

**ANTIBIOTIC SUSCEPTIBILITY PATTERN OF
SELECTED FOODBORNE ASSOCIATED BACTERIA
FROM COCKROACHES.**

By

Lawrence Kapansa

**A dissertation submitted to the University of Zambia in partial fulfilment of the
requirements for the Award of the Degree of Master of Science in One Health
Laboratory Diagnostic Sciences**

The University of Zambia

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DECLARATION

I, Lawrence Kapansa, declare that this dissertation represents my own work. This work has not been done in Zambia before and neither has it been published for any qualification at the University of Zambia or any other University. Various sources to which I am indebted are clearly indicated in the text and in the references.

SignedDate.....

CERTIFICATION OF APPROVAL

The University of Zambia approves the dissertation submitted by LAWRENCE KAPANSA, as fulfilling the partial requirements for the award of the Master of Science in One Health Laboratory Diagnostic Sciences by the University of Zambia.

.....
Supervisor	Signature	Date

.....
Examiner 1	Signature	Date

.....
Examiner 2	Signature	Date

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Examiner 3	Signature	Date

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ABSTRACT

Cockroaches appear to be suitable mechanical transmitters for a wide range of food borne pathogenic microorganisms due to their habit of feeding on excreta or garbage and occurrence in places where food is stored or handled. Therefore, they can play a significant role in the dissemination of microorganisms between the environment. The objective of the study was to determine the antibiotic susceptibility pattern of selected bacterial food-borne pathogens isolated from cockroach species. A total of 278 cockroaches were collected from 50 rooms at University of Zambia and 40 houses in Kalingalinga between November 2023 and January 2024. Two species of cockroaches namely *Periplaneta americana* and *Blattella germanica* were identified using identification keys. The distribution of these species was found to be statistically insignificant between the two study sites, with *B. germanica* being more prevalent, accounting for 66.7% of the cockroaches at UNZA and 58.2% at Kalingalinga.

Wash homogenates of the external and gut surfaces were used for culturing on selective and differential media. The isolated bacteria were identified using biochemical tests. Bacterial pathogens were isolated from both the external and gut surfaces of the cockroaches, with 39 foodborne-associated bacteria identified. These included *Escherichia coli* (12 isolates), *Salmonella species* (7 isolates), and *Staphylococcus aureus* (7 isolates) from *P. americana*, and from *B. germanica*, *Escherichia coli* (6 isolates), *Salmonella species* (3 isolates), and *Staphylococcus aureus* (4 isolates).

Of the 39 bacterial isolates, 79% were from the cockroach gut, while 21% were from the external surface, showing that the gut is a significant reservoir for pathogenic bacteria. The distribution of these pathogens was not statistically significant across the study sites or cockroach body parts.

Antibiotic susceptibility testing of the bacterial isolates revealed varying degrees of resistance. *S. aureus* showed high resistance to Penicillin G, while *Salmonella* and *E. coli* exhibited notable resistance to Ciprofloxacin and Cefotaxime, with 66.7% of all bacterial isolates being resistant to at least one class of antibiotics. Furthermore, 35.9% (N=39) of the isolates were multidrug-resistant (MDR), with the MDR rate being 39% (n=18) in *E. coli*, 40% (n=10) in *Salmonella* and 27% (n=11) in *S. aureus*.

Phenotypic testing detected Extended Spectrum Beta-Lactamase (ESBL)-producing bacteria among the isolates, with 8 *E. coli* and 3 *Salmonella* identified as potential ESBL producers. After screening on Cefotaxime/MacConkey agar, 3 *E. coli* and 2 *Salmonella* were confirmed as ESBL producers. PCR analysis revealed the presence of ^{bla}CTX-M and ^{bla}TEM genes in these ESBL-producing isolates, with ^{bla}CTX-M detected in 2 *E. coli* isolates and ^{bla}TEM detected in all 5 ESBL-producing isolates.

The findings from this study highlight the potential role of cockroaches as vectors for foodborne pathogens and multidrug-resistant bacteria in residential and institutional settings. Given the high percentage load of pathogenic bacteria, particularly in the gut of cockroaches, and the observed antimicrobial resistance, the results underscore the need for effective pest control strategies and prudent use of antibiotics to mitigate the spread of resistant bacteria. Moreover, the presence of ESBL-producing *E. coli* and *Salmonella* emphasizes the importance of ongoing surveillance of antimicrobial resistance in both healthcare and environmental contexts to protect public health.

DEDICATION

I would like to dedicate this work to my wife, Nancy Chapuswike Kapansa and daughter, Atalia Bupe Kapansa, who always motivates me to be resilient, hardworking, determined and focused.

ACKNOWLEDGEMENTS

I would like to thank the numerous people who contributed to the successful completion of my master's dissertation.

The Government of Zambia through the Africa Centre of Excellence for Infectious Diseases of Humans and Animal (ACEIDHA) at the University of Zambia, School of Veterinary Medicine is greatly appreciated for the scholarship provided to me during my studies.

Further gratitude goes to my supervisors Prof. Enala Mwase and Prof. Bernard Hang'ombe of the Department of Paraclinical Studies, School of Veterinary Medicine, at the University of Zambia, whose expertise, understanding, generous guidance and support made it possible for me to work on a topic that was of great interest to me. It was a pleasure working with them.

I am highly indebted and thoroughly grateful to Dr. Shawa for his immense interest in my topic of research, for providing me with the materials and links that I could not possibly have discovered on my own, for his kind words and suggestions. His office and laboratory were always open to do the research and was available whenever I ran into a trouble spot or had questions about my research or writing.

Let me also thank the laboratory staff of the University of Zambia, School of Veterinary Medicine under the Paraclinical Studies department and my colleagues, the participating communities of UNZA and Kalingalinga all of whom made this research possible.

Finally, I must express my very profound gratitude to my family and my wife, Nancy for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of research and writing this dissertation. This accomplishment would not have been possible without them.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
BG	<i>Blattella germanica</i>
BPW	Buffered peptone water
CTX	Cefotaxime
ESBL	Extended spectrum beta-lactamase
FBD	Foodborne diseases
IMP	Integrated Pest Management
MAC	MacConkey agar
MDR	Multi-drug resistance
MM	Master mix
PA	<i>Periplaneta americana</i>
PCR	Polymerase chain reaction
SE	<i>Staphylococcus enterotoxins</i>
SPP	Species
US	United States of America
UNZA	University of Zambia
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Globally, foodborne diseases (FBD) continue to place a significant cost on public health, the economy, and society. In order to promote evidence-based policies and assess the burden and distribution of foodborne illness, the World Health Organization (WHO) released the first estimates of the worldwide and regional disease burden resulting from 31 foodborne hazards in 2015 (WHO, 2015). Contamination can occur during the storage, transportation, and preparation of food for consumption (Kadariya et al., 2014). Although the majority of foodborne illnesses are minor and self-limiting, severe cases can happen, particularly in young children, the elderly, and people with compromised immune systems (Havelaar et al., 2015). According to Kapaya et al., (2018), diarrheal illnesses are among the top five causes of morbidity and death in Zambia across all age groups. Some foodborne pathogens can spread directly from animals to people, while others can do so by way of vectors including insects, food handlers, infected food items or surfaces used in food processing, or through contact with sponges, towels, or utensils. Furthermore, some foodborne infections may spread more easily by transborder movement of people and goods (Stein & Chirilă, 2017).

Cockroaches' dirty habits of feeding on fecal matter, living in sewer pipes and frequent presence in areas where food is handled or stored make them seem like ideal mechanical transmitters for a variety of food-borne pathogenic bacteria. Cockroaches can carry microorganisms externally on their cuticles or absorb them, which can lead to later excretion or regurgitation (Donkor, 2020). When cockroaches come into contact with food, they can readily contaminate it in this way (Donkor, 2020). Cockroaches are omnivorous species. They spread many dangerous microbes and illnesses, including typhoid, leprosy and dysentery, as they feed on a variety of foods and even human waste. These microbes eventually migrate to other organisms after spending a considerable amount of time living in the cockroaches' intestines (Turki Jalil et al., 2023).

Bacteria appear to be the primary cause of foodborne illness in the majority of countries and the causative agents behind two thirds of foodborne illness outbreaks that have been documented (Le Loir et al., 2003). Among the bacteria that can be found in food and can cause FBDs are: *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, and other pathogenic *Escherichia coli*; and a variety of *Vibrios*, particularly *Vibrio cholerae* and *Vibrio parahaemolyticus*. Some of these bacteria are eaten and then vomited or regurgitated, or they are carried by the cuticle of the insect. Additionally, it has been discovered that cockroaches harbour a wide range of bacteria in their digestive tracts that are pertinent to public health, including *Pseudomonas aeruginosa*, Enterobacteriaceae, *Streptococcus* spp., and *Staphylococcus aureus*. Furthermore, because of their connection to dangerous bacteria that have been identified through numerous investigations, cockroaches have been connected to a number of outbreaks of food-borne illnesses (Wannigama, 2014; Ifeanyi & Odunayo, 2015; Nazari et al., 2020; Molewa et al., 2022; Davari et al., 2023).

1.2 Rationale of the Study

Almost 60 species of yeast, 150 species of bacteria, 45 species of parasitic worms, and 90 species of protozoa can be mechanically or biologically transmitted to humans by cockroaches, making them an important biological agent (Tachbele & Ashenafi, 2006; Góralaska & Kurnatowski, 2013; Vazirianzadeh et al., 2014). Humans are infected by these harmful organisms, which can lead to a variety of illnesses including leprosy, dysentery, abscesses, acne, and urinary infections. For bacterial species, they can endure for several days on the body surfaces of cockroaches (Tachbele & Ashenafi, 2006; Góralaska & Kurnatowski, 2013; Vazirianzadeh et al., 2014).

In Zambia, cockroaches are among the most common pests found in offices, houses, restaurants, and hospitals (Mbata, 2018). However, there is currently no comprehensive study on the role of cockroaches as vectors of diseases in the country. Additionally, no studies have examined the pathogenic bacteria associated with cockroaches and their resistance patterns in Zambia, despite the fact that these pests are a primary source of bacterial infections and are linked to several drug-resistant strains in countries like India, Iran, Nigeria and have indicated this association (Wannigama, 2014; Ikechukwu et al., 2017; Nazari et al., 2020). Among the enteric pathogens and food poisoning microorganisms *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*

that have been reported in Zambia to be among the top causers of reported foodborne outbreaks (Kapaya et al., 2018; Dayo Omodele et al., 2021; Muligisa-Muonga et al., 2021).

This study aims to fill these gaps by identifying some selected foodborne bacterial pathogens associated with cockroaches found in hostels and homes in the University of Zambia and Kalingalinga settlement and determining their antimicrobial susceptibility pattern. The expected outcomes of this research will provide crucial data on the public health risks posed by cockroaches, informing strategies to control their population and reduce the spread of drug-resistant bacterial infections in Zambia.

1.3. General Objective

To determine the antibiotic susceptibility pattern of selected bacterial food-borne pathogens isolated from cockroach species captured from houses and student hostels in Kalingalinga and University of Zambia.

1.3.1 Specific Objectives

- i. To identify cockroach species in selected houses in Kalingalinga and student hostels at the University of Zambia.
- ii. To isolate and identify *Salmonella species*, *Escherichia coli* and *Staphylococcus aureus* from captured cockroaches.
- iii. To assess the drug-sensitivity patterns and detect associated antibiotic resistance genes of bacteria isolated from cockroaches.

1.4 Research questions

- i. What are the common species of cockroaches circulating in houses and rooms of Kalingalinga and University of Zambia?
- ii. What are the possible foodborne bacterial pathogens carried by the cockroach species circulating in these two study sites?
- iii. What is the antimicrobial susceptibility profile of foodborne bacteria associated with cockroaches from the two study sites?

1.5 Significance of the study

The concern about cockroaches' potential health problem in Zambia is that people might undermine the vector role of these pests. In view of the facts that the number of immunocompromised people and drug-resistant pathogenic strains are increasing simultaneously (Denegre et al., 2020), and the role of cockroaches as mechanical vectors and/or reservoir hosts to opportunists and real pathogens is unknown in Zambia, it is important to start investigations into this problem.

