BACTERIOLOGICAL EVALUATION OF BED LINEN AND MATTRESS IN POSTGRADUATE HOSTEL IN UNIVERSITY OF BENIN, UGBOWO CAMPUS

BY

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FEBRUARY, 2025.

CERTIFICATION

We certify that this work was carried out by **Bertha Osayanmon EDOBOR**in the Department of Microbiology, University of Benin, Benin City.

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CERTIFICATION OF THESIS

We attestand declare that the thesis titled **Bacteriological Evaluation of Bed Linen and Mattress in Postgraduate Hostel in University of Benin, Ugbowo Campus**has successfully passed the anti-plagiarism test and does not violate any copy right regulation.

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DEDICATION

This project work is dedicated to God Almighty, who has made it possible for me to be alive till this day and has provided for me and sustained me.

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TABLE OF CONTENTS

	PAGE
Title page	i
Certification	ii
Certification of Thesis	iii
Dedication	iv
Acknowledgements	v
Table of contents	vi
List of Tables	viii
List of Plates	viii
List of Figures	viii
Abstract	ix
CHAPTER ONE: INTRODUCTION	1
1.1 Aim and Objectives	4
CHAPTER TWO: LITERATURE REVIEW	5
2.1 Natural Fibres	5
2.2 Attack of Textile Materials by Microbes	5
2.3 Microbiological Assessment of Bedding Materials	7
2.4 Transmission of Diseases through Bed linen/Mattress	11
2.8 Sources of Contamination on Clothing and Household Linen	17
2.5 Occurrence of Pathogens on Clothing and Household Linen	18
2.6 Controlling Microbial Contamination of Bed linen	19
2.7 Cleaning of Mattresses and Bed linen	21

CHAPTER THREE: MATERIALS AND METHODS **22**

3.1 Study Area 22

3.2 Administration of Questionnaires 22

3.3 Sample Collection 22

3.4 Enumeration of Bacteria 22

3.5 Isolation of Pure Culture 23

3.6 Characterization of Bacterial Isolates 23

3.7 Virulence Factors 23

3.8 Antibiotic Susceptibility of Virulent Bacteria Isolates 24

3.9 Molecular Identification of Virulent Bacteria Isolates 25

3.10 Data Analysis 28

CHAPTER FOUR: RESULTS 29

CHAPTER FIVE: DISCUSSION 48

5.1 Discussion 48

5.2: Contributions to Knowledge 54

5.3 Conclusion 55

References 56

LIST OF TABLES

Table	Title	Page
4.1:	Demographic profiles of the students	30
4.2:	Student response rate on pathogenic bacteria contamination of bed linen and mattresses	31
4.3:	Total bacterial counts (x10^3 cfu/cm^2) of Bed linen (BB) from Postgraduate hostels	36
4.4:	Total bacterial counts (x10^3 cfu/cm^2) of Mattress (BM) from Postgraduate hostels	37
4.5:	Cultural morphological and biochemical characteristics of bacteria obtained from bed linen and mattresses	38
4.6:	Percentage occurrence of bacterial isolates from Bed linensamples	39
4.7:	Percentage occurrence of bacterial isolates from mattresssamples	42
4.8:	Molecular identification of bacterial isolates from bed linen	43
4.9:	Phenotypic virulence properties of the bacterial isolates from bed linen	44
4.10:	Antibacterial sensitivity of bacterial isolates obtained from Bed linen surfaces	45

LIST OF PLATE

Plate	Title	Page
4.1:	Plasmid DNA of multidrug resistance bacterial isolates	47

LIST OF FIGURE

Figure	Title	Page
4.1:	MAR index of bacterial isolates	46

ABSTRACT

Mattresses and bed linen are clearly recognized as potential reservoirs for microorganisms and could be vectors of disease transmission. Bedding materials include bed sheets, blankets, towels and personal clothing (night wares). Pathogenic microorganisms can be transmitted from bed linen to human. This study therefore, assessed pathogenic bacterial isolates from bed linen and mattresses in Postgraduate hostels in University of Benin, Ugbowo Campus

Samples were swabbed from bed linen and mattresses of Post-graduate Hostels in University of Benin, Ugbowo Campus with sterile swab sticks moistened in normal saline. Heterotrophic bacteria were cultured on nutrient agar, while hemolytic bacteria were isolated on sheep blood agar. Isolated pathogens were subsequently assessed for antibiotic resistance to common antibiotics using Kirby-Bauer Disc Diffusion method.

The total bacterial counts of bed linen ranged from 4.00±0.60 x 10^3 cfu/cm^2 - 82.1±3.91 x 10^3 cfu/cm^2 while the bacterial count from mattress ranged from 4.29±0.31 x 10^3 cfu/cm^2- 16.2±0.94 x 10^3 cfu/cm^2. Bacterial isolates identified in bedlinen and mattresses included three (3) Gram negative bacteria: *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* and two (2) Gram positive bacteria: *Bacillus mycoides* and *Staphylococcus aureus.* Frequency of occurrence of bacterial isolates from bed linen showed that *Escherichia coli* was the most occurring bacteria (36.4%) while the least occurring isolate was *B. mycoides* (5.45%). *S. aureus* (32.3%) was the most occurring bacterial isolates while the least was *B. mycoides* (10.8%) from mattresses. *Staphylococcus aureus*was positive for Dnase, lipase and hemolysin characteristics, *E. coli* and *Enterobacter cloacae* exhibited positive DNase and Lipase characteristics. *Staphylococcus aureus* had the highest multiple antibiotic resistance (0.36%), followed by *Enterobacter cloacae* (0.33%) while *E. coli* had least multiple antibiotic resistance (0.29%). Bed linen and mattresses harboured pathogenic bacteria. There is need to strengthen existing infection control strategies in the postgraduate hostels in order to minimise proliferation of bacteria and diseases occurrence in the hostels.

CHAPTER ONE

INTRODUCTION

Majority of University students engage in their busy academic schedules during the day and usually find comfort in their beds in the evening time till day break. Due to stress encountered during the school hours, most of them just jump on their beds to relax. In the process, microorganisms encountered from different contacts are being transferred to their beds. These microbes can as well find their way during the production of sweat through airborne contamination. The potential for spread from linen was demonstrated by Shiomori *et al*. (2002) who determined numbers of surface and airborne MRSA before, during and after bed making for 13 in patients with MRSA (Methillin resistance *Staphylococcus aureus*) infection or colonization in a Japanese hospital. Fungal spores released by soil fungi into the wind can be a natural mechanism of transportation. When clothing come in contact with these spores, it can serve as source of fungal infections (Fijan and Turk, 2012). Some individuals love pets so much that they allow them on their beddings not minding the microbial load on the animals. This can pose a serious public health risk because they may contain microorganisms that are both pathogenic to humans and resistant to several classes of antibiotics (El-Tras *et al*.,2015).

Students at academic institutions with inadequate and suboptimal sanitary practices are vulnerable to microbial infections and poor hygiene (Igudia *et al*., 2019). Asymptomatic colonization by multidrugresistant (MDR) organisms has been identified as the first step prior to subsequent infection (Jans*et al*., 2013). According to one study, undergraduate university students were found to harbour potentially harmful multidrug-resistant microorganisms (Chukwunwejim *et al*., 2018). Another study discovered the predominance of bacterial species as

compared to fungal isolates in bed linen from students who live in university hostels (Olowomofe *et al*., 2020).

Clothing materials have been shown to act as microorganism reservoirs, as these organisms can survive on such surfaces for periods ranging from a few seconds to several hours or days (Olowomofe *et al*., 2020; Gopal and Solabannavar, 2020). It has been reported that fabrics such as bed linen serve as a potential reservoir for both pathogenic and non-pathogenic microorganisms. When bed linen are heavily contaminated with potentially infectious agents, they can contain bacterial loads of 10^6 -10^8 Colony Forming Unit (cfu)/100 cm^2 (Koca *et al*., 2012)

One substantial life-style change over the last 30 years has been altered bedding – from feather/flock pillows and sheets/blankets, to mainly polyester pillows and quilts. With no need for feather containment, the covers on pillows are more porous with pore size increasing from 2 to >10 mL. Synthetic pillows are a risk for both prevalence and for severity of asthma. Butland *et al*. (2017) estimated that the increase in synthetic bedding could explain 50% of the increase in prevalence in wheezing. Fungi growing on bedding could be an environmental health risk. Adults may produce up to 100L of sweat in bed every year, which for approximately 8 h/24 is at approximately 30°C and high humidity – an ideal fungal culture medium. Fungal contamination of kapok pillows was noted in 1936, and associated with wheezing (Woodcock *et al*., 2014).

Skin is a home to diverse microorganisms normally associated with skin cells, sweat, sputum, and vaginal and anal excretions, some of which promote immunity or fight invaders. Most of these microbes are there to help to protect humans from pathogenic invaders and help the immune system to maintain delicate balance between protection and damaging inflammation. As we have microbes that are beneficial to our health, there are also pathogenic organisms which are

disease causing organisms and can also cause death due to their accumulation on the body (Creamer and Humphreys, 2008).

