ISOLATION AND IDENTIFICATION OF SOME MICROBIAL PATHOGEN AS SOCIATED WITH BEDBUGS

A PROJECT REPORT SUBMITTED

BY

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HND/23/SLT/FT/1043

BEING A RESEARCH POROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, INSTITUTE OF APPLIED SCIENC E, KWARA STATE POLYTECHNIC, ILORIN.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN SCIENCE LABORATORY TECHN OLOGY.

JULY,2025

CERTIFICATION

This is certify that this project is the original work carried out and reported by **SAKA AMINAT OMOTOYOSI** with matric number **HND/23/SLT/FT/0563** to the Department of Science Laboratory Technology, Microbiology unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfillment of the requirements for the Award of Higher National Diploma (HND) In Science Laboratory Technology

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DEDICATION

I dedicate this project to the Almighty God, whose guidance and strengt h have seen me through this journey. My deepest gratitude goes to My Husband who has provided all that was needed to complete this project. There was never lack or want.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to all those who have contr

ibuted to the successful completion of this project. Your support and gui

dance have been invaluable and I am thankful for your contributions to

my project work.

Special thanks to my supervisor (Mr Ibrahim A. Wahab) for his valuable i

nsights and feedback, which have significantly enriched my project. I als

o want to acknowledge the support of my project mates, whose encour

agement and assistance have been greatly appreciated.

Lastly, I express my gratitude to my family for their understanding and b

elief in my ability, your unwavering support has been a constant source

of motivation throughout this journey.

ABSTRACT

Bedbugs (Cimex lectularius), hematophagous ectoparasites, are increas ingly recognized not only as nuisance pests but also as potential carrier s of pathogenic microorganisms. This study aimed to isolate and identif y bacteria and fungi associated with bedbugs collected from residential homes, student hostels, and hospital wards within Ilorin metropolis, Nig eria. Bedbugs were collected using sterile techniques and processed thr ough homogenization and serial dilution. Samples were cultured on bloo d agar, MacConkey agar, and Sabouraud dextrose agar, followed by bioc hemical and morphological characterization of isolates. Results reveale d a high microbial load, especially in samples from hospital wards and s tudent hostels. Predominant bacterial isolates included Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, while fungal iso lates such as Aspergillus spp. and Candida spp. were also recovered. A ntibiotic susceptibility tests indicated varying levels of resistance amon g the bacterial isolates. These findings highlight bedbugs as potential re servoirs of both bacterial and fungal pathogens in urban environments, underscoring the need for integrated pest and infection control strategie

s in densely populated and healthcare-associated settings.

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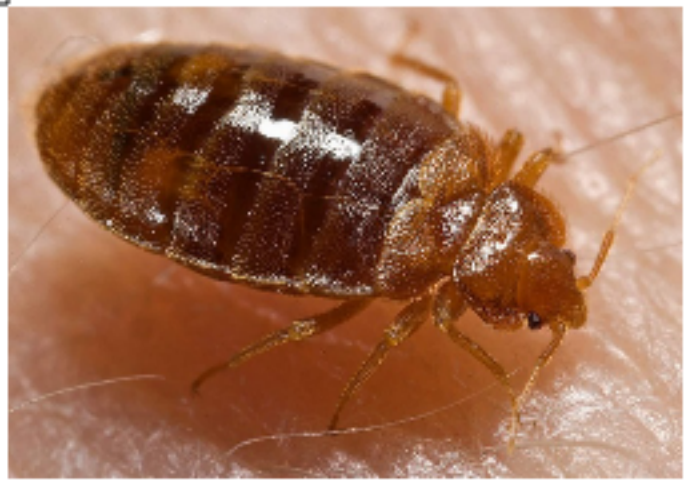
CHAPTER ONE

1.0 INTRODUCTION

Bedbugs (*Cimex lectularius*) are hematophagous ectoparasites that hav e re-emerged globally as pests of significant public health concern. Thes e small, flat, wingless insects feed primarily on human blood and are co mmonly found in homes, hotels, hospitals, and hostels, particularly in ar eas with high population densities and poor sanitation (Doggett *et al*., 2 020). Although not traditionally viewed as vectors of disease, bedbugs h ave increasingly been associated with the carriage of various pathogeni c microorganisms that can pose health risks to humans (Potts *et al*., 202 1).