With limited studies and reporting of foodborne diseases outbreak in Zambia, there is need to better understand the current distributions and possible future spread of their pathogens and potential vectors for successful surveillance and control programs. Thus, this study will provide crucial data on the public health risks posed by cockroaches, informing strategies to control their population and reduce the spread of drug-resistant bacterial infections

Antimicrobial resistance surveillance can also benefit from the identification of foodborne pathogens and drug resistance status by the detection of bacteria and their drug resistance pattern in circulation among possible vectors. Furthermore, this may shed light on potential outbreaks of foodborne illness in Lusaka, which may be helpful in modelling the dynamics of transmission and aiding in the mapping of disease distribution. Last but not least, the identification of foodborne bacterial pathogens linked to cockroaches will act as an early warning system for appropriate action, improving preparedness in the development of appropriate and sustainable pest control and preventive measures to reduce pest and vector associated disease outbreaks.

1.7 Ethical considerations

The approval to carry out this study was sought from the Excellence in Research Ethics and Science (ERES) Converge Ethics Committee IRB, reference number **2023-NOV-003 (Appendix 1)**. Written informed consent was obtained from room and house heads by means of standardised consent form. Permission was sought to enter the premises and set traps or capture cockroaches. Participation was voluntary and it could be withdrawn at any time without having to give reasons. The study's procedure was explained to the house or room owner before sample collection (Appendix 2).

CHAPTER TWO

LITERATURE REVIEW

2.1. Cockroach: Identification, Classification, and Distribution

Cockroaches belong to the Superorder Dictyoptera and the order Blattodea. The order consists of four families, Blaberidae, Blattellidae, Cryptocercidae and Blattidae, which comprise species that are somewhat important in medicine. *Blatta orientalis*, *Blattella germanica*, and *Rhyparobia maderae* are examples of representative species of Blattidae family (Burgess, 1993). Furthermore, the big cockroach species *Periplaneta americana*, a frequent annoyance in human habitations, belongs to the Blattidae family. Generally speaking, cockroaches are large, oval, dorso-ventrally flattened insects. Most adult cockroaches have two sets of wings, each of which has a vein network. Over the metathorax and abdomen, the wings are closed like scissors and have hardened to a leathery texture. The sole appendages on the abdomen are paired cerci at the posterior end, and the segments are clearly visible (Burgess, 1993).

The American cockroach, *P. americana*, which is big and has a dark-brown look, is one of the cockroach species that are frequently seen as pests in homes. Another type of cockroaches that live in human homes is the *B. germanica*, which is smaller and has a brown appearance. In contrast, the medium-sized oriental cockroaches have a dark brown to black coloration (Tatfeng et al., 2005).

The earlier indication of the distribution of *Blattella germanica* was that it was originated from Northeast Africa in Ethiopia (Rehn 1945) but recent studies have disputed this claim and has placed its origin in tropical Asia (Roth, 1985). This species is cosmopolitan and is virtually found in all parts of the world (Princis, 1969).

Periplaneta americana is believed to have originated from tropical Africa from whence it is believed to have been distributed to other parts of the world due to trade and commerce (Rehn, 1945). It has distributed to the tropical and subtropical parts of the world and it is more abundant in subtropical Africa and the Indian Ocean subcontinents (Nigam, 1933).



Periplaneta americana

Blattella germanica

Figure 2.1: Showing the two common cockroach species. Photo taken by Lawrence Kapansa

A total of 27 genera of cockroaches are reported to occur in Zambia. They are represented by 43 species and three subspecies. The province with the largest numbers of reported species and subspecies of cockroaches is the Southern Province. This is followed by Lusaka Province. Copperbelt Province is the third, while the least known province regarding this insect, is the Western Province. The occurrence of seven species of cockroaches in Zambia was confirmed during the biodiversity field surveys. The cockroach species were: *Blatta orientalis*, *Periplaneta americana*, *Blattella germanica*, *Supella supellectilium*, *Eustegasta poecila*, *Gyna maculipennis*, and *Gynopeltis cryptospila* (Mbata, 2018).

Cockroaches are affected by climatic conditions of the area. According to a study by Manyullei et al., (2022), it was found that the factors that affect the density of cockroaches are temperature and humidity, both in residential areas, hospitals, storage warehouses and ship raft rooms. Temperature has a positive correlation with cockroach density. The higher the temperature of a place, the higher the density of cockroaches. Humidity has a negative correlation with cockroach density. The more humid a place is, the higher the presence of cockroaches.

2.2 Biology and Control of Cockroaches

The life cycle of cockroaches, which are exopterygote insects, is referred to as indirect or incomplete. As shown in figure 2.2, after maturing and around one week following mating, the female cockroach lays eggs in an ootheca, or egg case, which serves as a protective covering

(Cochran et al., 1999). When the female cockroach reaches the height of her reproductive potential, she can lay up to two egg cases every week and a lifetime total of 10-84 egg cases and each egg case containing 14-40 eggs (Baumholtz et al., 1997). Depending on the species, the female may carry the case externally or internally. It is thought that the female leaves the ootheca alone after depositing it. Though they are smaller and lack developed wings and genitalia, the nymphs resemble adults as they emerge from the eggs. Following hatching, the nymph goes through six to sixteen moults in order to change into an adult (Cochran et al., 1999). When completely developed, an adult cockroach's lifespan can range from a few days to several years, contingent upon the species, surrounding circumstances, and particularly the accessibility of water (Baumholtz et al., 1997). Mature cockroaches will breed all year long if the conditions are favorable. Consequently, the cockroach poses a more alarming threat to humans as potential vector when compared with other vectors such as flies, which are dormant in winter (Baumholtz et al., 1997).

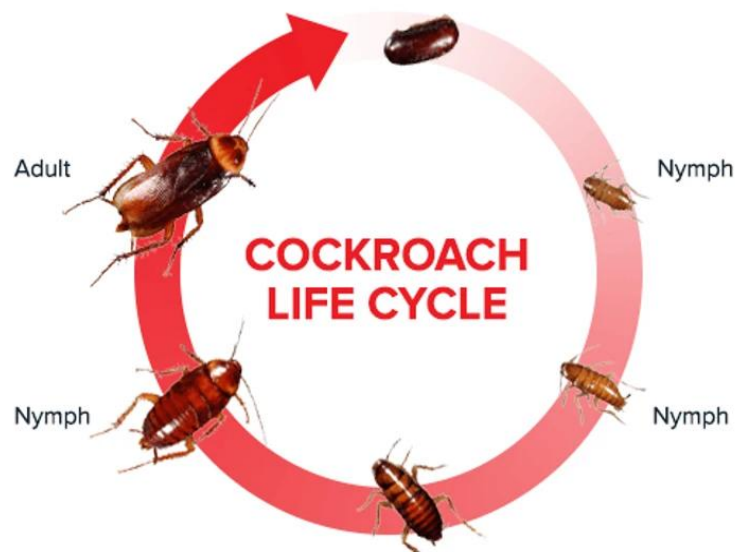


Figure 2.2: Lifecycle of a cockroach (© <https://www.rentokil.com/id/en/services/pest-control-services/cockroaches/cockroach-life-cycle>)

A correlation has been found at the home level between cockroach infestation and cleanliness standards. Cockroaches congregate in kitchen corners, particularly around refrigerators, and in bathrooms surrounding chests, near plumbing connections within or between rooms and/or apartments, according to several studies (Jeffery et al., 2012; Melton, 2012). Cockroaches leave

behind faecal pellets and shed skins during their nighttime foraging (Tatfeng et al., 2005). Cockroaches are prevalent in both households and food-handling establishments, with 54% infestation in households and 77% in food-handling establishments and they can serve as a possible vector for the transmission of drug-resistant bacteria and food-borne diseases, with bacterial species being more frequently identified in food-handling establishments compared to households (Wannigama, 2014).

Cockroach control and prevention strategies are essential for maintaining healthy environments due to the health risks associated with these pests. In order to adjust control strategies appropriately, effective approaches start with the identification and monitoring of cockroach species and then eliminating sources of food, water, and shelter, and above all sanitation measures are essential (Wang & Bennett, 2006). Applying chemical control sparingly is advised, taking pesticide safety and efficacy into account. A thorough strategy that combines several control techniques is provided by integrated pest management (IPM). These strategies include sealing entrance points, early detection and reporting of infestations and educating communities. By integrating these approaches, effective cockroach control and prevention programs can be achieved thereby promoting public health and well-being (Gondhalekar et al., 2021).

2.3 Medical Significance of Cockroaches

Despite the fact that most people think of cockroaches as pests, only around 1% of them—that is, no more than 50 species—are regarded as household pests. For example, cockroaches such as *B. germanica*, *B. orientalis*, *P. americana*, and *P. australasiae* can infest any area that offers the right conditions such as warmth, food, water, and shelter (Burgess, 1984). Numerous pathogenic microbes, including bacteria, fungi, helminths, and protozoa, have been linked to the spread of cockroaches (Ifeanyi & Odunayo, 2015). These species' presence in human environments raises questions about safety, especially as they have the potential to spread food-spoilage organisms and diseases. Cockroaches can easily go from unclean regions to places where food is prepared. There, they can disperse bacteria that can lead to illnesses and food deterioration (Ukoroiye et al., 2019). Cockroaches can readily contaminate food by releasing harmful organisms like fungi and bacteria that can cause food poisoning (Pai et al., 2005).

Cockroaches harbour a variety of parasitic organisms, including protozoans and helminthes. *Ascaris lumbricoides*, *Trichuris trichura*, *Taenia* spp., and *Strongyloides stercoralis* are among the helminthes (Chamavit et al., 2011; El-Sherbini & El-Sherbini, 2011). The recognised protozoans are *Entamoeba histolytica*, *Entamoeba coli*, *Balantidium coli*, and *Isospora belli* (Chamavit et al., 2011; El-Sherbini & El-Sherbini, 2011). *Candida*, *Aspergillus niger*, *Mucor*, *Rhizopus* spp., *Aspergillus fumigans*, and *Penicillium* spp. are among the medically significant fungifound in cockroaches (Salehzadeh et al., 2007). *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Citrobacter freundii*, *Enterobacter aerogenes*, and *Proteus mirabilis* are among the possible pathogens associated with cockroaches. According to a study in India, there was no discernible difference in the overall bacterial load on the outer surface of cockroaches found in homes (39.9%) and food-handling businesses (60.1%) (Wannigama et al., 2013).

German cockroaches (*Blattella germanica*) have been isolated from various environments including hospitals, food industries and landfill sites (Molewa et al., 2022). In a study conducted by Donkor (2019), it was discovered that cockroaches from hospitals and residences were carriers of microorganisms and involved in the etiology of nosocomial infections. Other notable harmful bacteria have also been isolated from cockroaches, and these include; *Shigella*, *Campylobacter*, *Salmonella*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Sh Jabber et al., 2015). Microorganisms are present in the faecal pellets and on the surfaces of cockroaches. They carry bacteria that cause food to deteriorate and spread food-borne illnesses with them wherever they feed or crawl around the house. When they are in households, the best precautions for food safety and quality are jeopardized (Ifeanyi & Odunayo, 2015).

According to a study by Narayanan & Selvam (2015), cockroaches, specifically *Blattella germanica*, were found to be potential reservoirs and vectors of food-borne pathogens, posing a health risk to humans. The study demonstrated that cockroaches could contribute to the dissemination and spread of food-borne pathogens and multiple drug resistance in human environments for an extended period of time. The results suggested that cockroaches, especially *B. germanica*, may

play a significant role in the transmission of drug-resistant pathogens in hospitals and food catering establishments.

2.4 Drug Resistance in Cockroach-Associated Bacteria

The decreasing number of innovative antimicrobial drugs in the pipeline has made the global problem of bacterial antibiotic resistance worse. As the number of resistant bacteria increases, the effectiveness of currently available antibiotics is declining. The therapeutic options available to antibiotic-resistant organisms are also severely restricted since these organisms typically display a multi-drug resistant (MDR) phenotype. Antimicrobial-resistant bacteria can spread via vectors such as humans, animals, and insects. Antimicrobial drug-resistant bacteria from animal dung and other decomposing organic substrates may be transported to residential areas by pests that thrive on decomposing organic material (Solomon et al., 2018).