Mattresses and bed linen are no exception in the case of presence of microorganisms. Mattresses and bed linen are clearly recognized as a potential reservoir for microorganisms and could be a vector of disease transmission (Nyinoh *et al*., 2018). Bedding materials includes bed sheets, blankets, towels, personal clothing, patient apparel, uniforms, gowns, drapes for surgical procedures (Fijan and Šostar. 2012). Since hospitals' linen comes in contact with various body fluids i.e. blood, urine, feces, saliva, sputum, therefore, pathogenic microorganisms are potentially to be stayed in it (Ot, 2018).

Studies evident that pathogenic microorganism can transmit from uniforms to patients and bed linen, or from dirty bed linen to staff uniforms (Wilson *et al*., 2017; Ot, 2018). Bed textiles have been considered as one of the possible vehicles of transmission of infections. Dried and streamed reused towels was found as the source of *Bacillus cereus* nosocomial infections among patients in the summer from 2001-2005 in Japan (Dohmae *et al*., 2018). Afroz*et al*. (2017) sought to identify the source of *Bacillus cereus* bacteremia outbreak at Jichi Medical University Hospital in 2006 and discovered that hospital linen were the main source of this contamination and *B. cereus* was transmitted from the linen to patients via catheter infection.

Teufel *et al*. (2018) used molecular genetics technique to isolate DNA as a quick and accurate way to estimate the number of organisms in textiles before and after cleaning in order to maintain the health and safety of patients and auxiliary bodies that use textile products in hospitals. The use of high quality standards contributes to the protection and safety of staff in the healthcare industry. Over the centuries, history has shown that health interventions and investments were always a good plan to improve the country's economy

The efficiency of the cleaning process, whether manual or automatic, moist or dry processes has great effect on the life cycle of textile. It maintains the health and safety of the individual if used in the optimal way, the effect of detergent and other additives used during washing process may adversely or positively affect both the mechanical and physical properties of the fabric and the consumer protection from certain illnesses (Enaamand Ghanem, 2016). On the other hand, the cleaning process includes a range of factors affecting the efficiency of the process. These factors include both the type and concentration of detergent, the washing water temperature, mechanical washing, additives of disinfectants, softeners, bleaches, the number of times, and the method of rinsing, as well as drying. Also, other affecting factors are knowledge of the contaminant and the properties of the fabric in terms of the nature of the surface of the fabric, including its capillary characteristic. All these characteristics and factors also affect the efficiency of the cleaning process (Hanan Al Gamel, 2018)

1.1 Aim and Objectives

This study was aimed at assessing pathogenic bacterial isolates from bed linen and mattress in postgraduate hostels in University of Benin (Ugbowo Campus)

The objectives of this study were to:

1. isolate, characterize and identify bacterial isolates associated with the mattress and bed linens using phenotypic and molecular techniques.
2. enumerate the bacterial load in the mattresses and bed linens.
3. determine the frequency of occurrence of the bacterial isolates.
4. evaluate the bacterial isolates for phenotypic virulence trait
5. determine the multiple antibiotic resistance pattern.

CHAPTER TWO

LITERATURE REVIEW

2.1 Natural Fibres

Textiles made from natural fibres are generally more susceptible to biodeterioration than are the synthetic (man-made) fibres (Agbulu *et al*., 2015). This is because their porous hydrophilic structure retains water, oxygen and nutrients, providing perfect environments for bacterial growth. Products such as starch, protein derivatives, fats and oils used in finishing of textiles can also promote microbial growth (Dixon, 2000). Micro-organisms may attack the entire substrate, that is the textiles fibres or may attack only one components of the substrate, such as plasticizer contained there in, or grow on dirt that has accumulated on the surface of a product. Nevertheless, even mild surface growth can make a fabric look unattractive by the appearance of unwanted pigmentation. Heavy infestation which results in rotting and breakdown of the fibres and subsequent physical changes such as loss of strength or flexibility may cause the fabric to fail in service (Bandiaga *et al*., 2008). The material is attacked chemically by the action of extracellular enzymes produced by the micro-organisms for the purpose of obtaining food. Plants fibres such as cotton, flax (Linen), jute and hemp are very susceptible to attack by cellulolytic (cellulose digesting) fungi. Indeed, the complete degradation of cellulose can be effected by enzymes, produced by the fungi and known as cellulases (Potera, 2001).

2.2 Attack of Textile Materials by Microbes

The inherent properties of the textile fibres provide room for the growth of micro-organisms. Beside the structures of the substrates and the chemical processes may induce the growth of

microbes. Humid and warm environments still aggravate the problem. Infection by microbes cause cross infection by pathogen and developments of odour where the fabric is worn next to skin (Agbulu *et al*., 2015). In addition, the staining and loss of the performance properties of textile substrates are the results of microbial attack. Garments of health care workers are important aspect of the environments that can easily become contaminated. Brook (2013) reported that 65% of nurses who had performed patient care activities on patients with methicillin-resistant *Staphylococcus aureus* (MRSA) in a wound or urine contaminated their nursing uniforms or gowns with MRSA. One critical factor for transmission of a micro-organisms from a person (patient or health care worker) to the fabrics and then to another person is the ability of that microbe to survive on that surface of the fabric. A few studies have examined the survival of gram-positive bacteria on various surface (Didymus, 2012; Leblance, 2013) etc. These researchers investigated the survival of these microbes and confirmed survival of days to months. Thus, act as a reservoir for these microbes. The spread of HIV and hypatitis viruses by contact of contaminated materials has created increase pressure for protection of personnel with functional clothing (Curtis *et al*., 2000). The author reported that an unpleasant odour develops when among other things; bacteria convert human perspiration into some foul smelling substances such as carboxylic acid, aldehydes and amines

In a study by Graham *et al*., (2008), the data indicated that Staphylococci and Enterococci can survive for extended periods of time on materials commonly worn by patients and health care workers and on various other fabrics in the hospital environment while Loren and Smith (2004) detected *S. aureus, Klebsiella pneumoniae* and *Acinetobacter baumanii* in hospital gowns. Neck ties worn by doctors at an intensive care unit produced heavy growths of coagulase negative staphylococcus on 2/5 ties tested (Dixon, 2000). *Candida, Aspergillus, Mucor*, and *Fusarium*

which are associated with nosocomial infections in patients survived long periods on fabrics and plastics which are routinely used in hospitals. These survival results indicate the potential for various fabrics and plastics to serve as reservoirs or vectors for fungi.

2.3 Microbiological Assessment of Bedding Materials

Microbes are the oldest form of life on earth. Without microbes, we could not eat or breathe but without us they will still survive, thus they form a part of our daily lives (Dodrill *et al*., 2011). Microbes are everywhere, there are more of them on a person's hand than there are people on the entire planet; they are in the air we breathe, the ground we walk on, the food we eat-they are even inside us. We could not digest food without them-animals could not either. Without microbes, plants could not grow, garbage would not decay and there would be a lot less oxygen to breathe. Interestingly, it has been noted that, less than one percent of the hundred percent of microbes cause diseases to humans, leaving the ninety-nine plus percentage as beneficial microbes (Adewoyin *et al*., 2013). A common word for these disease causing microbes is "germs", and examples are Rhinovirus, *Plasmodium falciparum, Trichophyton rubrum, Escherichia coli, Salmonella,* Enterococci*, Staphylococcus* and many others, that cause various illnesses such as cold, malaria, ringworm, colitis, salmonellosis, typhoid fever, urinary tract infections, skin infections like cellulitis, impetigo and many more that if ignored may result in death. All of which can survive on surfaces for varying degrees of time depending on soiling and moisture levels (Bashir and Ali, 2015).

Bedding materials can be contaminated by moulds, bacteria, viruses and any action that introduces contaminants into the surface. The concern for proper sanitation is necessary especially in commercial/industrial settings that accommodate a large number of people and

visitors because infections can cause both mild and severely irritating health conditions (Tambeker and Smith, 2007).

The industries are challenged by the presence of microorganisms and the negative effects they cause. Deterioration, defacement and odours are all dramatic effects which occur from the microbial contamination of environment. Bedding materials can act as a microbial "harbor", as most offer ideal environments for the proliferation of microorganisms that are harmful to buildings, textiles and humans. The ability to make surfaces resistant to microbial contamination has advantages in many applications and market segments. This is especially true in markets where many products have contributed a degree of aseptic sophistication beyond that required of consumer products. It has been found on mattress and bed clothing are contaminated with microorganisms (Fekety *et al*., 2001). Methycillin resistant *Staphylococcus aureus* (MRSA) has also be reported on bed linen surfaces (Duckworth and Jordens, 2000).

Escherichia coli can survive on gowns of bed linenas well as mattress surfaces (Blom *et al*., 2000). One study showed that VRE could live for up to 58 days on countertops (Bonilla *et al*., 2006). Contaminated mattress and mattress surfaces have unique microbial problems and their control is a complex task. The microbiological integrity of bed linenhas been the object of numerous studies ranging from bacterial loading of bed linento the evaluation of the barrier properties of environments (Fekety *et al*., 2001). The lessons learned from the historical use of sterilants and disinfectants are valuable today. The daily press has created a public frenzy by headlining even the most minor encounters with infectious diseases, resistant organisms, *E. coli* and flesh eating bacteria. All of this attention has resulted in heightened public concern about cross contamination issues and infection control in general (Ray *et al*., 2002). This increased public awareness has sent antibacterial and antimicrobial consumer product sales soaring. It is

also leading to extensive interest in the use of antimicrobial surfaces in a care facility environment. The desired performance of an antimicrobial treated surface is to significantly reduce levels of bacterial and fungal contamination, when compared to a similar untreated surface. Controlling and/or killing the microorganisms commonly associated with infections is a key component to maintaining an aseptic surface. Primary considerations regarding the selection of an antimicrobial are: its safety to the building occupants, that the antimicrobial activity remains unaffected by common cleaning procedures, and that the antimicrobial is not susceptible to inductive or mutative adaptation. Those surfaces that are handled by the staff, such as blankets, should also be expected to retain all of the original handling and appearance characteristics (Williams and Smith, 2006).