Fig. 1



Source: (Doggett *et al.*, 2020)

The resurgence of bedbug infestations, especially in developing countrie s, has triggered renewed scientific interest in understanding their biolog y and the microorganisms they may harbor. This is particularly importan t in urban centers like Ilorin, Nigeria, where overcrowding and limited acc ess to quality pest control contribute to the spread and persistence of b edbug populations (Ademola *et al.*, 2022). These insects typically hide in cracks, furniture, mattresses, and clothing, making eradication difficult w ithout targeted interventions. Previous studies have reported that bedbu gs can serve as mechanical or biological carriers of bacteria, fungi, and viruses. Among the bacterial species found in bedbugs are *Staphylococ*

cus aureus, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus* sp p., all of which are known to cause a range of infections in humans (Mas roujeh *et al.*, 2020). The detection of these microorganisms in bedbugs r aises concerns about their potential role in nosocomial infections and co mmunity-acquired diseases.

The microbial flora associated with bedbugs can be endogenous symbio tic bacteria within their gut or exogenous acquired from their environme nt or host's skin. The gut microbiota, in particular, plays a crucial role in b edbug digestion and survival, but can also act as a reservoir for antibioti c-resistant pathogens (Merlin *et al.*, 2023). This dual role highlights the i mportance of identifying and characterizing both beneficial and harmful microbes associated with bedbugs.

Ilorin metropolis, located in Kwara State, Nigeria, is a rapidly growing city with a diverse population and urban infrastructure that includes public a nd private residences, schools, and health centers. Many of these enviro

nments, especially those with limited hygiene practices, provide ideal br eeding grounds for bedbugs (Olatunji *et al.*, 2021). Despite anecdotal re ports of infestations, little scientific research has been conducted on the microbiological implications of bedbugs in this region.

The microbial composition of bedbugs in Ilorin is essential for public hea lth monitoring and infection control. The identification and isolation of m icroorganisms from these insects will provide data on possible reservoir s of pathogens and could guide targeted interventions (Obasi and Eze, 2 022). This research could also reveal the extent of microbial resistance, which is critical in an era of increasing antimicrobial resistance.

The ability of bedbugs to harbor and potentially transmit multidrug-resist ant organisms (MDROs) presents an urgent need for scientific inquiry. R ecent findings have shown the presence of *methicillin-resistant Staphyl ococcus aureus* (MRSA) and *extended-spectrum beta-lactamase* (ESBL) producing *E. coli* in bedbugs collected from hospital settings (Shariff *et a*

l., 2020). Such evidence indicates that bedbugs could be silent contributors to the burden of healthcare-associated infections.

In addition to bacteria, fungi such as *Aspergillus*, *Penicillium*, and *Candida* species have been isolated from the body surface and gut of bedbugs. These fungal species can exacerbate respiratory conditions and lead to opportunistic infections in immunocompromised individuals (Nwankwo *et al.*, 2023). Therefore, studying the fungal profile of bedbugs is equally important to obtain a complete picture of their microbiological relevance.

Bedbugs can migrate passively through clothing, luggage, and second-hand furniture, facilitating the spread of associated microorganisms across communities and borders. Their resilience to conventional insecticides makes them particularly difficult to eliminate, which contributes to persistent microbial contamination of infested environments (Abiola and Ahmed, 2021). These factors make bedbugs an important target for integ

rated vector and disease control programs.

In Nigeria, studies on bedbugs have largely focused on their distribution and pesticide resistance. However, there is a significant research gap in understanding the microbial ecology of bedbugs in Nigerian urban settin gs (Eze *et al.*, 2022). Filling this gap is necessary to develop informed pu blic health strategies, especially in environments with limited sanitation i nfrastructure.