Studies by Elgderi et al., (2006), Fotedar et al., (1991), Prado et al., (2006) showed that bacteria linked to foodborne illnesses that were found in and on cockroaches in Ethiopia, India, Brazil, and Libya were resistant to antimicrobial drugs that had been put to the test. The results of these investigations showed that between 30% and 100% of *S. aureus* isolates and between 14% and 100% of Enterobacteriaceae isolates were resistant to ampicillin, streptomycin, tetracycline, erythromycin, or trimethoprim-sulfamethoxazole. Moreover, it has been shown that cockroaches, which are common in homes, cafeterias, and hospitals, serve as hosts to bacteria that are resistant to drugs. Certain investigations have connected methicillin-resistant strains of *S. aureus* and extended-spectrum lactamase-producing organisms to epidemics that are associated to cockroaches (Abdolmaleki et al., 2019; Ikechukwu et al., 2017; Obeng-Nkrumah et al., 2019).

2.5 Common bacteria associated with food poisoning

Among the bacteria that can be found in food are: *Salmonella*, *Shigella*, *Clostridium perfringens*, *Bacillus cereus*, *Campylobacter*, and *Staphylococcus aureus*; pathogenic *Escherichia coli*; and a variety of *Vibrios*, particularly *Vibrio cholerae* and *Vibrio parahaemolyticus* (Bhunja, 2018). Unfortunately, some of these harmful bacteria have been isolated from cockroaches thus far, including *Shigella*, *Campylobacter*, *Pseudomonas aeruginosa*, *Salmonella*, and *K. pneumoniae*. It has been established that several bacteria that are commonly linked to these insects can induce

food poisoning, diarrhoea, and dysentery in humans. Cockroaches' stomachs may hold onto toxic strains of *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae* for a few days (Kassiri et al., 2014).

2.5.1 *Salmonella* species

As a member of the Enterobacteriaceae family, *Salmonella* is a gram-negative bacillus that is typically facultatively anaerobic, non-sporulating, non-capsulate, motile, oxidase negative, aerogenic, non-lactose fermenting, urease-negative and citrate-utilizing. With approximately 2800 serovars now identified, *Salmonella* is the most common type of bacteria in the environment. *Salmonella enteritidis*, *S. montevideo*, *S. typhimurium*, and *S. enterica* subspecies *enterica* are the most commonly linked to foodborne disease outbreaks.

In countries where enteric notifiable illnesses and foodborne disease outbreak surveillance records are maintained, salmonellosis typically ranks first or second in terms of outbreaks and case counts. An estimated 644 786-1,679,667 foodborne illnesses and 378 fatalities occur in the US annually (Bhunia, 2018). *Salmonella* is the most common cause of foodborne illness-related deaths in the US. Although the symptoms of salmonellosis are typically mild and the gastroenteritis lasts for several days, there may be consequences of a previous disease (Bhunia, 2018).

Salmonella typhi is the primary cause of salmonella infections in Lusaka, Zambia, according to a study by Yamba et al., (2022). Additionally, the study also revealed a high percentage of multi-drug resistance (MDR), *S. typhi*, evolution and genetic diversity, and the formation of resistance to cephalosporins and ciprofloxacin (Yamba et al., 2022).

Extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* have been increasingly reported as a serious concern due to their role in antibiotic resistance. Cockroaches, often found in close proximity to human dwellings and healthcare facilities, have been identified as potential carriers of ESBL-producing *Salmonella*. These insects can harbor and disseminate antibiotic-resistant bacteria, thereby contributing to the spread of resistance genes within environments where they thrive (Ikechukwu et al., 2017).

These findings underscore the importance of controlling cockroach populations in critical environments such as hospitals, restaurants, and homes to mitigate the dissemination of ESBL-producing *Salmonella*.

2.5.2 *Escherichia coli*

Escherichia coli is a gram-negative, rod-shaped, straight, non-sporing, non-acid fast bacilli that can be found alone or in pairs. Typically, cells have a rod-like shape and measure 1-3 $\mu\text{m} \times 0.4-0.7 \mu\text{m}$ (micrometre), which is equivalent to 1 μm in length, 0.35 μm in width, and 0.6-0.7 μm in volume. Because of its peritrichous flagellar configuration, it is motile; very few strains are not. While 37°C is the ideal temperature for *E. coli* growth, many lab strains can thrive at as high as 49°C). Under ideal circumstances, reproduction can occur in as little as 20 minutes. There are both motile and non-motile fimbriated strains. Certain strains of *E. coli* that were isolated from extraintestinal illnesses have been found to contain a polysaccharide capsule. By employing negative staining techniques, which create a brilliant halo against a dark background, the *E. coli* capsules are easily visible. They only have one or two peptidoglycan layers in their thin cell wall (Köhler & Dobrindt, 2011).

According to a study by Bumbangi et al., (2022), in Ndola and Lusaka districts of Zambia on the community wide spread of MDR *E. coli* in young children, they reported that MDR is highly prevalent in both healthy and diarrheal children, and that there are multiple unique patterns involving high-priority and critically important antibiotics that are regarded as essential for treating serious bacterial infections in people. The study also revealed potential PDR- *E. coli* carriage in children in good health, emphasizing the potential role this group may play in harbouring and passing on resistance genes to other infections.

Recent studies in Zambia have pointed out an increase in the number of drug-resistant *E.coli* according to a study by Yamba et al., (2022) there findings from a study conducted in Lusaka, Zambia using a one health approach indicated that of the 215 *E. coli* isolates, 66.5% were found to be multidrug-resistant (MDR), and 42.8% were producers of extended-spectrum beta-lactamase (ESBL). The majority of the isolates exhibited resistance to ampicillin (81.4%), sulfamethoxazole/trimethoprim (70.7%), ciprofloxacin (67.9%), levofloxacin (64.6%),

ceftriaxone (62.3%), and cefuroxime (62%). The study concluded that there was a high resistance of *E. coli* to commonly used antibiotics in humans, and the presence of MDR and ESBL-producing *E. coli* poses a significant public health concern, necessitating urgent action.

The *bla*_{CTX-M} and *bla*_{TEM} genes, which confer resistance to beta-lactam antibiotics, have also been reported in *Escherichia coli* isolated from cockroaches (Ikechukwu et al., 2017; Obeng-Nkrumah et al., 2019). These findings suggest that cockroaches may serve as reservoirs or vectors for the spread of these antibiotic resistance genes, potentially contributing to the dissemination of multidrug-resistant *E. coli* in various environments, including healthcare settings.

2.5.3 *Staphylococcus aureus*

Staphylococcus aureus is a facultative anaerobic Gram-positive coccus. It is coagulase and catalase positive, non-motile. The cells are round and can form clusters like grapes or be single or paired cocci. *Staphylococcus* species' pentaglycine bridges are specially broken by lysostaphin, which selectively cleaves the staphylococcal cell wall while being resistant to lysosomes (Le Loir et al., 2003). Staphylococcal food poisoning is caused by some strains of *S. aureus* that have the ability to create staphylococcal enterotoxins (SEs).

Staphylococcus aureus is a well-known pathogen due to its resistance to treatment, invasive nature, and toxin-mediated pathogenicity. Common symptoms of staphylococcal poisoning include diarrhoea, vomiting, nausea, and abdominal pain. The symptoms emerge fast (between 30 minutes and 8 hours), and after 24 hours, a spontaneous decline is typically noticed (Le Loir et al., 2003). Warm-blooded mammals are thought to be the primary *Staphylococcus* species reservoir (Le Loir et al., 2003), they have been reported to be recovered from the gut and body surface of cockroaches collected from hospitals and residential houses in different parts of the world in an attempt to establish the vector role of the insect in the transmission of the pathogen (Abdolmaleki et al., 2019; Islam et al., 2016). *Staphylococcus aureus* has been linked to several food-borne disease outbreaks. In a study on foodborne disease outbreak investigation experience in a College in Lusaka, Zambia, in 2017 by Kapaya et al., (2018), *Staphylococcus aureus* was one of the isolates implicated in the recorded outbreak.

S. aureus strains are usually resistant to a variety of drugs. MRSA has become a major issue in hospitals and homes. Documented data demonstrated that approximately 50-70% of the *S. aureus* strains isolated from hospitals and homes were MRSA (Isaac et al., 2014; Safarpour et al., 2017). According to study in Bangladesh by Islam et al., (2016), *S. aureus* strains isolated from cockroach samples had the highest percentage of resistance to the antibiotics erythromycin (58%), kanamycin (23%), penicillin (71%), oxacillin (45.50%), cephalothin (11%), and clindamycin (38%).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Research Design

This study utilized a cross-sectional design and was conducted from November 2023 to January, 2024.

3.2 Study Area

The study was carried out at the University of Zambia student hostels and Kalingalinga compound of Lusaka district, Zambia. Figure 3.1 shows the two areas marked 1 and 2 indicating the University of Zambia and Kalingalinga, the two study sites respectively.



Figure 3.1: Map showing UNZA and Kalingalinga (Imagery © 2024 google)

3.3 Sample size calculation

There were no published data on the percentage of cockroaches in houses in Zambia. No pilot study was done prior to this project, and therefore the sample size was calculated using the modified Cochran (1977) formula for smaller population as shown below.

$$n = (z^2 pq)/e^2$$

$$n = (1.96)^2 (0.50) (1-0.50)/ (0.05)^2$$

$$\Rightarrow n = (1.96 \times 1.96) (0.50) (0.50)/ (0.05 \times 0.05)$$

$$n = 384.16 \approx 384$$

Where n=sample size

Z=considering a confidence level of 95%, the z-value is read from the normal distribution table

$$Z=1.96$$

p=estimate proportion of an attribute present in a given population (for maximum sample size p is usually 50%= 0.5

$$q=1-p$$

e=error of margin=5% or 0.05

The modified Cochran formula

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Here n_0 is Cochran's sample size recommendation,

N is the population size, and

n is the new, adjusted sample size.

Assuming there are just 1000 cockroaches in the target population, the sample size is

$$385 / (1 + (384 / 1000)) = 278$$

The sample size was reduced from 385 to 278 in the research because the study's objectives were narrowly focused, allowing for sufficient data collection with a slightly smaller sample size while still meeting the research goals. Additionally, the population of cockroaches was found to be relatively homogenous in terms species and the pathogens they carried, meaning that reducing the sample size did not significantly impact the variability or reliability of the results. Furthermore,

there was a noticeable unwillingness or difficulty in obtaining samples from certain areas, which necessitated the adjustment. As a result, the sample size was reduced to ensure that the study remained both scientifically robust and practically manageable.

3.4. Cockroach collection

A purposive sampling method was applied for selecting study houses/rooms in each targeted area. In each area, only adult cockroaches were collected as samples. Another house/room was selected if the household head or owner was not willing to participate in the study. For houses in Kalingalinga, two food baited pitfall traps made of plastic containers and smeared on the bottom with peanut butter and crumbs of bread were set in the kitchen area and near the toilet overnight. While for UNZA, one food baited pitfall trap was set in the room and another near the garbage areas of the hostel on the same floor overnight. The traps were set approximately for 16-18 hours once in each target house/room and if no cockroach was caught then another house or room was picked. The cockroaches were also picked with gloved hands, placed in sterile and labeled universal containers according to the procedure by Paul et al., (1992). The collected cockroaches were then transported to the Microbiology Laboratory Unit under Paraclinical Studies department, School of Veterinary Medicine. To prevent microbial load degradation, the captured cockroaches were kept alive during transfer to the laboratory.

3.5 Cockroach Identification

Identification of cockroaches to species level was done based on keys provided by Burgess (1993) and an online file (Beccaloni, 2014), see appendix E.

3.6 Processing of cockroaches

The collected cockroaches were killed in a sterile jar using chloroform-soaked cotton wool. A single cockroach was placed in a sterile test tube using a pair of sterilized forceps. The external body surfaces were washed by vortexing in 5ml sterile physiological saline for two minutes, and the wash was taken as an external body wash sample. After external body washing, the cockroaches were soaked in 90% ethanol for five minutes to decontaminate their external surfaces and were dried for two minutes. They were then rewashed with sterile saline to remove traces of ethanol, and the alimentary tracts were aseptically dissected out using sterilized entomological

dissecting needles. The instruments were dipped in ethanol and flamed between dissections (Betre, 2004; Naher et al., 2018).