Infections acquired by human may have resulted from contact with a carrier of the pathogen directly or indirectly through bed linen. Improper/unhygienic ventilation system can continually be a source of nosocomial infection (Alyife and Al-Ghamdi*,* 2009). Sneezing has been described as the most vigorous mechanisms of generating millions of air bone microbial infections into the environment (Pasquaria and Smith, 2000). Droplets can contaminate mattress surfaces, smaller ones are rapidly evaporated into a non-volatile form where they remain suspended in the air thereby serving as a source of infection when inhaled by occupants of the hospital beddings. Hospital staffs may contaminate mattressby handling them. Acquired infections are an important cause of morbidity and mortality in hospitals in both the developing and developed worlds. The rate of this infection varies from 5-10% in the developed countries to 25% or more in developing countries. These infections are mostly caused by microorganisms or surfaces contaminated by the microorganisms or air currents and dust containing microbial infections nuclei (Odimayo and Oluyege,2008). Bacteria that can be found on bed lineninclude; *Mycobacterium tuberculosis*,

Bacillus anthracis, *Bordetella pertusis* which cause pulmonary tuberculosis, pulmonary anthrax and whooping cough respectively (Sadharsanam and Smith*,* 2008). Infections can cause severe pneumonia, infections of the urinary tract, bloodstream and other parts of the body. The microorganisms implicated can enter the body through wounds, catheters as well as by inhalation (Prescott *etal*., 1999). In the tropics, researchers have identified microorganisms such as: *Staphylococcus aureus, Escherichia, coli, Candida* spp., *Streptococcus* spp.*, Klebsiella*, *Penicilliuim, Aspergillus* and *Bacillus* spp are some of the most commonly isolated microorganisms from hospital environments (Ekhaise *et al*., 2010). Moulds are particularly important as a source of textile contaminants because of their ability to produce harmful spores and mycotoxins (Prigane Smith, 2004; Odimayo and Oluyege, 2008).

There can be failures of high level disinfection, but in general these processes are something that institutions monitor closely. In general, high level disinfection has come under more scrutiny and has more stringent process control than cleaning and low level disinfection (Fekety and Smith, 2001).

The ability of bacteria to adhere to textile compromises the hygiene of the environment. Surface physicochemical properties of the bacterial cell as well as of the materials such as hydrophobicity and roughness, are determinant during the initial attachment phase. Bacterial adhesion is also affected by the nutrient availability in the surrounding medium and the growth stage of the bacterial cells themselves and by the pH and temperature of the medium, cell structures including EPS (Extracellular Polymeric Substances) and flagella and ionic concentration (Guoxiang and Zhang, 2009).

2.3.1 *Escherichia coli*

Escherichia coli is a Gram negative rod-shaped, non-sporulating bacterium that is commonly found in the gut of humans and other warm-blooded organisms and forms part of the micro flora of these organisms. It is a facultative anaerobe which is specifically known to be found in the lower intestines. Most strains of *E. coli* generally are motile with peritrichous flagella. Most *E. coli*strains are harmless, but some, such as serotype O157:H7 can cause serious food poisoning in humans (Vogt and Dippold, 2005).Some harmful strains known are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enterotoxigenic *E. coli* (ETEC) which are classified based on their virulence, cause symptoms ranging from mild afebrile diarrhea to a severe cholera-like syndrome of watery stools without blood or mucus, bloody diarrhea, severe anaemia, and in severe cases, kidney failure. People have different ways of responding to *E. coli* infections; some people do not notice any infections and may spread it to others without knowing, while most people will also develop symptoms early enough. Strains found in the normal flora of the gut constituting the harmless forms, benefit their hosts by producing vitamin K2, or by preventing the establishment of pathogenic bacteria within the intestine (Hudault *et al.,* 2001). However, E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them ideal indicator organisms of fecal contamination of environmental samples.

The presence of *Escherichia coli*in bed linens and mattresses is a growing public health concern due to its potential to cause infections and diseases. *E. coli*, a common bacterium found in the human gut, can become pathogenic when it contaminates environments such as bedding. Recent studies have highlighted the widespread presence of E. coli in household and institutional bedding. For example, a study by Odonkor and Addo (2018) found that 35% of bed linens sampled from hospitals in Ghana tested positive for E. coli, with higher contamination rates in

linens used by patients with gastrointestinal infections. Similarly, Smith *et al.* (2020) reported that 25% of mattresses in long-term care facilities in the United States harbored E. coli, often due to inadequate cleaning practices.In domestic settings**,** Khan *et al*. (2021) found that bed linens washed at low temperatures (below 40°C) were more likely to be contaminated with E. coli, as the bacteria can survive and multiply in such conditions. These findings underscore the importance of proper hygiene practices in both household and institutional environments.

2.3.2 Enterococcus

Giraffa, (2003) and Foulquié *et al*. (2006) describe Enterococci as coccus-shaped, Gram-positive, facultative anaerobic, oxidase-negative, non-endospore forming, catalase negative bacteria that occurs in chains, pairs or singly. Enterococci are known to tolerate a wide range of environmental conditions. They inhabit the alimentary tract of humans, and are also isolated from environmental and animal sources (Fisher, 2009).The genus Enterococcus consists of 41 species of which *Enterococcus faecalis* and *Enterococcus faecium* are commonly isolated from faeces as they are inhibitors of the gastrointestinal tract in humans and animals. Enterococci are opportunistic pathogens known to cause clinical human infections such as endocarditis, meningitis and bacteremia (Ryan and Ray, 2004). They are also able to form biofilms in the prostate gland, thus their eradication becomes difficult. Enterococci enter food from other primary habitats such as faeces, soil, plants and water. However, Enterococci have a history of being safe to use in food fermentations (Cortés *et al*., 2006). Baron *et al*. (1994) give sulphonamide and amoxicillin as antibiotics that Enterococci are sensitive to, thus making them capable to be used in the treatment of urinary tract infections. Ampicillin and penicillin are also antimicrobial agents available for the treatment of Enterococci infections, which work by

suppressing their (Enterococci infections) growth. Hand hygiene however, is one way of controlling Enterococci infections.

Enterococcus species, particularly *Enterococcus faecalis* and *Enterococcus faecium*, are common contaminants in bed linens and mattresses. These bacteria are known for their resistance to antibiotics and ability to survive in harsh environments. A study by Odonkor and Addo (2018) found that 20% of hospital bed linens in Ghana tested positive for *Enterococcus species*, with higher contamination rates in intensive care units (ICUs)

2.3.3*Staphylococcus* species

Staphylococcus is a genus of Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters (Reynolds *et al*., 2004).The *Staphylococcus* genus includes at least 40 species. Of these, nine have two subspecies, one has three subspecies, and one has four subspecies (Harris *et al*., 2002). Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora (Madigan and Martinko, 2005). *Staphylococcus* species can be differentiated from other aerobic and facultative anaerobic, Gram-positive cocci by several simple tests. *Staphylococcus* species are facultative anaerobes (capable of growth both aerobically and anaerobically). All species grow in the presence of bile salts. All species were once thought to be coagulase-positive, but this has since been disproven (Reynolds *et al*., 2004). Growth can also occur in a 6.5% NaCl solution. On Baird Parker medium, *Staphylococcus* species grow fermentatively, except for *S. saprophyticus*, which grows oxidatively.

A study conducted in Saudi Arabia on domestic environment and surfaces found *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* contaminated floor, cabinet and door handles (Bahannan and Abdel-Salam, 2002; Dufrenne *et al*., 2001; Regnath *et al*., 2004). *Staphylococcus*

species are resistant to bacitracin (0.04 U disc: resistance = <10mm zone of inhibition) and susceptible to furazolidone (100µg disc: resistance = <15mm zone of inhibition). Further biochemical testing is needed to identify to the species level. When these bacteria divide, they do so along two axes, so form clumps of bacteria. *Staphylococcus* can cause a wide variety of diseases in humans and animals through either toxin production or penetration (Hennekinne *et al.,* 2012).*Staphylococcus species*, including *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), are frequently found in bed linens and mattresses. These bacteria are a major cause of skin and soft tissue infections. Smith et al. (2020) reported that 30% of mattresses in long-term care facilities in the United States were contaminated with *Staphylococcus species*.

2.3.4*Pseudomonas* species

Pseudomonas is a genus of Gram-negative, rod-shaped, non-spore forming, catalase-positive, oxidase-positive, motile having one or more flagella, aerobic Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species (Meyer *et al*., 2002). The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches (Madigan and Martinko, 2005). *Pseudomonas* species also typically give a negative result to the indole test, does not ferment glucose, betahemolytic (on blood agar), methyl red negative, Voges–Proskauer test negative, and citrate positive.