The use of microbiological and molecular techniques to isolate and iden tify microorganisms in bedbugs can provide high-resolution data on spe cies composition, diversity, and antibiotic susceptibility. Culturing metho ds on nutrient agar, MacConkey agar, and Sabouraud dextrose agar allo w for selective growth of bacteria and fungi, while biochemical and mole cular tests offer definitive identification (Agboola *et al.*, 2023). These too ls will be critical in achieving the objectives of this study. This research a ims to isolate, identify, and characterize bacteria and fungi present in be

dbugs collected from different parts of Ilorin metropolis. Sampling will ta rget residential areas, student hostels, and clinics to capture a broad mi crobial spectrum. By comparing the microbial profiles across locations, t he study may highlight differences in infestation-related risks and health implications.

The results of this study will provide valuable insights for clinicians, publi c health officers, and environmental health practitioners. In particular, th e findings may influence disinfection protocols in hospitals and homes, and support the development of evidence-based pest control policies. T his is especially relevant as the WHO calls for integrated strategies to co mbat vector-borne diseases (WHO, 2023). Ultimately, this research unde rscores the intersection between entomology and microbiology in disea se ecology. Bedbugs, once considered only nuisance pests, are now incr easingly recognized as potential carriers of pathogens. By identifying an d isolating microbial pathogen associated with bedbugs in Ilorin, this pro ject contributes to the growing body of knowledge needed to address hi

dden threats in urban ecosystems.

CHAPTER TWO

2.0 Materials and Methods

2.1 Materials

The materials used in this study included both biological and laboratory i tems necessary for microbial isolation and identification. Bedbugs were collected from infested environments within Ilorin metropolis. Sterile sa mple containers with tight-fitting lids were used to safely transport the b edbugs to the laboratory. A 70% ethanol solution and a UV light source were employed for surface sterilization of the insects. A mortar and pes tle or tissue homogenizer was used to grind the bedbugs, while sterile di stilled water served as the diluent during serial dilution and homogeniza tion. The culture media used included blood agar, MacConkey agar, and Sabouraud dextrose agar, each selected for their ability to support the gr owth of different microbial species. Petri dishes, an incubator set to vari ous temperatures, a light microscope, and a set of biochemical test kits (Gram staining, catalase, and oxidase tests) were essential for microbial

observation and characterization. Additionally, a PCR machine was inclu
ded for molecular identification where applicable.

2.2 Sample Collection

Bedbugs were collected using sterile forceps and transferred into sterile,
screw-capped containers. The collections were made from locations kn
own to have persistent infestations, including homes, student hostels, a
nd hospital wards. Each container was labeled with details such as date,
time, and location of collection. Care was taken to avoid contamination
by wearing gloves and ensuring minimal human contact with the sampl
es. The containers were immediately sealed and transported to the micr
obiology laboratory for further processing within 2–4 hours to preserve t
he microbial integrity of the specimens.

2.3 Sampling Site

The sampling was conducted in Ilorin metropolis, the capital of Kwara St ate, Nigeria. The metropolis was selected due to frequent reports of bed bug infestations in both residential and institutional settings. Specific sa mpling sites included densely populated student hostels, low-income re sidential areas, and selected hospital wards known for recurring pest co mplaints. These locations were chosen to represent a variety of socio-e nvironmental conditions that might influence bedbug-associated microbi al diversity. GPS coordinates were notated for traceability, and permissio n was obtained from residents or facility administrators before sample c ollection.

2.4.0 Media Preparation

Culture media used for microbial isolation were prepared following the manufacturers' instructions. Blood agar was used to support the growth of fastidious organisms, MacConkey agar for Gram-negative enteric bac teria, and Sabouraud dextrose agar for fungi. Each medium was weighe

d and dissolved in distilled water, sterilized by autoclaving at 121°C for 1 5 minutes, and poured aseptically into sterile Petri dishes. The plates w ere allowed to solidify at room temperature, labeled, and stored in a refri gerator at 4°C until use. All media were quality-checked using control or ganisms before inoculation of the test samples.