The excised guts were then homogenized in 5 ml of sterile normal saline according to the procedure by Fotedar et al., (1991). A total of 556 samples consisting of 278 external body surface wash and 278 gut homogenates were analyzed.

3.7 Isolation and Identification of Bacteria

3.7.1 Quality control Process

The study's methodology included producing culture media using aseptic procedures and validating sterility by incubating aliquots at the right temperature for 18-24 hours. American Type Culture Collection (ATCC) 23922 (*Escherichia coli*) and ATCC 23925 (*Staphylococcus aureus*) strains were used as quality control organisms and subcultured to guarantee their viability and purity, and the prepared media was inoculated with a standardised inoculum size of 0.5 MacFarland standard. The media (Blood agar, MacConkey and XLD) were incubated at 37°C for 18-24 hours, and growth features such as colony shape and haemolysis patterns were observed.

3.7.2 Culturing of specimens

In order to isolate the bacteria, 1 ml of the gut and external homogenate samples were suspended independently into 9 ml of sterile buffered peptone water and the mixture was incubated aerobically at 37°C for 18 to 24 hours (Fotedar et al., 1991). After incubating the inoculated buffered peptone water, a loopful was individually inoculated on MacConkey agar (Himedia, Mumbai, India) and Xylose Lysine Deoxycholate agar (Oxoid, UK) for the isolation and detection of *Salmonella* and *E. coli*, which were then incubated at 37°C for 18 to 24 hours. Blood agar (Himedia, Mumbai, India) was also utilized to identify *Staphylococcus aureus* and incubated under the same conditions.

3.8.3 Identification of Bacteria

The isolated colonies were then purified by subculturing a colony on a purity plate of Mueller Hinton agar (Himedia, Mumbai, India) and then the growth from the purity plate was identified by using standard microbiological techniques such as colony morphology, Gram staining, and a

battery of biochemical tests that included catalase, coagulase, oxidase, citrate utilization, indole production, urease, motility, the reaction in triple iron sugar agar, gas, H₂S production and polyvalent antisera as described by Cheesbrough, (2000).

3.8 Antibacterial Sensitivity Testing:

For antimicrobial susceptibility testing, antibiotic discs were prepared, and the test media were inoculated with the control strains. The antibiotic discs were placed on the inoculated media with adequate spacing, and the plates were incubated for 18-24 hours. Following incubation, the zones of inhibition around the antibiotic discs were measured and compared to expected quality control ranges provided by CLSI (2024).

In-vitro antibiotic susceptibility of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* was determined by the standard Kirby-Bauer disc diffusion technique using Oxoid discs; cefotaxime (30 µg), penicillin G (10 µg), sulphamethoxazole-trimethoprim (25 µg), chloramphenicol (30 µg), gentamicin (10 µg) and ciprofloxacin (5 µg), azithromycin (30 µg), tetracycline (30 µg). Few colonies of each test isolate were suspended in sterile normal saline to match 0.5 MacFarland turbidity standards. Precisely 0.1 ml of the suspension was then evenly spread on Mueller Hinton agar (HiMedia, Mumbai, India). The inoculated plates were allowed to air-dry prior to the placing of the discs (Vineetha et al., 2015). The diameters of the inhibition zones were measured in millimeters (mm) using a ruler after 24 hours' incubation at 37°C. The interpretation of the inhibition zones as susceptible or resistant was according to standard guidelines (CLSI, 2024) and an online platform (WHONET, 2023 version).

3.9 Screening and Detection of ESBL-Positive Isolates

Isolates resistant to cefotaxime from the disc diffusion method were considered potential ESBL producers and were further screened for the production of the ESBL phenotypically on MAC/CTX using the standard method of preparation. A discrete colony of possible ESBL-producing bacteria was picked from a pure culture plate and streaked on MacConkey agar plate supplemented with 1mg/L cefotaxime. The plate was incubated aerobically at 37°C for 18-24 hours. After the incubation, the colonies from the plates that had growth were then further analyzed using PCR.

3.10 PCR Analysis of ESBL Genes

The DNA of the ESBL-producing isolates was extracted using ZR DNA Miniprep Kit (Zymo Research Corporation, USA) according to manufacturer's instructions.

The DNA extraction protocol involved several steps to isolate DNA from cell colonies. First, the sample was added to ZR Bashing Bead™ Lysis Tubes along with Zymo BIOMICS™ Lysis Solution. After securing the tubes in a bead beater and processing, centrifugation was performed to separate the supernatant. This supernatant was then transferred to a Zymo-Spin™ III-F Filter for further purification. Following this, the DNA was bound to a Zymo-Spin™ IICR Column and washed with specific buffers. Subsequent elution of the DNA was achieved by adding Zymo BIOMICS™ DNase/RNase Free Water to the column. The eluted DNA was then subjected to additional filtration using Zymo-Spin™ III-HRC Filters, resulting in DNA suitable for downstream applications such as PCR.

DNA quantification was performed using a NanoDrop spectrophotometer following standard protocols. The instrument was first powered on and set to the Nucleic Acid mode. A blank measurement was obtained by loading 2 µL of nuclease-free water onto the lower pedestal, lowering the arm, and selecting the “Blank” option to establish a baseline reading. Following this, 2 µL of the DNA sample was pipetted onto the pedestal, and the absorbance was measured at 260 nm to determine the DNA concentration. The instrument also provided absorbance ratios (A260/A280 and A260/A230) to assess DNA purity, with A260/A280 values around 1.8 indicating pure DNA. Between each measurement, the pedestal was thoroughly cleaned with a lint-free tissue to prevent cross-contamination. The process was repeated for each sample, with the blank being periodically recalibrated. Once measurements were complete, the pedestal was cleaned, and the instrument was powered down as per manufacturer instructions.

The presence of *bla*CTX-M, and *bla*TEM genes was investigated by PCR using the primer pairs shown in Table 3.1. PCR amplification was performed in volume of 12.5 µl which consisted of 1.0 µL 100 ng DNA, 6.25 µl of *Thermococcus kodakarensi* (KOD) one MM, 1.5 µl of each primer pair and 2.25 µl nuclease free water, all in a 0.2 ml PCR tube. The PCR cycling profile used for

the reaction consists of an initial step at 96 °C for 5 minutes, 35 cycles of 94°C for 30 seconds, 58°C (*bla*TEM and *bla*CTX-M) for 1 minute and 62°C for 1 minute and a 10minute final extension at 72°C. The PCR products were subjected to electrophoresis. The PCR products (5 µl) and 1 µl of the gene ladder 100bp were placed on the prepared 2% agarose gel and stained with ethidium bromide and the resulting bands were visualized under the gel documentation system, comprising a charged coupled device camera and a transilluminator, facilitated the capture of images by illuminating the stained molecules with ultra violet after running it for 45 minutes at 100V.

Table 3.1: Primers used for the detection of *bla*CTX-M and *bla*TEM genes in *Escherichia coli* and *Salmonella*.

Primer	Sequences (5' → 3')	Expected size	reference
TEM-F	CCCCGAAGAAGCTTTTC	480bp	(Cai et al., 2016)
TEM-R	AGAAGTGGCCTGCAACTTT		
CTX-MA1	CGATGTGCAGCACCAGTAA	544bp	(Pokhrel et al., 2014)
CTX-MA2	CCGCAATATGATTGGTGGTG		

3.11 Data Analysis

Data on the cockroach types, percentage distribution, and bacteria species isolated was entered into Excel. All results were analyzed at 95% confidence interval and the p-values was considered significant if it was <0.05.

The variables that were used in the Chi-Square analysis was location as the independent variable and cockroach species as a dependent variable. For bacteria percentage distribution, the location of bacteria on the cockroach was used as independent variable and number and species of bacteria as dependent variables.

CHAPTER FOUR

RESULTS

4.1 Identification of cockroaches

In this study, 278 cockroaches were collected from Fifty (50) rooms and forty (40) houses sampled from the University of Zambia and Kalingalinga respectively from November 2023 to January, 2024. Cockroaches were identified as *Periplaneta americana* and *Blattella germanica* using identification keys of Burgess (1993) and an online file (Beccaloni, 2014), refer to appendix E. Figure 4.1 shows the morphological differences of the two species collected from the two study sites.



Blattella germanica

Periplaneta americana

Figure 4.1: Cockroach species collected from the two study sites. Photo taken by Lawrence Kapansa

The two collected cockroach species were distributed across the two study sites as shown in Table 4.1. The distribution of these species was not statistically significant for both within and between study sites.

Table 4. 1: Numbers of cockroach species collected from the two selected study sites.

Cockroach species	Location		Total
	UNZA	Kalingalinga	
<i>Periplaneta americana</i>	40	66	106
<i>Blattella germanica</i>	80	92	172
Total	120	158	278
p-value	0.557077	0.641245	0.15133

From the two identified cockroaches, *B. germanica* was more abundant in the two study sites. The overall percentage distribution of the two cockroach species was 38.1% and 61.9% for *P. americana* and *B. germanica* respectively, as shown in Figure 4.2.

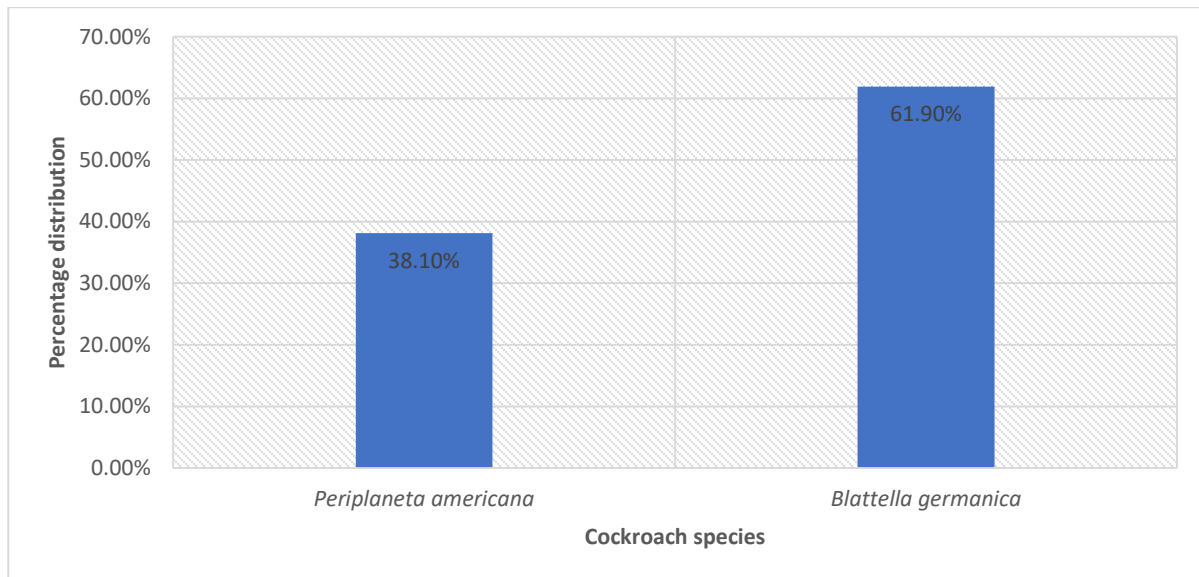


Figure 4.2: Overall percentage of cockroach species from the study sites

4.2. *Escherichia coli*, *Salmonella* species and *Staphylococcus aureus* isolation and identification

Eighteen lactose fermenting isolates from MacConkey agar and XLD were identified as *E. coli* using biochemical tests. All samples were characterized as Gram-negative rods. Biochemical reactions showed that the samples were positive for lactose fermentation, negative for urea hydrolysis, and negative for citrate utilization. In the Sulphur Indole Motility (SIM) test, they were negative for hydrogen sulfide production but positive for indole production and motility. The Triple Sugar Iron (TSI) test results indicated an acidic slant and butt with positive gas production. Finally, all samples tested negative for oxidase.

4.2.1 *Salmonella* isolation and identification

Ten non lactose fermenting isolates from MacConkey agar and hydrogen sulphide producers on XLD were identified as *Salmonella* species using biochemical and serological tests. The *Salmonella* species isolated were characterized as Gram-negative rods. Biochemical reactions showed that they were negative for lactose fermentation, urease activity, and citrate utilization. The SIM test results indicated positive motility but negative for both hydrogen sulfide production and indole production. In the triple sugar iron agar test, the slant was alkaline while the butt was acidic, with no gas production observed. Additionally, the oxidase test results were negative. These biochemical characteristics aligned with the typical profile for *Salmonella* species.

