe, A. A. and Olabode, P. O. (2009). Antimicrobial potentials of indigenous Lactobacillus strains on Gram-negative indicator bacterial species from *Clarias gariepinus* (Burchell,

- 1822): Microbial inhibition of fish-borne pathogens. *African Journal of Microbiology Research*, **3**(12): 870- 876.
- Okpo, N. O., Abdullahi, I. O., Whong, C. M. Z. and Ameh, J. B. (2016). Occurrence and antibiogram of *Staphylococcus aureus* in dairy products consumed in parts of Kaduna State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, **9**(2):225-229
- Olatoye, I. O. (2010). The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. *African Journal of Biotechnology*, **9**(8):1196-1199.
- Onwugamba, F. C., Fitzgerald, J. R., Rochon, K., Guardabassi, L. Alabi, A., Kühne, S., Grobusch, M. P. and Schaumburg, F. (2018). The role of ‘filth flies’ in the spread of antimicrobial resistance. *Travel Medical Infection Discussion*, 22:8–17.
- Rahuma, N., Khalifa S. G., Aissa, R. B. and Elamaari, A., (2005). Carriage by the housefly (*Musca domestica*) of multiple-antibiotic resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya.
- Service, M. (2012) Medical Entomology for Students. United Kingdom: Cambridge University Press; 140–141.
- Smallegange, R. C. and Den Otter, C. J. (2007). Houseflies, annoying and dangerous. *Emerging pests and Vector-borne diseases in Europe*, 17:281–292.
- Songe, M. M, Hang’ombe B. M, TJD K-J. and Grace, D. (2017). Antimicrobial Resistant Enteropathogenic *Escherichia coli* and *Salmonella* spp. in Houseflies Infesting Fish in Food Markets in Zambia. *International Journal of Environmental Research and Public Health*. **14**(1):21.

- Spanggard, B., Jorgenses, F. G. and Huss, H. H. (1993). Antibiotic resistance in bacteria isolated from three freshwater farms and an unpolluted stream in Demark. *Aquaculture*, 115: 195-207.
- Stewart-Greco, V.S., Brown, E. E., Parr, C., Kalab. M., Jacobs, M. R. and Yomohovian, R. A. (2012). *Serratia marcescens* strains implicated in adverse transfusion reaction form biofilms in platelet concentrates and demonstrate reduced detection by automated cultures. *Vox Sang.* **102**(3):212–20.
- Szalanski, A. L., Owens, C. B., Mckay T., and Steelman, C. D. (2004). Detection of Campylobacter and Escherichia coli O157:H7 from filth flies by polymerase chain reaction. *Medical and Veterinary Entomology*, **18**(3):241–246
- Tatfeng Y.M., Usuanlele, M.U., Orukpe, A., Digban, A.K., Okodua, M., Oviasogie, F. and Turay. A. A. (2005). Mechanical transmission of pathogenic organisms: The role of cockroaches. *Journal of Vector and Disease*, 42: 129-134.
- Tsagaan, A., Kanuka, I. and Okado, K. (2015). Study of pathogenic bacteria detected in fly samples using universal primer-multiplex PCR. *Mongolian Journal of Agricultural Science*; **15**(2):27–32.
- Wang, W. (2013). Houseflies as Potential Vectors for Antibiotic Resistant Bacteria. Graduate Program in Food Science and Nutrition: The Ohio State University.
- WHO. (2008). First Inter-Ministerial Conference on Health and Conference in Africa: Health security through Health environment.
- Williams, J. O. and Hakam, K. (2016). Microorganisms associated with dump sites in Port Harcourt Metropolis, Nigeria. *Journal of Ecology and the Natural Environment*, **8**(2) 9-12

Yujing, W., Fan, L., Xinyue, K., Xiangyu X., and Wenwen, C., (2022). Odor Characteristics and health risks during food waste bioconversion by housefly (*Musca domestica* L.) larvae. *American Biology*, 25:86-89.

Zurek, L. and Ghosh, A. (2014). Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits. *Applied Environmental and Microbiology*, 80:3562–3567.