Out door environment are potential places for harboring and spreading pathogenic bacteria including *Pseudomonas aeruginosa*, *K. pneumoniae*, *Bacillus* spp. and *Staphylococcus epidermidis*. According to Kusmaningrum *et al*. (2002) and Tumwine *et al*. (2003), these

pathogens survive on surfaces for hours or days, depending on the species. They also stated that wiping of surfaces (physiccal removal) tends to transfer and spread microorganisms from one surface to the other (Ojima *et al*., 2002). Bacteria are readily spread from cloths during wiping (Cogan *et al*., 2002; Ojima *et al*., 2002; Gorman *et al*., 2002). *Pseudomonas* spp., an opportunistic pathogen causes UTI, respiratory tract infection, dermatitis, soft tissue infection and joint infection, gastrointestinal infections and a variety of systemic infections. *Pseudomonas* spp. can also be found in household drains of showers and gate pass (Regnath *et al*., 2004). Its prediction to moist environment makes it more possible to exist in surfaces and dust beans. Once infection with pseudomonas is established; it is hard to control since this organism is frequently resistant to many commonly used antibiotics (Qarah, 2006; Humphrey *et al*., 2001).

Most *Pseudomonas* spp are naturally resistant to penicillin and the majority of related beta lactam antibiotics, but a number are sensitive topiperacillin, imipenem, ticarcillin, or ciprofloxacin (Reynolds *et al*., 2004). Aminoglycosides such as tobramycin, gentamicin and amikacin are other choices for therapy. This ability to thrive in harsh conditions is a result of their hardy cell walls that contain porins. Their resistance to most antibiotics is attributed to efflux pumps, which pump out some antibiotics before they are able to act.

Infectious species include *P. aeruginosa*, *P. oryzihabitans*, and *P. plecoglossicida*. This pathogenesis may in part be due to the proteins secreted by *P. aeruginosa*. The bacterium possesses a wide range of secretion systems, which export numerous proteins relevant to their pathogenesis (Hardie, 2009).*Pseudomonas* species, particularly *Pseudomonas aeruginosa*, are opportunistic pathogens commonly found in moist environments, including bedding. A study by Khan *et al*. (2021) found that 15% of bed linens in households with poor hygiene practices tested positive for *Pseudomonas species*.

2.3.5*Bacillus* species

Bacillus is a genus of gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic). They will test positive for the enzyme catalase when there has been oxygen used or present. Ubiquitous in nature, *Bacillus* includes both free-living (non-parasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of the Firmicutes (Madigan and Martinko, 2005). *Bacillus* species are almost ubiquitous in nature, e.g. in, soil, but also occur in extreme environments such as high pH (*B. alcalophilus*), high temperature (*B. thermophilus*), or high salt (*B. halodurans*). Two *Bacillus* species are considered medically significant: *B. anthracis*, which causes anthrax, and *B. cereus*, which causes food poisoning similar to that caused by *Staphylococcus* (Reynolds *et al*., 2004). Colonies are usually large, spreading, and irregularly shaped. Under the microscope, the *Bacillus* cells appear as rods, and a substantial portion of the cells usually contain oval endospores at one end, making it bulge.

Bacterial contamination in surfaces spread out through air (Alwakeel, 2007). *Bacillus subtilis* is widely distributed and ubiquitous throughout the environments, particularly in soil and air. It has been shown a capacity to grow over a wide range of temperatures and can inhabit the kitchen environments (Iurlina *et al*., 2006). Tesfaye and Ketema (2016) reported that gate pass are among the possible sources of contaminants in an establishments. They reported that the isolated genera were dominated by *Pseudomonas* spp., (16.9%), *Bacillus* spp., (11.1%), *Micrococcus* spp.,

(10.6%), *Streptococcus* spp., (7.8%) and *Lactobacillus* spp. (6%). The high microbial counts (aerobic mesophilic bacteria, coliforms, Enterobacteriaceae, and yeast and molds) reveal the existence of poor kitchen sponge sanitization practice (Tesfaye and Ketema, 2016).

Studies conducted to evaluate microbial safety of public environment showed that bacterial profiles of chairs, windows, gates, tables and walls surfaces were significantly contaminated by bacteria (Speirs *et al*., 2005). Several other bacterial infections associated with contaminated outdoor environment are *Listeria, Campylobacter Bacillus cereus, Staphylococcus aureus,*and *Escherichia coli* (Dufrenne *et al.,* 2001). *Bacillus species*, including *Bacillus cereus*, are spore-forming bacteria that can survive in harsh conditions, making them persistent contaminants in bedding. Odonkor and Addo (2018) reported that 10% of hospital bed linens in Ghana were contaminated with *Bacillus species*

2.3.6*Klebsiella* species

Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule (Hemalata and Virupakshaiah, 2016). *Klebsiella* species are found everywhere in nature. This is thought to be due to distinct subline ages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects, animals, and humans (Brisse, 2006; Lagier *et al*., 2015).

Klebsiella species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. *Klebsiella* species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens (Vos and Didelot, 2009). *Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea,

and soft tissue infections (Lagier *et al*., 2015). *Klebsiella* species have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies (Sieper *et al*., 20012). The majority of human *Klebsiella* infections are caused by *K. pneumoniae*, followed by *K. oxytoca*. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer (Vos and Didelot, 2009), and the most common cause of nosocomial respiratory tract and premature intensive care infections, and the second-most frequent cause of Gram-negative bacteraemia and urinary tract infections. This antimicrobial resistance is thought to be attributable mainly to multidrug efflux pumps (Ogwa *et al*., 2005). They have the ability to colonize the bathroom, including air, waste baskets, showers, and various surfaces, as well as the skin of users and cleaners through which they spread to other indoor environments (Jadhav *et al*., 20012).*Klebsiella* species, particularly *Klebsiella pneumoniae*, are known for their role in healthcare-associated infections (HAIs). A study by Smith *et al*. (2020) found that 25% of mattresses in long-term care facilities were contaminated with *Klebsiella species*.

2.4 Transmission of Diseases through Bed linen/Mattress

Clothing and household linen (sheets, pillows and towels etc) have the potential to act as vehicles for spread of infection in home and everyday life settings. This can occur where family members, or others, share bed linen or share towels (not only in the home but elsewhere e.g. in sports changing rooms). Clothes have the potential, just as any other hand contact site, to be a component in the chain of infection transmission during normal daily activities. There are also additional points where clothing can spread infection. The first is where contaminated items are handled before and during laundering. Secondly, if the laundry process fails to eliminate contamination, this can then be spread to other items in the laundry load. If laundry is left damp,

this encourages microbial survival and there is the chance for growth of residual micro-organisms, such that clothes can then become a source of microbes.

Infectious agents that have the potential for spread via bed clothing and mattress include enteric bacteria such as *Salmonella, Shigella, Campylobacter, E. coli* (including *E. coli* O157) and *C. difficile* and respiratory and enteric viral strains such as norovirus, rotavirus, adenovirus and astrovirus. It also includes respiratory (cold and flu) viruses such as rhinovirus, influenza virus, respiratory syncytial virus etc. *S. aureus* (including MRSA), yeasts (such as *Candida albicans*) together with dermatophyte fungal strains such as and viral strains such as herpes (Graham and Smith, 2008; Abdulrahman and Faiza, 2017).

2.4.1 Rashes

A rash is a change of the human skin which affects its color, appearance, or texture. A rash may be localized in one part of the body, or affect all the skin. Rashes may cause the skin to change color, itch, become warm, bumpy, chapped, dry, cracked or blistered, swell, and may be painful. The causes, and therefore treatments for rashes, vary widely (Wright *et al*., 2012). Diagnosis must take into account such things as the appearance of the rash, other symptoms, what the patient may have been exposed to, occupation, and occurrence in family members. The diagnosis may confirm any number of conditions. The presence of a rash may aid diagnosis; associated signs and symptoms are diagnostic of certain diseases. For example, the rash in measles is an erythematous, morbilliform, maculopapular rash that begins a few days after the fever starts. It classically starts at the head, and spreads downwards (Shapiro, 2014).

Since clothing is in close contact with the skin most of the day, it’s no surprise that mattress clothing can cause skin problems Any kind of fiber can bring on a rash, but an individual is more likely to get textile dermatitis from clothes made with synthetics such as polyester, rayon, nylon,

spandex, or rubber. Formaldehyde resins used to make clothing wrinkle-free or dirt-repellent can cause problems. So dyes, glues, and chemicals used on bed linen are capable of causing skin rash (Boyd *et al*., 2007).An allergic person to nickel might get a red, itchy reaction when hard textile bed linentouches their skin.