4.2.2 *Staphylococcus aureus* isolation and identification

The biochemical characteristics of 11 *Staphylococcus aureus* isolated from collected cockroaches revealed consistent results across multiple specimens. The identified specimens all exhibited the morphological trait of being Gram-positive cocci arranged in clusters. Biochemically, these isolates uniformly tested positive for both catalase and coagulase, indicating the presence of enzymes that decompose hydrogen peroxide and cause blood plasma clotting, respectively. Additionally, all specimens were negative for the oxidase test, signifying the absence of cytochrome c oxidase. On blood agar, these *S. aureus* isolates consistently display beta hemolysis, characterized by a clear zone of hemolysis around the colonies, indicative of complete lysis of red blood cells. These results confirmed the typical biochemical profile of *Staphylococcus aureus*.

A total of 39 foodborne associated bacteria were isolated from the 556 external and gut homogenates as shown in Table 4.2. From UNZA, a total of 17 bacteria were isolated comprising 11 from *P. americana* and 6 from *B. germanica* while from Kalingalinga, a total of 22 bacteria comprising 15 isolated from *P. americana* and 7 from *B. germanica*.

Table 4. 2: Number of bacteria isolated from *Periplaneta americana* and *Blattella germanica* from both external and gut surfaces.

Isolates	UNZA		Kalingalinga		Total
	PA	BG	PA	BG	
<i>Escherichia coli</i>	4	2	8	4	18
<i>Salmonella</i> species	3	2	4	1	10
<i>Staphylococcus aureus</i>	4	2	3	2	11
Total	11	6	15	7	39

KEY: PA= *Periplaneta americana*, BG= *Blattella germanica*

4.3 Distribution of isolated bacteria from the two collected collect species

A total of 18 *E. coli* of which 4 were from the external body surface and 14 from the gut homogenate as shown in figure 4.3. From the total of 10 *Salmonella* isolates, 9 were isolated from gut and 1 was from external body surface of the cockroaches. 11 *S. aureus* were isolated and of the 11 isolates, 3 were from external surface and 8 were from gut of the cockroaches. The distribution of the bacteria on the body surfaces of the cockroach was not statistically significant and had a p value was 0.668

The overall isolation rate was 21% and 79% for the external and gut homogenate respectively and that of the isolated selected bacteria was 26% for *Salmonella*, 28% for *S. aureus* and 46% for *E. coli* where n is 39. As shown in figure 4.3, the majority of the pathogens were isolated from the gut of the cockroaches.

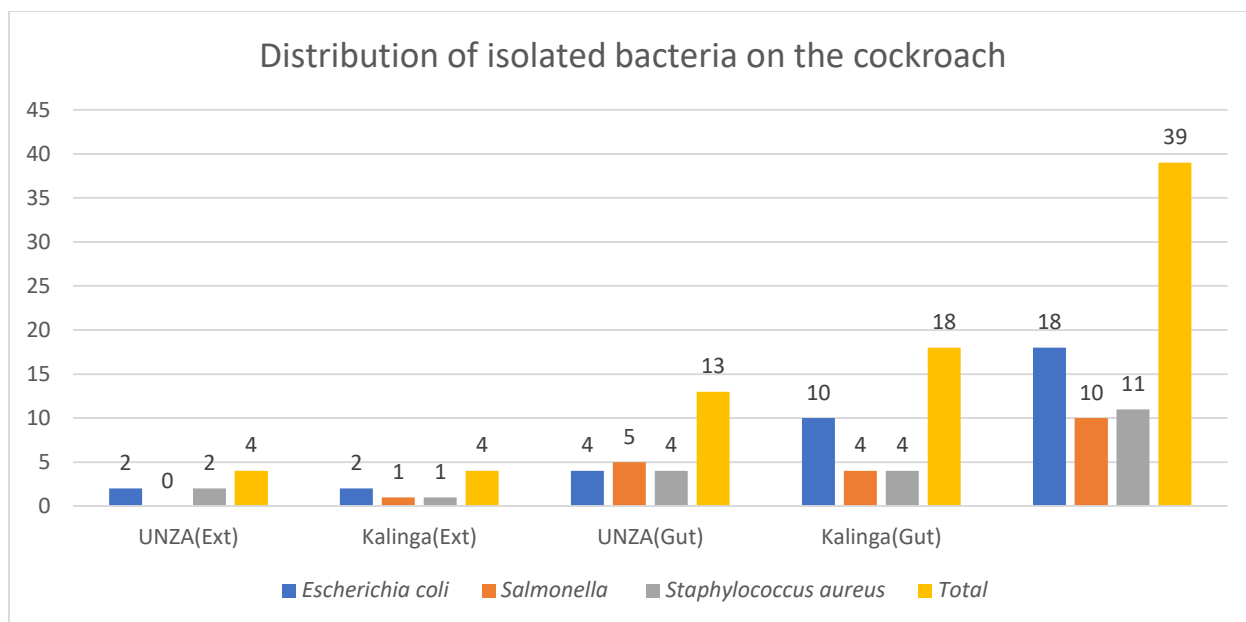


Figure 4.3: Showing number of bacteria isolated from cockroach external and gut surfaces

4.4 In Vitro Drug Susceptibility Test

4.4.1 Phenotypic drug susceptibility testing of isolated bacteria against selected antibiotics

A total of 11 *S. aureus*, 10 *Salmonella* and 18 *E. coli* isolates were tested against different Gram-negative and Gram-positive battery of antibiotics as shown in appendix D. It was observed that 66.7% (n=39) of all the isolated bacteria were resistant to at least 1 class of antibiotics and 35.9% (n=39) isolates were multi-drug resistant.

4.4.1.1 *Staphylococcus aureus* antibiogram

Staphylococcus aureus was subjected to a battery of 8 antibiotics as shown in figure 4.4. Four isolates were resistant to Penicillin G, one was resistance to Gentamicin and chloramphenicol, two to tetracycline and Ciprofloxacin, three to Cefotaxime and no isolate was resistant to Azithromycin and sulfamethoxazole-trimethoprim. It was observed that the *S. aureus* isolated were highly resistant to Penicillin.

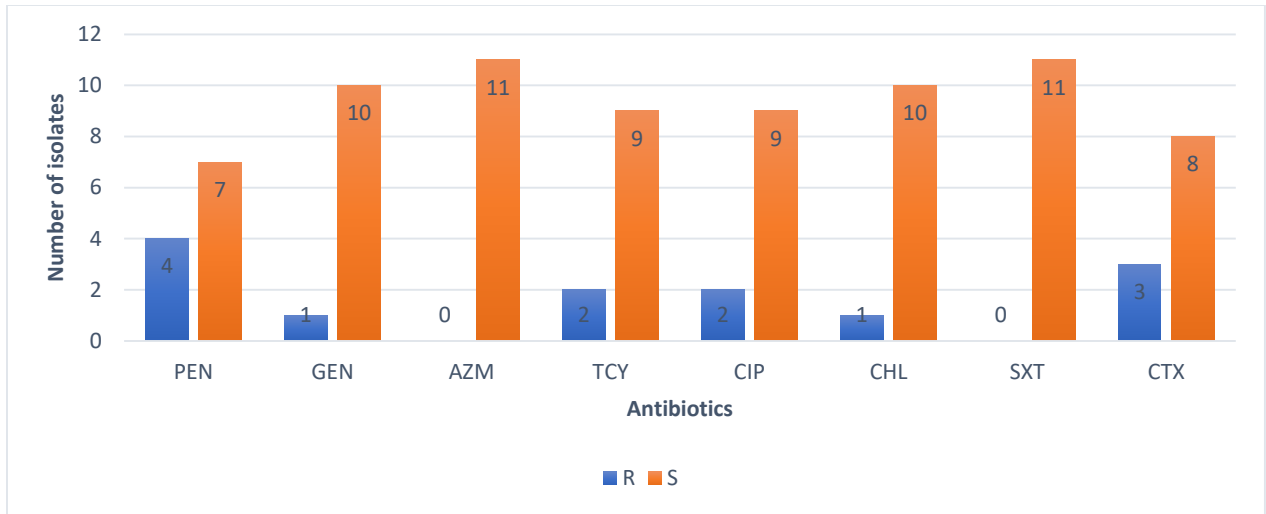


Figure 4.4: *Staphylococcus aureus* antibiogram against selected antibiotics.

Key: R=Resistant, S=Susceptible, PEN=Penicillin, GEN=Gentamicin, AZM=Azithroymicin, TCY=Tetracycline, CIP=Ciprofloxacin, CHL=Chloramphenicol, SXT=Sulphamethaxole-Trimethoprim, CTX=Cefotaxime

4.4.1.2 *Salmonella* antibiogram

Two isolates of *Salmonella* exhibited resistance to ampicillin, one to gentamicin, and six to ciprofloxacin and cefotaxime, while none showed resistance to tetracycline, chloramphenicol, or sulfamethoxazole-trimethoprim, as shown in Figure 4.5. It was observed that *Salmonella* was highly resistant to ciprofloxacin and cefotaxime a third generation cephalosporin.

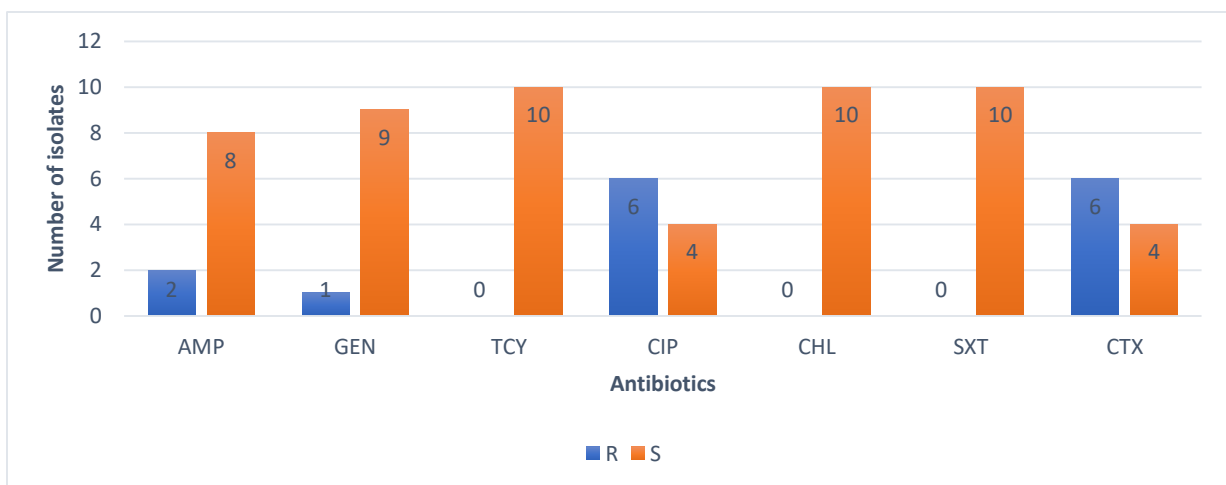


Figure 4.5: *Salmonella* species antibiogram against selected antibiotics

Key: R=Resistant, S=Susceptible, PEN=Penicillin, GEN=Gentamicin, AZM=Azithroymicin, TCY=Tetracycline, CIP=Ciprofloxacin, CHL=Chloramphenicol, SXT=Sulphamethaxole-Trimethoprim, CTX=Cefotaxime

4.4.1.3 *Escherichia coli* antibiogram

Five isolates of *E. coli* as shown in figure 4.6 were resistant to ampicillin and gentamicin, two to tetracycline and sulfamethoxazole-trimethoprim, four to ciprofloxacin, one to chloramphenicol and eight to cefotaxime. It was observed that *E. coli* was highly resistant to cefotaxime a third generation cephalosporin, followed by ampicillin, gentamicin and ciprofloxacin.