2.4.1 Sample Preparation

Upon arrival in the lab, bedbugs were washed in sterile water to remove superficial contaminants and then surface-sterilized with 70% ethanol fo r 10–15 minutes or exposed to UV light for the same duration. They wer e then rinsed in sterile water and transferred to a sterile mortar. A small volume of sterile distilled water was added, and the bedbugs were hom ogenized using a sterile pestle until a uniform suspension was achieved. This homogenate was subjected to serial dilution using 1 mL of homoge nate into 9 mL of sterile water to obtain dilutions ranging from 10^{-1} to 10 $^{-3}$. These dilutions were used for microbial plating.

2.5 Bacterial Isolation and Identification

From each dilution, 100 µL was aseptically plated onto prepared blood a gar, MacConkey agar, and Sabouraud dextrose agar using a sterile spre ader. Plates were incubated at 25°C, 30°C, and 37°C for 24 to 48 hours, depending on the media. Colony growth was observed and recorded ba sed on morphological characteristics such as color, shape, elevation, an d margin. Distinct colonies were sub-cultured to obtain pure isolates. Ea ch isolate was then subjected to Gram staining, catalase test, and oxida se test to aid in bacterial identification. Further identification was perfor med using conventional biochemical tests and, where applicable, molec ular methods such as polymerase chain reaction (PCR).

2.6 Antibiotic Susceptibility Testing

Antibiotic susceptibility of bacterial isolates was assessed using the Kirb y-Bauer disk diffusion method on Mueller-Hinton agar. Bacterial suspens ions were adjusted to 0.5 McFarland standard and evenly spread on the surface of the agar. Antibiotic discs including ampicillin, ciprofloxacin, tet

racycline, gentamicin, and ceftriaxone were placed on the plates. The pl ates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines to classify isolates as sensitive, intermediate, or resistant.

2.7 Data Analysis

All recorded observations from colony morphology, biochemical tests, a nd antibiotic susceptibility results were tabulated. The frequency of diffe rent microbial isolates was calculated and presented using descriptive s tatistics such as percentages and means. Statistical analysis was carrie d out using software like SPSS to determine significant differences in mi crobial loads between different sampling sites. Graphs and charts were used to visually represent data for ease of interpretation. Molecular dat a (where applicable) were analyzed using gel electrophoresis and comp ared against known DNA ladders and marker strains.

2.8 Quality Control

Quality control was maintained throughout the experimental process. All culture media were tested with control strains (*E. coli*, *S. aureus*) to ens ure proper growth before use. Sterility of reagents and tools was confir med before each session. Autoclaved materials were checked for prope r temperature and pressure settings. Negative controls were included d uring biochemical and molecular analyses to rule out contamination. Da ta entries were double-checked for accuracy and reliability. All procedur es were carried out under aseptic conditions to minimize external conta mination and ensure reproducibility of results.

CHAPTER THREE

3.0 RESULT

3.1 Colony Count of Microbial Isolates from Bedbugs

The table below shows the average colony count obtained from differen t sampling locations after serial dilution and incubation on nutrient and s elective media. Higher microbial loads were observed in samples from s tudent hostels and hospital wards.

Table 1: Average Colony Count (CFU/mL) from Bedbug Samples

Sampling Lo cation	Dilution F actor	Blood Agar (CFU/mL)	MacConkey Ag ar (CFU/mL)	SDA (CFU/ mL)
Residential Homes	10^{-2}	2.3×10^3	1.8×10^3	1.5×10^3
Student Hos tels	10^{-2}	4.1×10^3	3.9×10^3	2.8×10^3
Hospital Wa	10^{-2}	5.6×10^3	4.7×10^3	3.2×10^3

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Note: SDA = Sabouraud Dextrose Agar

3.2 Morphological Characteristics of Isolates

Colonies grown on different media were observed for morphological cha racteristics. Variations in colony color, shape, elevation, and consistency provided clues to microbial diversity.

Table 2: Cultural and Morphological Characteristics of Isolates

Isolate Code	Media Type	Colony Color	Shape	Elevation	Consistency	Presumptive ID
B1	Blood Agar	Creamy white	Circular	Raised	Smooth	*Staphylococcus* sp.
B2	MacCon	Pink	Circular	Flat	Moist	*E. coli*