Scabies sometimes also can be spread by contact with items such as clothing, bedding, or towels that have been used by a person with scabies, but such spread is very uncommon unless the infested person has crusted scabies

Clothing can be a cause of occupational dermatitis. The source of dermatitis can be the fabric itself, chemical additives used in processing the fabric and hardware and fasteners. The physical or occlusive effect of clothing can result in dermatitis. Contaminated clothing from workplace chemicals, friction from clothing rubbing the skin, or heat retention from perspiration-soaked clothing in hot working environments can cause distinctive dermatologic conditions. Textile fiber can be natural, synthetic or a combination of the two materials. Natural fiber is proteinaceous such as silk or wool or made from cellulose plant material such as cotton or linen. Man-made fibers such as rayon and acetate are cellulose polymers. Synthetic polymers include nylon, polyester, fiberglass, rubber and spandex (Agbulu *et al*., 2015). All fibers can cause irritant and allergic contact dermatitis although allergic contact dermatitis is rare. For instance, people with atopic dermatitis may find some fabrics such as wool irritating their skin lesions but it is not an allergy. Both irritant and allergic contact dermatitis can have the same morphology and pattern of lesions and may require patch testing to distinguish the difference between the two conditions. The distribution of the skin reaction is usually where the garments fit snugly and is worse in areas of friction and perspiration (Pratt and Taraska, 2000). Allergic or irritant reactions to clothing is more often a result of the rubber materials, formaldehyde finishing resins, chemical

additives, dyes, glues and tanning agents used in processing the fabric or clothing or metallic fasteners. Elastic in clothing material such as brassieres and the waistband in underwear or pants can cause contact dermatitis where there is contact of the clothing item with the skin. The areas involved are subjected to friction and perspiration, which contributes to the uncomfortable skin lesions. Another common example is dermatitis on the hands caused by use of protective latex gloves. The reaction can be an irritant contact dermatitis from occlusion of the skin by the gloves and constant exposure to the irritant effects of hand washing or the less common allergic reaction to the chemicals used in processing latex (rubber additives, anti-oxidants, preservatives, etc.) or the latex protein. The health care industry where frequent handwashing and protective glove use is prevalent has significant problems with occupational irritant and allergic contact dermatitis (Bloomfield*et al*., 2011). Textile formaldehyde resins are used in materials such as cotton or cotton/polyester blend fabrics to make the fabric wrinkle-resistant. There are nine formaldehyde resins in current use in the United States and most do not release a significant amount of formaldehyde as older formulations in the past. An allergic reaction may actually be due to the resin and not the released formaldehyde (Olajubu *et al*., 2017). Allergic reactions to the dyes used in fabrics are more common than a reaction to the fabric material that has been dyed. The specific type of fabric to be dyed determines the type of dye used. Most reported allergic reactions have been to dispersal dyes with azo and anthraquinone structures. These dyes are loosely held on the fabric structure and easily rubbed off on the skin (Soni and Sheretz, 2006)

Contaminated clothing can result in skin rashes. Clothing that is contaminated with oils, greases, coal tar, pitch or creosote can cause acne and folliculitis from occlusion, heat and friction. This problem is common in auto mechanics, roofers, asphalt paving workers and workers in the oil industry and coal tar plants (Fowler, 1999). Pesticide residues that have soaked clothing or

footwear can result in severe dermatitis (Ertek *et al.,* 2002). Solvent-soaked clothing can cause severe chemical burns. Hands contaminated with chemicals before putting on occlusive, protective gloves can also result in severe irritant dermatitis. Urushiol, an oil in poison oak and ivy plants, can leave a residue on clothing and shoes. If the oil is not removed from the clothing by laundering or washing the shoes with soap and water, allergic contact dermatitis can result from handling or wearing the clothing and footwear (Muthaini *et al.,* 2010). Fine metallic dust particles such as antimony trioxide and arsenic trioxide that are capable of imbedding into the clothing fabric can result in irritant skin reactions (Burrows and Charbonneau, 2000). When there is sweat combined with the metallic dust particles, pruritic, erythematous, papular lesions develop where the clothing is in contact with the skin. Workers at smelting furnaces where there is high heat, dust and using heavy protective clothing are susceptible to these irritant rashes (Kanerva, 1999)

2.8 Sources of Contamination on Clothing and Household Linen

Within the home, the primary sources of contamination on bed linenare from the users own body flora, from handling of contaminated cloths, touching contaminated surfaces and from contact with other people or household pets. Whereas organisms shed via skin scales or via faeces will mainly contaminate underclothing in contact with the skin, contamination from e.g. nasal secretions or during sleep or from nursing care of infected family members is more likely on outer mattresses and bed linen. The potential for spread of pathogens to mattresses and bed linenfrom infected sources in relatively high numbers (Agbulu*et al.*, 2015). Data presented in this report show that an infected person can shed large number of enteric pathogens on mattresses and bed linen. Awumah (2014) estimate that, of the 100 to 500 g of faeces excreted

per day by the average American, approximately 0.1 g of residual faecal material remains on the clothing of any person. Up to 10^7 infectious influenza particles per ml may be found in nasal secretions on mattresses. People who carry *S. aureus* can shed the organism in large numbers during normal daily activities, most usually associated with skin scales. It is estimated that around 106 skin squames containing viable organisms are shed daily from normal skin (Abdulrahman and Faiza, 2017). Awe and Abuh, (2016) reported that clothing act as a barrier to dispersal, from which it must be concluded that these organisms are retained on the inner surfaces of underclothing. It is possible that transmission of contamination onto clothing and household linen from contaminated sources occurs not by direct contact, but via the hands, but apart from 2 studies using laboratory models which indicate the potential for this to occur, no useful studies of the risk of transfer via hands to clothing were identified.

2.5 Occurrence of Pathogens on Clothing and Household Linen

The potential for infection transmission via clothing and household linen is shown by a range of field studies carried out to assess microbial contamination on clothing during daily wear and household linen during use. The majority of these studies have been carried out in hospital settings in situations where there are patients known to be infected with organisms such as *S. aureus* (including MRSA), *Clostridium difficile*, etc (Takashima *et al*., 2004). These studies show that in this situation, the causative pathogens are quite frequently isolated from clothing (either that of the patient or of the medical staff caring for them) and bed linen. It must be concluded however that the same potential for infection transmission must occur in the home where a family member is carrying and shedding pathogenic organisms. There are also a number

of reports where clothing and linen in hospitals, and in the general community, were sampled at random. These studies show that the most common isolates are species such as staphylococci, micrococci, corynebacteria etc which are part of the normal skin flora. Gram negative pathogens are also reported, but less frequently (probably because they need a moist environment for survival), although a number of studies show that spp. such as *Salmonella*, *P. aeruginosa* are sometimes found on clothing and household linen, although probably in small numbers (Neely and Marley, 2000; Loren and Smith, 2004; Graham and Smith, 2008).

2.6 Controlling Microbial Contamination of Bed linen

Since the organisms use the environment as a reservoir, it is imperative that adherence to hand hygiene protocols be followed. The use of universal precautions, especially gloves and gowns, is an important tool in the prevention of infection, however, gowns and gloves can become contaminated as well so workers who work in public setting must be prudent in their disposal. Effective disinfection of the mattresses, bed linenand environment must take place. Some strains of bacteria can be more virulent and resistant to normal cleaning methods. Cleaning products containing chlorine appear to be most effective (Gould and Freeman, 2003).

Since it's known that surfaces are contaminated, it is essential to promote hand hygiene immediately before and after using mattresses and bed linen. Workers in public settings should remember to protect themselves after tasks that involve gross soiling, contact with faeces. Many industries/companies and commercial settings have put equipment cleaning wipes in their work places to make spot cleaning of equipment and surfaces easy. Frequent feedback and illustrations of the places that pathogens are found can help raise awareness, and convenient location of

cleaning supplies along with the expectation that all staff members are responsible for keeping a clean environment can help improve cleanliness (Dumford*et al*., 2009).

Besides proper hand hygiene, good contact control is essential for preventing the transmission of organisms. Microorganisms evade us because they are invisible, and many of the currently problematic microbes (Staphylococci, Enterococci, *Acinetobacter, Clostridium*) survive longer than some other organisms on what appear to be completely clean surfaces (NeelyMaley, 2000; Kim *et al*., 2001). Because microorganisms cannot be seen with the naked eyes, it is easy to forget that they are there. It is advisable to train personnel in terms of good contact control and good work habits. To prevent transmission of infection in public settings, people should insure that they rigorously adhere to recommendations for hand hygiene and that the environment is clean, clutter free.

Factors that may be pertinent to the processing environment may include, but are not limited to:

Open doors: Doors should remain closed as much as possible. This will prevent entrance of excess outside air and fluctuation of ambient temperature and humidity. People carry a number of microorganisms on their person. The movement of personnel is associated with higher contamination levels (Rahkio and Korkeala, 2007).

Fan operation: Fan operation causes increased air flow. Air flow will affect air contamination and, in the absence of walls, corners, or other means of separation, microorganisms may be moved by the air flow into clean areas (Rahkio and Korkeala, 2007). Usually, fan operation is triggered by a rise in temperature. Research (Cundith *et al*., 2002) has indicated that the use of air filtration units installed into the fan system can reduce the amount of airborne bacteria.

Ambient humidity: Extrinsic factors such as humidity and temperature affect the growth of microorganisms (Helm-Archer *et al*., 2004). Increased humidity increases the likelihood of

survival of microorganisms in the air. Humidity can increase in the processing facility from the entrance of outside or warm air into a much cooler environment and vise versa. Both of which can be controlled in the processing facility by keeping doors closed and limiting employee traffic.

Ambient temperature: In general, microbes usually grow best at temperatures above that typical of refrigeration (~45°F). Therefore maintaining cool temperatures is very important. Temperature within the facility can be maintained by the same means as humidity. Likewise, increase in ambient temperature also heightens the survival of microbes in the air.