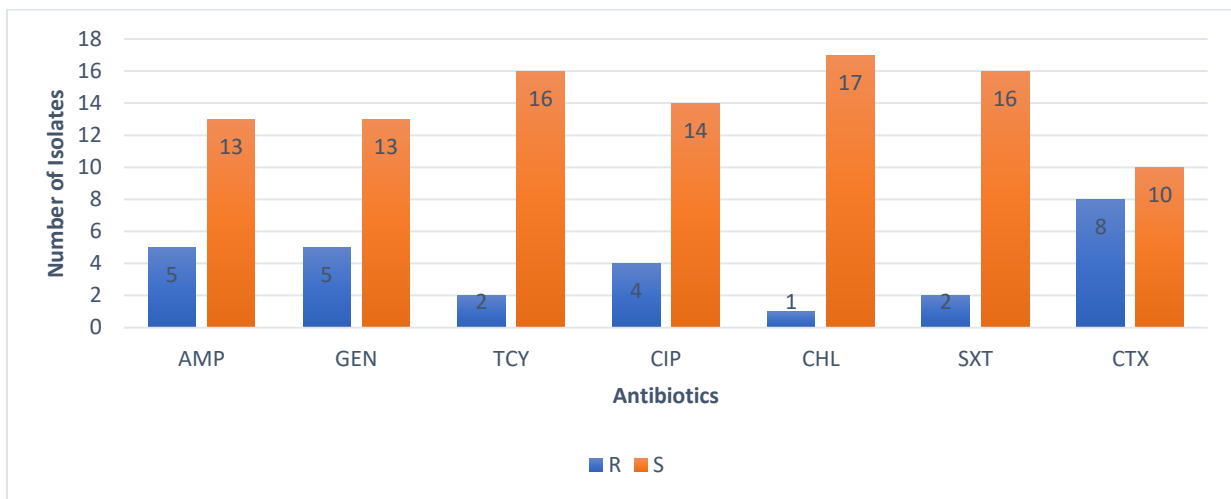


Figure 4.6: *Escherichia coli* antibiogram against selected antibiotics

Key: R=Resistant, S=Susceptible, PEN=Penicillin, GEN=Gentamicin, AZM=Azithroymicin, TCY=Tetracycline, CIP=Ciprofloxacin, CHL=Chloramphenicol, SXT=Sulphamethaxole-Trimethoprim, CTX=Cefotaxime

4.5 Multi-drug resistance associated with the isolated foodborne bacteria

The proportion of multidrug resistance associated with the isolated bacteria as shown in figure 4.7 shows that *E. coli* had the highest proportion of isolates which had a number of isolates being multidrug resistance to a number of selected antibiotics with the highest being resistant to at least three classes of antibiotics and the least was observed in *S. aureus* being resistant to only two classes of antibiotics.

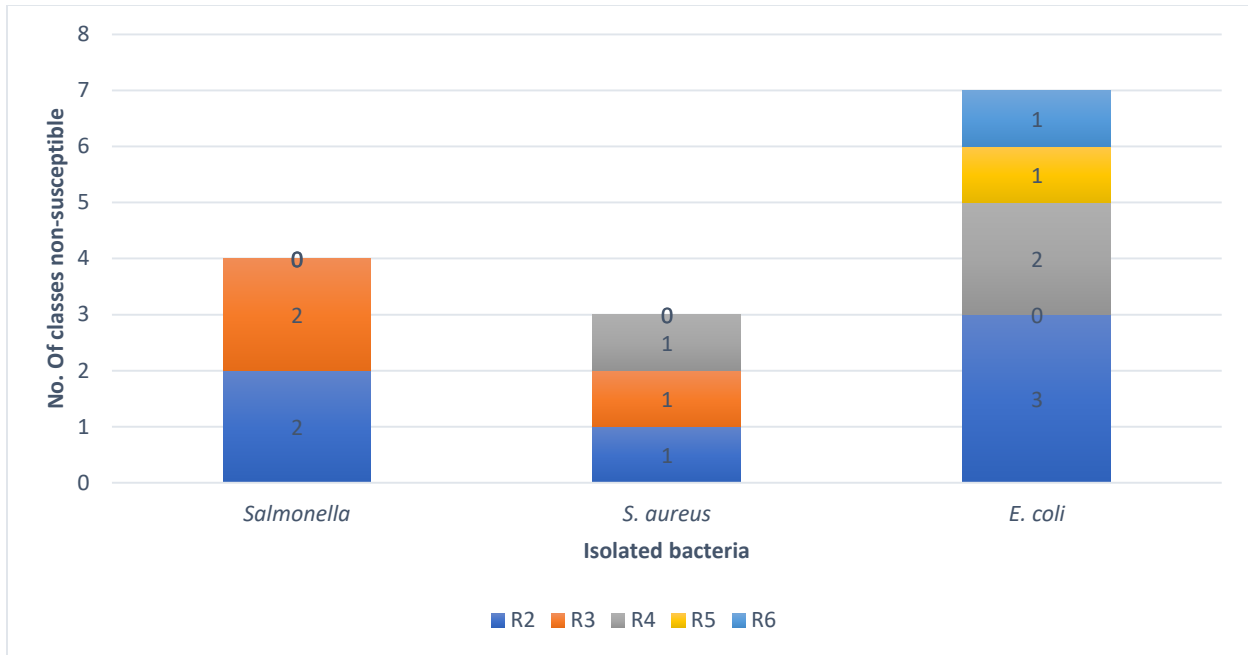


Figure 4.7: Proportion of MDR from isolated bacteria

Key: R2=Resistant to 2 antibiotics, R3=Resistant to 3 antibiotics, R4=Resistant to 4 antibiotics, R5=Resistant to 5 antibiotics and R6=Resistant to 6 drugs.

The overall proportion of MDR was at 39% (n=18) in *E. coli*, 40% (n=10) in *Salmonella*, and 27%(n=11) in *S. aureus* as shown in Figure 4.8. The difference in the overall proportion of MDR can be attributed to the difference in number of isolates.

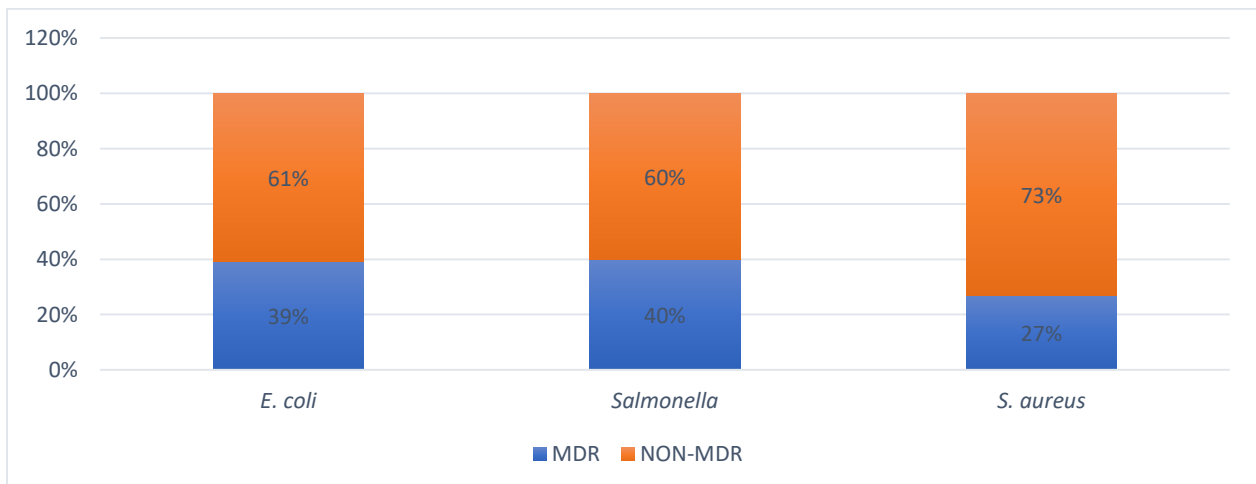


Figure 4. 8: Proportion of MDR isolates from bacteria isolated from the two cockroaches.

4.4.2 In-vitro antibiotic susceptibility of ESBL-producing *Escherichia coli* and *Salmonella*.

After entering the antibiogram results into WHONET 2023 Version and data was analyzed, there were 8 *E. coli* and 3 *Salmonella* that were detected as possible ESBL-producing Enterobacteriaceae phenotypically. And after screening the isolates on Cefotaxime/MacConkey agar, there were 3 *E. coli* and 2 *Salmonella* that were obtained as possible ESBL-producing bacteria as shown in Table 4.3.

Table 4. 3: Frequency of ESBL-producing bacteria

Isolate	Number positive for ESBL (%) phenotypically	Number of positive for ESBL (%) after screening	Number Positive for CTX-M (%)	Number Positive for TEM (%)
<i>E. coli</i>	8/18 (44.4)	3/8 (37.5)	2/3 (66.7)	3/3 (100)
<i>Salmonella</i>	3/10 (30)	2/3 (66.7)	0	2/2 (100)

4.4.3 Detection of bla^{CTX-M} and bla^{TEM}

Agarose gel electrophoresis of the PCR products for bla^{CTX-M} , and bla^{TEM} are shown in Figures 4.9 and 4.10 respectively, *Bla* Frequency of occurrence of the genes among the 5 ESBL-producers were; 2 (40.0 %) for CTX-M, and 5 (100.0 %) for TEM. Co-existence patterns observed were; $bla^{CTX-M} + bla^{TEM}$ in 2 of the *Escherichia coli*. In addition, bla^{CTX-M} alone was detected in 2 of the *Escherichia coli* and was not observed in the 2 *Salmonella* isolates. The bla^{TEM} gene was detected in all the isolates as shown on the gel in Figure 4.9

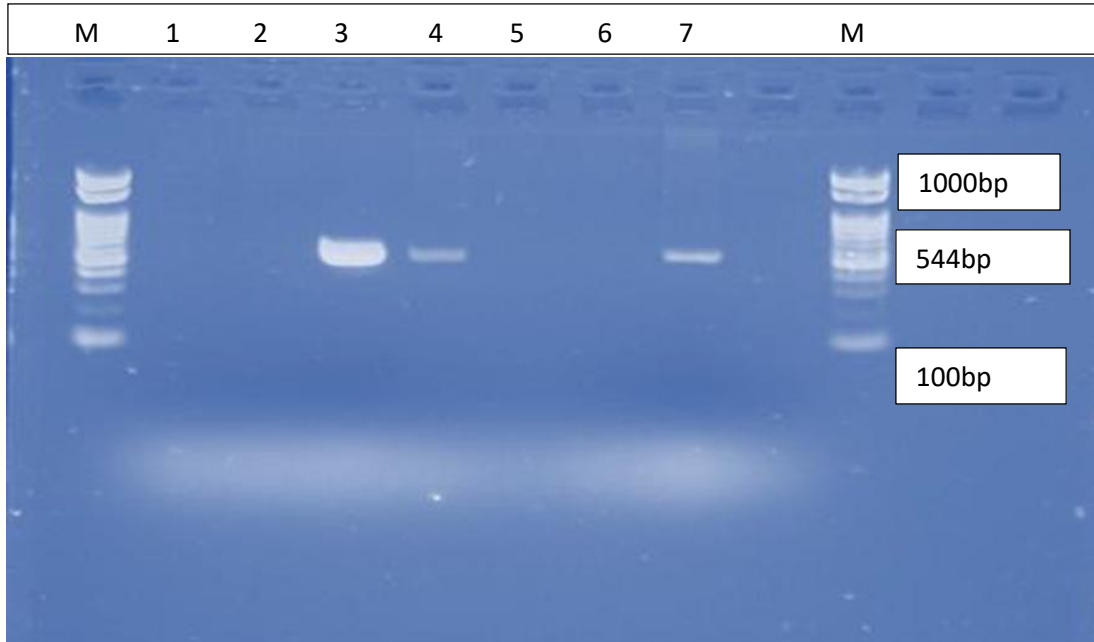


Figure 4.9: Gel Electrophoresis of PCR Products of CTX-M Genes.

Lane M is the 100 base pair DNA ladder, while Lanes 1-2 = ESBL-producing *Salmonella*, Lanes 3-5 = ESBL-producing *E. coli*, Lane 6=Negative control and Lane 7=Positive control

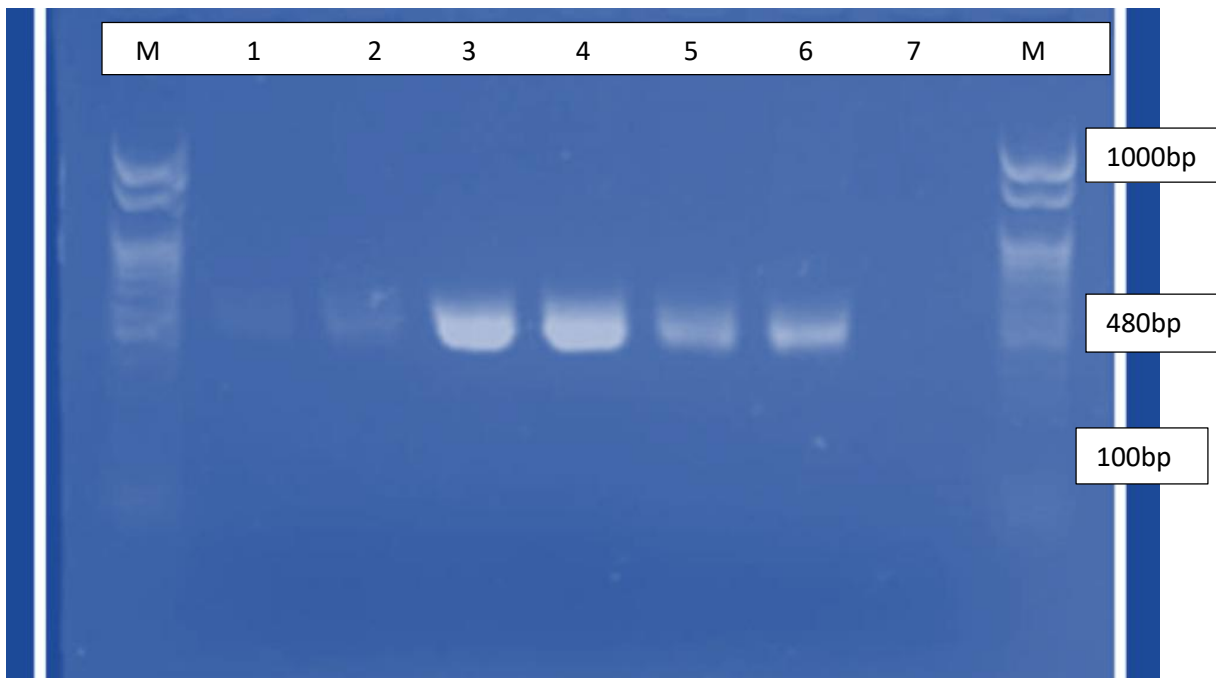


Figure 4.10: Gel Electrophoresis of PCR Products of TEM Genes. Lane M is the 100 base pair DNA ladder, while Lanes 1-2 = ESBL-producing *Salmonella*, Lanes 3-5 = ESBL-producing *E. coli*, Lane 6=Positive control and Lane 7=Negative control

CHAPTER FIVE

DISCUSSION

5.1 Distribution of Cockroach Species

The results presented in this study shed light on several important aspects regarding the distribution, and antibiotic susceptibility of selected foodborne pathogens isolated from cockroaches in student hostels at the University of Zambia and residential areas of Kalingalinga, Lusaka, Zambia.

During the study period of between November 2023 and January 2024, the study identified two predominant cockroach species, *Periplaneta americana* and *Blattella germanica*, in the sampled locations. It should be noted that, it is not yet established if other species of cockroaches occur outside this period. Although there was a higher percentage of *B. germanica*, both species were found in significant numbers. The study found that *B. germanica* and *P. americana* were prevalent in UNZA hostels and Kalingalinga residential areas, with *B. germanica* being more prevalent than *P. americana*, indicating a widespread presence in urban environments (Akter et al., 2023; Naher et al., 2018). Similarly, *P. americana* and *B. germanica* were found to be the two dominating species in previous studies of a similar nature conducted in Iranian regions (Davari et al., 2023; Nazari et al., 2020). The results of Pai et al., (2003) showed that in clinical environment most cockroaches are

B. germanica and in non-clinical places, most cockroaches are *P. americana* but this difference was not statistically significant which is in agreement with the study. The study by Turki Jalil et al., (2023) however, showed a different distribution of bacteria in hospital, restaurants and homes with the highest frequency of 67 % samples being *P. americana*, and the frequency of *Blattella germanica* and *Blatta orientalis* was 19 % and 14 %, respectively. It was not feasible to compare this data with local studies because no previous research had been conducted on the identification, percentage, and vector potential of cockroach species in Zambia.

5.2 Isolation and Identification of Bacterial Pathogens

The study isolated and identified *E. coli*, *Salmonella* species, and *S. aureus* from both cockroach species. These bacteria were distributed on the external body surfaces of these cockroaches as well as in the gut. The findings are in agreement with other studies that reported the potential role of invertebrate vectors in the spread of several infections and which established that cockroaches' external body parts like antennae, mouth parts and legs as well as their gut surface, which retain, in most cases, pathogens without loss in their virulence, are ideally suited for disease dissemination (Ifeanyi & Odunayo, 2015; Naher et al., 2018; Molewa et al., 2022). The distribution of these pathogens on the cockroaches which are well known foodborne pathogens and are often associated with cockroach infestations (Bhunias, 2018; Donkor, 2020; Rizki & Susanna, 2020) indicates potential health risks associated with cockroach infestations in both residential and institutional settings.

The distribution of these pathogens varied between the two study sites and body surfaces, with different percentages observed for each species with no statistical significance observed on the distribution of the isolated pathogens which is in agreement with a study done in Varanasi, India, where there was no discernible difference in the overall bacterial load on the outer surface of cockroaches found in homes (39.9%) and food-handling businesses (60.1%) (Wannigama et al., 2013). Though the distribution was not statistically significant, it was observed that, *P. americana* harboured more foodborne bacteria pathogens having an overall isolation rate of 66.7% (26/39) compared to *B. germanica* which had 33.3% (13/39). This difference can be attributed to the fact that *P. americana* is mostly associated with sewer, septic tanks, poultry houses and animal cages where these isolated bacteria can easily be found (Pai et al., 2005; Melton, 2012). These results are in agreement with the findings from a study by Davari et al. (2003) in which they reported that *P. americana* had the highest percentage of bacteria compared to *B. germanica*.

Notably, a total of 39 foodborne-associated bacteria were isolated, highlighting the potential role of cockroaches as carriers of various pathogens. In addition, 79% of isolated bacteria were recovered from the gut of cockroaches and this finding is in agreement with other studies that reported the distribution of bacteria to be more concentrated in the internal surface of the cockroach than the external (Vazirianzadeh et al., 2014; Adejumo et al., 2016; Solomon et al., 2018). This is

an interesting finding in a way that resistant bacteria might be well protected from the action of disinfectants which are applied during routine cleaning.

Furthermore, there is a possibility for these bacteria to replicate in the gut of cockroaches and be released to the environment during feeding or defecation. In the event that other factors, like a susceptible host and a path of entry, are met, these bacteria have a high potential to cause serious illness that could be challenging to cure. The isolation rate of pathogenic bacteria including *E. coli*, *Salmonella* species and *S. aureus* from this study is similar to the work of Solomon et al. (2018) and Al-Marjani et al. (2017) who reported similar percentage of pathogenic bacteria isolated from cockroaches that infested residential houses, restaurants and hospital environments.

The results highlight the significance of taking cockroaches into account when looking at food-borne outbreaks and the requirement for improved control techniques to stop them from being linked to food-borne infections.

5.3 In Vitro Drug Susceptibility Testing

The antibiotic susceptibility profiles of the isolated bacteria revealed varying degrees of resistance, consistent with reports of antimicrobial resistance in foodborne pathogens worldwide (WHO, 2020). Phenotypic drug susceptibility testing revealed varying levels of resistance among the isolated bacteria. *Staphylococcus aureus* showed resistance to Penicillin G, Gentamicin, and Chloramphenicol, while *Salmonella* species exhibited high resistance to Ciprofloxacin and Cefotaxime. *Escherichia coli* displayed resistance to multiple antibiotics, with notable resistance to Cefotaxime, Ampicillin, and Gentamicin. This finding is in agreement with a scoping review by Molewa et al. (2022) which indicated that cockroaches may be a potential vector for a diverse range of pathogenic bacterial agents and that most bacterial agents isolated are antidrug-resistant and antibiotic-resistant which is considered the greatest threat to public health in the current period.

The high resistance observed in *Salmonella* species and *E. coli*, particularly to ciprofloxacin and third-generation cephalosporins, underscores the importance of prudent antibiotic use and surveillance of antimicrobial resistance in both clinical and environmental settings.

5.4 Multi-Drug Resistance (MDR) Patterns

The study identified a significant proportion of MDR isolates, especially among *E. coli* and *Salmonella* species. This aligns with global concerns regarding the emergence and spread of MDR bacteria, emphasizing the need for comprehensive strategies to mitigate antimicrobial resistance in both healthcare and environmental settings (O'Neill, 2016). A significant proportion of the isolated bacteria exhibited multi-drug resistance, particularly *E. coli* and *Salmonella* species this is in line with other reports from Ghana, Ethiopia and other countries (Brown & Alhassan, 2015; Moges et al., 2016; Solomon et al., 2018). The high percentage of MDR strains underscores the importance of prudent antibiotic use and infection control measures to combat antibiotic resistance.

5.5 ESBL-Producing Enterobacteriaceae

In this study, phenotypic ESBLs activity was detected in 5 (18%) out of the total number of 28 isolates from the cockroaches comprising of 18 *Escherichia coli* and 10 *Salmonella* species after screening on CTX/MAC agar and confirmed via PCR and agarose gel electrophoresis. Similar findings have been observed and reported that cockroaches serve as reservoirs for ESBL-producing *Escherichia coli* and *Salmonella* species, exhibiting significant resistance to antibiotics, indicating the potential role of these insects in the dissemination of multidrug-resistant bacteria among human populations (Adejumo et al., 2016; Ikechukwu et al., 2017). The therapeutic choices for ESBL-producing bacteria pathogens are severely restricted because all cephalosporins are often avoided if a clinical isolate is found to be an ESBL producer. There was relatively low percentage of ESBL-producing *Escherichia coli* 17% (n=18) and *Salmonella* 20% (n=10) that were resistant to cefotaxime as observed in this study.

The detection of ESBL-producing *E. coli* and *Salmonella* highlights the potential role of cockroaches in the dissemination of antibiotic resistance genes in the environment. In this study, co-existence of bla CTX-M + TEM was observed in 2 of the *E. coli*. Co-existence of beta-lactamase genes in isolates from cockroaches similar to our findings have been reported in other studies (Loucif et al., 2016; Ikechukwu et al., 2017; Obeng-Nkrumah et al., 2019). The presence of CTX-M and TEM genes in these isolates underscores the importance of understanding the molecular mechanisms underlying antibiotic resistance and their implications for public health.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

1. This study has provided valuable insights into the distribution, and antibiotic susceptibility of foodborne pathogens carried by cockroaches.
2. Two major cockroaches' species *Periplaneta americana* and *Blattella germanica* were identified as potential mechanical vectors of foodborne pathogens.
3. The cockroaches can act as reservoirs of antimicrobial resistance pathogens as observed for ESBL-producing *Escherichia coli* and *Salmonella* species.
4. The isolates exhibited significant resistance to the antibiotics tested; highlighting the negative impact of increasing and inappropriate use of antimicrobial agents both in human and veterinary medicine in our environment.
5. The isolated ESBL-producing Enterobacteriaceae carried two important antibiotic resistance genes and exhibited the co-existence of bla CTX-M + TEM genes.

6.2 Recommendations

The findings of this study underscore the potential role of cockroaches as vectors for foodborne pathogens and antibiotic-resistant bacteria. Therefore, the study recommends a broader study, incorporating multiple seasons and mixed household structures, to generate more comprehensive and representative data on cockroach species composition and the distribution of associated pathogens across different settings in Zambia. Furthermore, there is need to establish how long cockroaches are able to harbour these pathogens. Finally, there is need to establish mechanisms of resistance gene transfers in the associated foodborne pathogens in cockroaches.

6.3 Study limitations

The study was conducted at only two sites and over a limited period, which covered just one season. These factors may have influenced the results. The small number of study locations means that the findings may not fully represent the diversity of environments across Zambia. Additionally, the short duration and single-season focus may have prevented the observation of seasonal variations in both cockroach species and the pathogens they carry.