Research (Helm-Archer *et al*., 2004) has shown that these factors account for most of the variation in microbial contamination of product by means of airborne microbes. if these factors are not controlled, it can lead to other factors affecting microbial survival in air. Such factors include: time of year, time of day, outside temperature, and outside humidity. Processors can prevent the emergence of other factors which may or may not be controllable by controlling known factors. Practices as simple as closing doors and limiting employee traffic are effective means to ensure limited air contamination within the facility.

2.7 Cleaning of Mattresses and Bed linen

Mattresses and bed linenmay transfer germs. If these mattresses and bed linenare touched, they can get onto hands and transferred by contact to another person. Wash hands with water and detergent before touching mattresses and bed linen, cobwebs should be removed from walls and proper ventilation should be ensured in environments (Shawk and Tarek, 2018).

Inanimate objects should be cleaned regularly with a limescale remover. Bleach should not be used as it can stop good bacteria breaking down solids. Surfaces that have come into contact with solid and liquid waste should be cleaned. Waste from the surface using paper or a disposable cloth should also be cleaned. Surfaces should be cleaned with hot water and detergent using a

fresh cloth or paper towel to get rid of dirt, then put household bleach onto the surface using a fresh cloth or paper towel to kill any germs. Disposable gloves should be worn when come into contact with waste and wash hands after gloves have been taken off.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in University of Benin (UNIBEN). UNIBEN is a public university located in Benin City, Edo State, Nigeria. It is among the universities owned by the Federal Government of Nigeria and was founded in 1970. The school currently has two campuses with fifteen faculties including a central library called the John Harris Library. The building in UNIBEN are sparsely built, they are not close to each other.

3.2 Administration of Questionnaires

In the survey some predisposing factors were observed in regards to the proximity of the restroom to the bedrooms, level of ventilation, population of student per room and the architectural design of the hostel.A well-structured questionnaire bothering on age, sex, level of student, method of bed-linen washing and method of mattress sanitation was administered to

students in postgraduate hostels for this study. The questionnaire was numbered against the specimen container used to obtain the mattress and bed linen swab sample.

3.3 Sample Collection

Ten rooms were selected in different postgraduate hostels in Ugbowo Campus, one sampleeach were taken from the upper part of bed linen and mattressfrom each room in Keystone, Hall 7 and Akinbola hostel. Sterile swab sticks moistened in normal saline were used to pick samples from the mattress and bed linen. The swab sticks were immediately transported to the laboratory for microbial analyses.

3.4 Enumeration of Bacteria

Enumeration of the microorganism was carried out in accordance to the methods described by Cheesbrough (2006). Each swab sample were immersed in peptone water in a test tube to resuscitate the organisms. Serial dilution of the sample was aseptically carried out by pipetting 1 ml from the already inoculated peptone water into a test tube (10^{-1}) containing 9 mL of sterile distilled water. The process was repeated until a dilution of 10^{-3} is obtained and an aliquot of 1 ml from the 10^{-3} dilution was dispensed onto petri plates. Cooled already prepared Nutrient agar was added and allowed to solidify. The agar plates was incubated by inversion at 37^0C for 24 hr. Upon incubation, the plates were examined for growth; developed colonies were counted while distinct colonies were sub cultured. The mean counts were recorded and expressed in colony forming units per centimeter square of the sample (cfu/cm^2).

3.5 Isolation of Pure Culture

The discreet colonies on nutrient agar plates were streaked on freshly prepared agar using the streak plate technique, and further Gram stained (Cheesbrough, 2010).

3.6 Characterization of Bacterial Isolates

The heterotrophic bacterial isolates were identified and characterized on the basis of their cultural, morphological and biochemical characteristics (Sharma *et al.*, 2009).

3.7 Virulence Factors

The bacterial isolates were subjected to various tests to determine the presence of virulence factors.

3.7.1 Haemolytic activity

The production of hemolysin was determined using 5 % sheep blood agar. The bacteria were streaked onto blood agar plates and incubated for 24 hr at 37 ºC. The presence of greenish coloration halos around the colonies indicates production of α-hemolysin, while complete clear zone indicates production of β-hemolysin (Akinjogunla and Enabulele, 2010).

3.7.2 Gelatinase activity

Gelatin hydrolysis test determines the ability of the bacterium to break down gelatin protein. The test tube containing the nutrient gelatin medium was stabbed five times (half an inch) to inoculate a heavy inoculum of test bacterial isolate (18 to 24 hours). For 48 hours, incubate the inoculated test tube at 30 °C with a test tube containing uninoculated medium. Every day, the test tubes were put in the fridge for 30 minutes, or until the control test tube solidified. The test tubes were gently tilted to monitor the test organism's liquefaction after 30 minutes of cooling. Even after refrigeration at 4°C, partial or entire liquefaction of the inoculated test tube indicated a positive result; complete solidification of the inoculated test tube after refrigeration was indicative of a negative result (Alnahdi, 2012).

3.7.3 Deoxyribonuclease (DNase)

The method described by Pimenta *et al*. (2008) was used. DNase agar (containingtoluidine blue and methyl green) plates were inoculated by spotting a loopful of overnight pure cultures on to

the surface of the tryptone soy broth, followed by aerobic incubation at 37°C for 48h. The plates were flooded with 0.1 % 1 N HCl. The development of a red color or a zone of clearing indicated a positive result (Tille and Forbes, 2014).

3.7.4 Coagulase test

A dilution of the plasma at a ratio of 1 in 6 in saline (0.85% NaCl) was prepared, and 1 mL of the diluted plasma was put into test tubes. The isolated colonies of the test organisms were emulsified in 1 ml of diluted rabbit plasma to create a milky suspension. The test tube was incubated at 30 °C in free air for 4 h. The negative tubes were left at room temperature overnight for further examination. Any size fibrin clot was classified as positive, and no clot as negative (Tille and Forbes, 2014).

3.8 Antibiotic Susceptibility of Virulent Bacteria Isolates

The agar disc diffusion technique was used as described by the Clinical Laboratory Standards Institute (2017). Colonies of each pure isolate was suspended in sterile water and matched with Mcfarland standard (0.5). This was streaked on sterile Muller Hinton agar plates aseptically using sterile inoculating wire loop. The appropriate multi-discs containing minimum inhibitory concentrations (MIC) of Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxacillin (30 µg), Rocphin (25 µg), Ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg) and Erythromycin (10 µg) were aseptically placed (impregnated) firmly onto the surface of the dried plates using sterile forceps. The plates were left at 37°C for 1 hr to allow diffusion of the different antibiotics from the disc into the medium. The plates were incubated at 37°C for 24 hr. Interpretation of results was done measuring the length of the zone of inhibition as diameter. The interpretation of the measurements as sensitive or resistant was done by comparing it to the manufacturer’s standard zone interpretative table.

3.9 Molecular Identification of Virulent Bacterial Isolates

3.9.1 DNA extraction

Virulent bacterial isolates were resuspended in 200µL of sterile deionized water, boiled for 15 min and centrifuged for 5 min at 12 000 ×g. The supernatant was stored at -20°C for further use as genomic DNA template for PCR. DNA quality was first confirmed by agarose gel electrophoresis with 5µL of each DNA preparation on a 0.7% Tris-Acetate-EDTA agarose gel using 0.5µg/ml ethidium bromide solution and DNA was visualized with ultraviolet transilluminator. The DNA concentration was estimated using a spectrophotometer by diluting it in distilled sterile water (1:100) and reading the absorbance at 260 nm (A260) and 280 nm (A280). The concentration of DNA was estimated by the A260 considering that 1 absorbance unit equals 50 µg/ml of double stranded DNA. The quality was evaluated by the A260/A280 ratio (Sambrook *et al*., 1989).

3.9.2 Polymerase Chain Reaction

PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60 s and 72°C for 1 minute 30 s; and a final termination at 72°C for 10 mins and chill at 4°C Agarose gel

3.9.3 Integrity of amplified DNA

The Mb gene fragment was checked on a 1% Agarose gel to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µL of 0.5 g/mL Ethidium bromide. A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify

for 20 mins to form the wells. The Tris-acetate-EDTA (1XTAE) buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 μL) of 10X blue gel loading dye was added to 4μL of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 mins, visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products was estimated by comparison with the mobility of a 100 base pair (bp) molecular weight ladder that was ran alongside experimental samples in the gel(Ahmed *et al.,* 2013).

3.9.4 Purification of Amplified Product

After PCR, the amplification was confirmed by agarose gel electrophoreses. The gel was prepared with 1× Tris-Acetate EDTA-Buffer. To every 100 ml 1× TAE, 1 g Agarose (Sigma) was added in an Erlenmeyer flask and heated for 5 min in a microwave at 650 Watts. After cooling at approximate 50°C every 100 ml of this mixture was receive 1 μL of 10 mg ml-1 ethidiumbromide. This solution was poured into an electrophoresis tray and left in rest for 30 min until the gel had solidified. The gel was subsequently transferred to the gel electrophoresis chamber filled with 1× TAE solution. From every PCR sample obtained, 5 μL was taken and transferred into the wells of the agarose gel. In the first slot of the agarose gel the 1 kb DNA Ladder (Promega) was loaded. After transferring all samples to the gel, the electrophoresis analysis was performed for 60 min at 120 V. To visualize and analyze the DNA bands, the gel was placed on ultraviolet transilluminator(Ahmed *et al*., 2013).

3.9.5 PGPRs DNA Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of

BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 was used for all genetic analysis

3.9.6 Plasmid profiling (TENS method)

An Overnight bacterial culture of 1.5ml was poured into a microfuge and spun at top speed for 1min to pellet the cells. The supernatant was poured out, and ~150 µl of media was kept in the tube, the cells will be resuspended by vortexing, 300 µl of TENS buffer was added, the tubes was inverted 3-4 times gently for the cells to lyse completely, 150 µL of 3M NaOAc (pH 5.6) was added and the tubes was inverted 3-4 times gently, it was spun at maximum speed for 5 min to pellet the white precipitate. Clear supernatant was pipetted into a clean tube and 900 µL of 95% ethyl alcohol (ETOH)was added and the tube was inverted to mix and spun at maximum speed for 2 min to pellet DNA. The solution was poured out and 500 µL of 70% ETOH was added to wash the pellet by vortexing, it was spun again for 1 min and 70% ETOH was poured, and the DNA pellet was left to dry and the DNA was dissolved in 50 µL 10mM Tris (pH 8) (Birmboin and Doly, 1979).

3.9.7 Plasmid curing and subsequent evaluation of antibiotic susceptibility testing

The characterized bacterial isolates were inoculated into 10 ml of nutrient broth containing 100µg/mL of the mutagen (acridine orange) as described by the modified methods of Sheikh *et al*. (2003) and Yah *et al*. (2007). The mixture was incubated overnight at 37 ^{O}C for 24hr upon incubation, each mutagen-exposed culture was plated on nutrient agar medium and incubated at 37 ^{O}C for 24 hr. Colonies were randomly selected from each of the plates per isolate, for sensitivity testing upon incubation at overnight in 5 ml nutrient broth and diluted to 10^{-2} in sterile distilled water, and 0.1 ml of the diluted (10^{-2}) inoculum was seeded onto solidified nutrient agar. Thereafter, multi antibiotic discs containing different antibiotics were firmly placed onto the

surface of the agar plates using forceps. The plates were incubated at 37 ^{0}C for 24 hr and visible zones of inhibition was measured. Distances less than 14 mm was recorded as resistant (R), while distances which ranged from 14 mm to 17 mm was reported as intermediate (I). Zones of inhibition greater than 17 mm were recorded as susceptible (S) for the respective isolates (Prescott and Harley, 2005).

3.10 Data Analysis

The data generated were analyzed by one –way ANOVA (analysis of variance) using Genstat 12th edition analytical package as well as non-parametric t. test. Differences in mean were compared by Duncan's multiple range tests (Ogbeibu, 2015).

CHAPTER FOUR

RESULTS

The demographic characteristics of the respondents used for this study based on age and sex are presented in Table 4.1. Majority of the respondents were within 20-25 years (50.0%) with females being the dominant respondents (64.71%) compared to male respondents (35.29%).

Table 4.2 shows students' response rate on evaluation of pathogenic bacteria from bed linen and mattresses. The result revealed that most of the respondents sometimes (50.0%) do not have visitors frequently, majority of the rooms in the hostels had at least 2-3 occupants (63.16%), with 77.8% of the respondents having at least 2 bed linen. The result shows that majority of the respondents change their bed linen at least 2-3 times (52.63%) and also wash their bed line 2-3 times (52.63%) per weeks. Majority of the respondents indicated that they wash their bed linen with detergent and water (88.89%) in order to prevent infection (11.11%). The result also shows that most of the respondents use disinfectant on bed linen (84.21%). Most of the respondents indicated that they are the only onesthat lay on their bed (57.89%) and wait till when their bed linen is dirty before they wash it (52.63%). Student's response also shows that their bed linen is less than one year (88.89%). Majority of the respondents do not lay on their mattress without a bed linen (94.74%), do not use disinfectant on mattress (94.74%) but take their bath before

sleeping on mattress (89.47%). Majority of the respondents were aware that mattress and bad linen can be reservoir for bacterial infection (73.68%). Most of the respondents sweep the floor at least once (63.16%) per day while majority of the respondents place their mattress on a mattress stand (100%).

Table 4.1: Demographic profiles of postgraduate Students employed for the study

Variables	Percentage
Age group	
20-25	50.00
25-30	18.75
30 years and above	31.25
Gender	
Male	35.29
Female	64.71

Table 4.2: Students'response rate on pathogenic bacteria contamination of bed linen and mattresses

Question	Frequency
How often do you have visitors	
Never	11.11
Occasionally	5.56
Sometimes	50.00
Often	33.33
None of the above	0.00
Do you have occupants in room?	
Just me	15.79
2-3	63.16
4-6	21.05
7 and above	0.00
Do you have a bed linen?	
Yes	94.74
No	5.26
Number of bed linen	
1	11.11
2	77.78
3	5.56
4 and above	5.56
Number of time you change bed linen per week	
Once	36.84
2 -3 times	52.63
Can't Say	10.53
How many times do you wash your bed linen in a week?	
Once	36.84
2 -3 times	52.63
Whenever I feel like	10.53
Why do you wash bed linen?	

Prevention of Rashes	0.00
To Prevent Infection	11.11
Odour Prevention	5.56
Normal Practice	83.33
What do you wash bed linen with	
Detergent and water	88.89
Bar soap and water	5.56
Water only	5.56
Do you use disinfectant on bed linens	
Yes	15.79
No	84.21
Question	**Frequency**
Number of persons that lay on the bed	
One Person	57.89
2- 3 persons	21.05
3-4 persons	0.00
4 and above	21.05
When do you wash bed linen?	
When it is dirty	52.63
Dirty or Not, I wash at specific time	47.37
How old is your bed linen?	
Less than one year	88.89
One year	0.00
Two years	11.11
Three years and above	0.00
Do you lay on your mattress without bed linen?	
Yes	5.26
No	94.74
Do you use disinfectant on mattress?	
Yes	5.26
No	94.74
Do you take bath before sleeping on your mattress	
Yes	89.47
No	5.26
Sometimes	5.26
Awareness that mattress and bad linen can be reservoir for bacterial infection	
Yes	73.68
No	26.32
Sometimes	0.00
Number of times participants sweep the floor per day	
Once	63.16
2-3 times	36.84

None at all	0.00
Where do you place your mattress?	
Mattress stand	100.00
On the floor	0.00

Table 4.3 shows the total bacterial counts of bed linen from Postgraduate hostels. The total bacterial counts of bed linen sample 1 ranged from 16.45±1.17 x 10^3 cfu/cm^2 (Hall 7, Male) to 72.2±2.89 x 10^3 cfu/cm^2 (Hall 7, female), sample 2 ranged from 10.4±0.20 x 10^3 cfu/cm^2 (Keystone, Male) to 62.3±2.19 x 10^3 cfu/cm^2 (Akinbola, Male), bacterial counts in sample 3 ranged from 12.7±0.31 x 10^3 cfu/cm^2 (hall 7, Female) to 32.3±0.89 x 10^3 cfu/cm^2 (Akinbola, Male), sample 4 ranged from 15.2±0.31 x 10^3 cfu/cm^2 (Keystone, Female) to 30.0±0.88 x 10^3 cfu/cm^2 (Akinbola, Male), sample 5 ranged from 7.9±0.19 x 10^3 cfu/cm^2 (Keystone, male) to 53.3±2.27 x 10^3 cfu/cm^2 (Akinbola, Male), bacterial counts in sample 6 ranged from 6.66±1.66 x 10^3 cfu/cm^2 (hall 7, Female) to 82.3±3.91x 10^3 cfu/cm^2 (Akinbola, female), sample 7 ranged from 6.66±2.60 x 10^3 cfu/cm^2 (hall 7, Female) to 82.3±3.91x 10^3 cfu/cm^2 (Akinbola, female).

Sample 8 ranged from 13.33±3.48 x 10^3 cfu/cm^2 (hall 7, Male) to 30.0±1.48 1x 10^3 cfu/cm^2 (Keystone, male), sample 9 ranged from 7.75±0.89 x 10^3 cfu/cm^2 (Keystone, Male) to 48.00±6.35 x 10^3 cfu/cm^2 (Hall 7, Female), sample 10 ranged from 18.66±3.48 x 10^3 cfu/cm^2 (Akinbola, female) to 44.66±8.41 x 10^3 cfu/cm^2 (Hall 7, Female), bacterial counts in sample 11 ranged from 14.7±0.31 x 10^3 cfu/cm^2 (Keystone Female) to 48.33±6.01 x 10^3 cfu/cm^2 (Hall 7, female), sample 12 ranged from 4.00±0.60 x 10^3 cfu/cm^2 (Keystone male) to 25.7±3.41 x 10^3 cfu/cm^2 (Keystone, female), sample 13 ranged from 9.00±0.55 x 10^3 cfu/cm^2 (Akinbola Female)

to 72.0±2.86 x 10^3 cfu/cm^2 (Keystone, female), sample 14 ranged from 7.00±1.15 x 10^3 cfu/cm^2 (Akinbola male) to 44.3±1.65 x 10^3 cfu/cm^2 (Keystone, female), sample 15 ranged from 7.00±1.15 x 10^3 cfu/cm^2 (hall 7 Female) to 72.0±2.86 x 10^3 cfu/cm^2 (Keystone, female) while bacterial counts in sample 16 ranged from 7.33±1.45 x 10^3 cfu/cm^2 (hall 7 Female) to 25.5±1.41 x 10^3 cfu/cm^2 (Keystone, male). Bed linen samples 6 from Keystone female hostel had the highest bacterial count (82.1±3.91 x 10^3 cfu/cm^2) while bed linen from Keystone male hostel had the least bacterial count (4.00±0.60 x 10^3 cfu/cm^2). There were significant differences in bacterial counts of bed linen from all the postgraduate hostels ($P<0.05$).