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APPENDICES

Appendix A: Research concept note approval letter



**THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
OFFICE OF THE ASSISTANT DEAN (POSTGRADUATE)**

Telephone: 293727
Telegrams: UNZA LUSAKA
Telex: UNZALU ZA 44370
Fax: 293727/253952
School Fax: 293727
Vet. Clinic Telephone: 291515

P.O. Box 32379
Lusaka, Zambia

Your Ref:

Our Ref:

24th March, 2023

Lawrence Kapansa
University of Zambia
Department of Paraclinical Studies
LUSAKA

Dear Kapansa

SUBJECT: APPROVAL OF RESEARCH CONCEPT NOTE

I am writing on behalf of the Board of Graduate Studies to inform you that your research concept note entitled- *'Profiling of bacterial foodborne pathogens associated with cockroaches in Lusaka, Zambia: distribution and antimicrobial susceptibility profile'* was tabled and considered on 23rd March, 2023 by the Board of Graduate Studies of the School of Veterinary Medicine. I am therefore, pleased to inform you that your research concept was subsequently approved by the Board.

On behalf of the Board, I wish you success as you proceed with your research proposal development and all relevant related research activities.

Yours sincerely

Dr. Andrew Kataba (PhD)

ACTING ASSISTANT DEAN (PG), SCHOOL OF VETERINARY MEDICINE

Cc Director, DRGS
 Dean, School of Veterinary Medicine
 Assistant Registrar, School of Veterinary Medicine
 Head, Paraclinical Studies
 File

Appendix B: Ethics approval letter



Plot No. 272, Cnr Olive Tree Meanwood Road,
Meanwood Ibex
Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493 220
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

5th February, 2024.

Ref. No. 2023-Nov- 003

The Principal Investigator
Mr. Lawrence Kapansa
The University of Zambia
School of Veterinary Medicine
Lusaka, Zambia

Dear Mr. Kapansa

RE: PROFILING OF BACTERIAL FOODBORNE PATHOGENS ASSOCIATED WITH COCKROACHES IN LUSAKA, ZAMBIA: DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE.

Reference is made to your protocol submission. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Ordinary	Approval No. 2023-Nov-003
Approval and Expiry Date	Approval Date: 5 th February, 2024	Expiry Date: 4 th February, 2025
Protocol Version and Date	Version - Nil.	4 th February, 2025
Information Sheet, Consent Forms and Dates	<ul style="list-style-type: none"> English. 	4 th February, 2025
Consent form ID and Date	Version - Nil	4 th February, 2025
Recruitment Materials	Nil	4 th February, 2025
Other Study Documents	Questionnaires	4 th February, 2025
Number of participants approved for study		4 th February, 2025

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered

to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- A reprint of this letter shall be done at a fee.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB



Dr. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Sc., PhD
CHAIRPERSON

Appendix C: Participant consent form

Title: Profiling Of Bacterial Foodborne Pathogens Associated with Cockroaches in Lusaka, Zambia.

Consent to take part in research

I..... voluntarily agree to participate in this research study.

I understand that even if I agree to participate now, I can withdraw at any time or refuse to allow the researcher to collect samples without any consequences of any kind.

I understand that I can withdraw permission to use specimen/data from my place in which case the data will be deleted.

I have had the purpose and nature of the study explained to me in writing and I have had the opportunity to ask questions about the study.

I understand that participation involves setting of traps in my house to catch cockroaches

I understand that I will not benefit directly from participating in this research.

I understand that all information gathered for this study will be treated confidentially.

I understand that in any report on the results of this research my identity will remain anonymous. This will be done by changing my name and disguising any details of my interview which may reveal my identity or the identity of my place.

I understand that signed consent forms and data will be retained in the University of Zambia Veterinary laboratory until the exam board confirms the results of the dissertation.

I understand that I am free to contact any of the people involved in the research to seek further clarification and information.

Fomu Yachivomerezo cha Wotenga Mbali

Kafukufu Wofotokoza za Tizilombo ta Mabakteriya Topezeka mu Mphemvu Tomwe Timayambisa Matenda Kudzera mu Chakudya Komaso Kufufuza za Mankhwala Ochiritsira Matenda a Mabakteriya awa mu Lusaka, Zambia

Kuvomereza Kutenga Nawo Mbali pa Kafukufuku

Ine..... ndikuvomereza mwakufuna kwanga kuti nditenge nawo mbali mu kafukufuku ameneyi.

Ndamvetsetsa kuti ngakhale nditavomera kutenga nawo mbali pano, ndikhoza kusiya nthawi iliyonse kapena kukana kuti ofufuza atole zitsanzo popanda zotsatira zamtundu uliwonse.

Ndamvetsetsa kuti nditha kuchotsa chilolezo chogwiritsa ntchito chitsanzo/zonse zomwe anatenga pamalo anga ndipo zonse zomwe anatenga ndikulemba za pamalo panga zizafufutidwa.

Ndamvetsetsa cholinga komanso mtundu wa kafukufukuyu munjira yolembe ndipo ndinali ndi mwayi wofunsa mafunso okhudza kafukufukuyu

Ndamvetsetsa kuti kutenga nawo mbali mkafukufukuyu kuli mnjira yotchera misampha mnyumba mwanga kuti agwire mphemvu.

Ndamvetsetsa kuti sindipindulapo mwachindunji pochita nawo kafukufukuyu.

Ndamvetsetsa kuti zonse zomwe zasonkhanitsidwa pa kafukufukuyu zisungidwa mwachinsinsi.

Ndamvetsetsa kuti mu lipoti lililonse la zotsatira za kafukufukuyu sindidzadziwika. Izi zizachitika posintha dzina langa ndikubisa tsatanetsatane wa kuyankhulana kwanga komwe kungadzawulule dzina langa kapena malo anga.

Ndamvetsetsa kuti mafomu ovomereza osainidwa ndi zonse zasonkhanitsidwa mukafukufukuyu zikasungidwa mu nyumba ya zoyezayeza za kafukufuku wamatenda (laboretare) pa sukulu yaukachenjede ya Zambia (University of Zambia) mpaka bungwe la mayeso litatsimikizira zotsatira za buku la kafukufukuyu.

Ndamvetsetsaso kuti ndili womasuka kulumikizana ndi aliyense mwa anthu omwe akukhudzidwa ndi kafukufukuyu kuti andifotokozere zambiri.

kachenjede ya Zambia (University of Zambia) mpaka bungwe la mayeso litatsimikizira zotsatira za buku la kafukufukuyu.

Ndamvetsetsaso kuti ndili womasuka kulumikizana ndi aliyense mwa anthu omwe akukhudzidwa ndi kafukufukuyu kuti andifotokozere zambiri.

Signature of participant

Date

I believe the participant is giving informed consent to participate in this study.

Signature of researcher

Date

APPENDIX D: Antimicrobial Susceptibility Results for Isolated bacteria

SAMPLE ID	NO. OF CLASS TESTED	NO. OF CLASS NONSUSCEPTIBLE	ANTIBIOTICS									
			PEN (10ug)	AMP (25ug)	GEN (10ug)	AZM (30ug)	TCY (30ug)	CIP (5ug)	CHL (30ug)	SXT (25ug)	CTX (30ug)	
Sau 1	8	1	R	NE	S	S	S	S	S	S	S	S
Sau 2	8	1	R	NE	S	S	S	S	S	S	S	S
Sau 3	8	0	S	NE	S	S	S	S	S	S	S	S
Sau 4	8	2	R	NE	S	S	S	S	S	S	S	R
Sau 5	8	3	S	NE	S	S	R	R	R	S	S	S
Sau 6	8	0	S	NE	S	S	S	S	S	S	S	S
Sau 7	8	4	R	NE	R	S	S	R	S	S	S	R
Sau 8	8	0	S	NE	S	S	S	S	S	S	S	S
Sau 9	8	1	S	NE	S	S	R	S	S	S	S	S
Sau 10	8	1	S	NE	S	S	S	S	S	S	S	R
Sau 11	8	0	S	NE	S	S	S	S	S	S	S	S
Sal 1	7	1	NE	S	S	NE	S	S	S	S	S	R
Sal 2	7	2	NE	S	S	NE	S	R	S	S	S	R
Sal 3	7	1	NE	S	S	NE	S	R	S	S	S	S
Sal 4	7	3	NE	R	S	NE	S	R	S	S	S	R
Sal 5	7	3	NE	R	S	NE	S	R	S	S	S	R
Sal 6	7	1	NE	S	S	NE	S	R	S	S	S	S

Sal 7	7	2	NE	S	R	NE	S	R	S	S	S
Sal 8	7	1	NE	S	S	NE	S	S	S	S	R
Sal 9	7	0	NE	S	S	NE	S	S	S	S	S
Sal 10	7	1	NE	S	S	NE	S	S	S	S	R
Eco 1	7	2	NE	S	R	NE	S	S	S	S	R
Eco 2	7	1	NE	S	R	NE	S	S	S	S	S
Eco 3	7	0	NE	S	S	NE	S	S	S	S	S
Eco 4	7	0	NE	S	S	NE	S	S	S	S	S
Eco 5	7	0	NE	S	S	NE	S	S	S	S	S
Eco 6	7	1	NE	S	S	NE	S	S	S	S	R
Eco 7	7	2	NE	S	S	NE	S	S	R	S	R
Eco 8	7	4	NE	R	S	NE	R	R	S	S	R
Eco 9	7	0	NE	S	S	NE	S	S	S	S	S
Eco 10	7	5	NE	R	R	NE	S	R	S	R	R
Eco 11	7	4	NE	R	R	NE	S	R	S	S	R
Eco 12	7	6	NE	R	R	NE	R	R	S	R	R
Eco 13	7	0	NE	S	S	NE	S	S	S	S	S
Eco 14	7	0	NE	S	S	NE	S	S	S	S	S
Eco 15	7	2	NE	R	S	NE	S	S	S	S	R
Eco 16	7	0	NE	S	S	NE	S	S	S	S	S
Eco 17	7	0	NE	S	S	NE	S	S	S	S	S
Eco 18	7	1	NE	S	S	NE	S	S	R	S	S

KEY: Sau= *Staphylococcus aureus*, Sal= *Salmonella species*, Eco= *Escherichia coli*, S= Susceptible, R= Resistant, NE= Not Evaluated (Gram specific antibiotics), PEN=Penicillin, AMP=Ampicillin, GEN=Gentamicin, AZM=Azithromycin, TCY=Tetracycline, CIP=Ciprofloxacin, CHL=Chloramphenicol, SXT= Sulfamethoxazole-Trimethoprim, CTX=Cefotaxime.

Appendix E: Cockroach identification features

Cockroach species-specific features (Based on Burgess, 1993)

Species	Body Size	Body Color	Antennae Length	Wing Presence	Key Diagnostic Feature
<i>Blattella germanica</i>	Small (<2 cm)	Light brown	Long	Full wings, rarely flies	Two dark parallel stripes on pronotum
<i>Periplaneta americana</i>	Large (>4 cm)	Reddish-brown	Long	Full wings, can glide	Yellow band around pronotum
<i>Blatta orientalis</i>	Medium (2-4 cm)	Dark brown/Black	Long	Short wings (males), none (females)	Shiny, dark body; prefers cooler climates
<i>Supella longipalpa</i>	Small (<2 cm)	Light brown	Long	Full wings	Light-colored bands across wings and abdomen

Features for Cockroach Identification (Based on Beccaloni, 2014)

Species	Body Shape	Antennae Structure	Pronotum Shape	Leg Morphology	Other Distinguishing Features
<i>Blattella germanica</i>	Slender	Segmented evenly	Oval, shield-like	Spiny	Two dark longitudinal stripes on thorax
<i>Periplaneta americana</i>	Robust	Long, whip-like	Convex, oval-shaped	Spiny	Yellow band around pronotum
<i>Blatta orientalis</i>	Broad, flattened	Long, whip-like	Flat, shield-like	Spiny, thick legs	Dark glossy color, wings absent in females
<i>Supella longipalpa</i>	Slender	Long, whip-like	Flat, oval-shaped	Spiny, slender legs	Bands on wings and abdomen