The total bacterial counts of mattress from Postgraduate hostels are shown in Table 4.4. Bacterial counts in sample 1 ranged from 10.2±0.51 x 10^3 cfu/cm^2 (Hall 7, female) to 14.5±1.43 x 10^3 cfu/cm^2 (Akinbola, female), sample 2 ranged from 8.33±0.28 x 10^3 cfu/cm^2 (Keystone, female) to 14.6±1.70 x 10^3 cfu/cm^2 (Akinbola, female), bacterial counts in sample 3 ranged from 7.72±1.20 x 10^3 cfu/cm^2 (Keystone, male) to 14.4±1.01 x 10^3 cfu/cm^2 (Akinbola, Female), bacteriual count in sample 4 ranged from 4.29±0.31 x 10^3 cfu/cm^2 (Akinbola, Female) to 13.0±0.25 x 10^3 cfu/cm^2 (Hall 7, Male), sample 5 ranged from 7.43±1.01 x 10^3 cfu/cm^2 (Akinbola, female) to 12.5±3.51 x 10^3 cfu/cm^2 (Keystone, Male), bacterial counts in sample 6 ranged from 8.27±0.33 x 10^3 cfu/cm^2 (Keystone, Female) to 13.99±0.13 x 10^3 cfu/cm^2 (Akinbola, female).

Sample 7 ranged from 5.23±0.67 x 10^3 cfu/cm^2 (Akinbola, Female) to 10.0±1.01 x 10^3 cfu/cm^2 (Hall 7, male), sample 8 ranged from 7.72±1.20 x 10^3 cfu/cm^2 (Akinbola, Male) to 11.0±0.77 x 10^3 cfu/cm^2 (Hall 7, female), sample 9 ranged from 7.58±0.55x 10^3 cfu/cm^2 (Akinbola, Male) to 16.94±2.52 x 10^3 cfu/cm^2 (Akinbola, Female), sample 10 ranged from 10.5±1.05 x 10^3 cfu/cm^2 (Keystone, female) to 14.5±1.0 x 10^3 cfu/cm^2 (Hall 7, male), bacteria counts in sample 11 ranged from 5.71±0.22 x 10^3 cfu/cm^2 (Akinbola, male) to 14.5±0.95 x 10^3 cfu/cm^2 (Hall 7, male), sample

12 ranged from 9.09±0.00 x 10^3 cfu/cm^2 (Keystone, male) to 16.2±0.94 x 10^3 cfu/cm^2 (Akinbola, female), sample 13 ranged from 5.26±0.62 x 10^3 cfu/cm^2 (Keystone, Female) to 13.5±0.72 x 10^3 cfu/cm^2 (Hall 7, male), sample 14 ranged from 6.76±0.76 x 10^3 cfu/cm^2 (Hall 7, male) to 14.3±0.90 x 10^3 cfu/cm^2 (Keystone, female).

Sample 15 ranged from 8.00±0.23 x 10^3 cfu/cm^2 (Keystone, Female) to 14.3±0.48 x 10^3 cfu/cm^2 (Akinbola, male) while bacterial counts in sample 16 ranged from 6.84±0.27 x 10^3 cfu/cm^2 (Keystone, Female) to 14.7±0.76 x 10^3 cfu/cm^2 (Akinbola, male). Mattress samples 12 from Akinbola male hostel had the highest bacterial count (16.2±0.94 x 10^3 cfu/cm^2) while mattress from Akinbola male hostel had the least bacterial count (4.29±0.31 x 10^3 cfu/cm^2). There were significant differences in bacterial counted of bed linen from all the postgraduate hostels ($P<0.05$).

Table 4.5 shows the cultural morphological and biochemical characteristics of bacteria obtained from bed linen and mattresses. Bacterial isolated include *Escherichia coli, Enterobacter cloacae, Bacillus mycoides, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The percentage occurrence of bacterial isolates from bed linensamples is shown in Table 4.6. *S. aureus* was the highest occurring bacterial isolate (33.3%) from Keystone (male) hostel, followed by *E. coli* (28.6%) from female hostel while *E. cloacae, B. mycoides* and *P. aeruginosa* were the least occurring bacterial isolates (13.3%) from Keystone (male) hostel. The occurrence of bacterial isolates in bed linen from Hall 7 shows that *E. coli* (60%) was the most occurring bacterial isolates while *E. cloacae* (10%) was the least occurring bacterial isolates. Bacteria frequency of occurrence in bed linen from Akinbolahostel shows that *E. coli* (44%) was the highest occurring bacterial isolate while *B. mycoides* (11.1%) was least occurring bacterial

isolate. Out of the 110 bacteria isolated, *E. coli* (36.4%) was the most occurring bacterial isolates while *B. mycoides* (5.45%) was the least occurring bacterial isolate.

Table 4.3: Total bacterial counts (x10^3 cfu/cm^2) of bed linen (BB) in Postgraduate hostels used for the study

Material	Keystone		Hall 7		Akinbola	
	Male	Female	Male	Female	Male	Female
BB1	68.0±1.83^a	35.6±3.55^a	16.45±1.17^b	72.2±2.89^a	26.0±0.71^b	17.3±1.76^a
BB2	10.4±0.20^a	54.5±3.74^b	14.75±0.17^a	15.7±0.20^a	62.3±2.19^b	12.3±0.8^b
BB3	27.0±0.20^a	14.2±0.42^b	24.0±1.38^a	12.7±0.31^b	32.3±0.89^a	19.7±3.18^a
BB4	29.0±1.46^a	15.2±0.31^b	16.66±0.8^b	16.7±0.81^b	30.0±0.88^a	24.3±2.96^{ab}
BB5	7.9±0.19^a	41.5±3.62^b	9.00±5.50^a	30.0±9.03^b	53.3±2.27^b	13.3±0.88^b
BB6	15.6±0.20^a	82.1±3.91^b	8.00±1.73^a	6.66±1.66^a	76.66±4.4^b	23.0±3.79^a
BB7	7.5±0.18^a	39.3±3.59^b	10.33±2.01^a	6.66±2.60^a	24.66±0.9^b	18.0±1.73^a
BB8	30.0±1.48^b	15.7±0.32^a	13.33±3.48^a	14.33±2.60^a	24.33±3.3^b	27.3±2.03^{ab}
BB9	7.75±0.89^a	40.6±2.61^b	37.66±7.66^b	48.00±6.35^b	31.00±2.08^b	32.1±3.00^b
BB10	37.0±1.57^b	19.4±3.29^a	42.66±2.03^b	44.66±8.41^b	27.33±9.49^b	18.66±3.48^a
BB11	28.0±1.45^b	14.7±0.31^a	36.33±13.38^b	48.33±6.01^b	21.66±6.69^b	18.00±4.04^a
BB12	4.00±0.60^a	25.7±3.41^b	24.66±4.66^b	21.00±3.46^b	13.66±3.17^a	16.00±2.65^a
BB13	22.0±1.34^a	72.0±2.86^b	35.00±8.08^a	17.00±0.57^a	18.33±6.01^a	9.00±0.55^a
BB14	44.3±1.65^b	15.71±3.20^a	7.00±2.88^a	9.33±3.92^a	7.00±1.15^a	28.66±2.90^b
BB15	25.5±1.41^a	72.0±2.86^b	15.00±2.30^a	7.00±1.15^a	10.66±0.33^a	8.00±2.08^a
BB16	25.5±1.41^a	24.9±2.40^a	19.33±2.96^a	7.33±1.45^b	8.66±0.66^b	14.7±0.33^b

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript within row are not significantly different from each other (P>0.05). Mean values with different superscript within row are significantly different from each other (P<0.05).

Table 4.4: Total bacterial counts (x10^3 cfu/cm^2) of mattresses (BM) in Postgraduate hostels used for the study

Material	Keystone		Hall 7		Akinbola	
	Male	Female	Male	Female	Male	Female
BM1	13.5±0.72^a	11.3±0.34^a	12.2±0.11^a	10.2±0.51^a	11.7±0.51^a	14.5±1.43^c
BM2	8.76±0.76^a	8.33±0.28^a	11.5±0.63^a	12.6±0.20^a	14.1±0.28^a	14.6±1.70^b
BM3	7.72±1.20^a	11.4±0.52^a	9.51±0.31^a	9.98±0.24^a	9.07±0.60^a	14.4±1.01^c
BM4	7.58±0.55^a	9.41±0.52^a	12.9±0.45^b	10.7±0.66^b	13.0±0.25^a	4.29±0.31^a
BM5	12.5±3.51^a	8.54±1.23^a	11.0±4.20^a	9.58±3.51^c	10.4±0.79^a	7.43±1.01^b
BM6	8.53±0.52^a	8.27±0.33^a	9.07±0.43^a	12.0±1.00^a	13.5±0.72^c	13.99±0.13^b
BM7	9.20±0.99^a	9.55±0.05^a	10.0±1.01^a	8.50±0.55^a	8.76±0.76^a	5.23±0.67^a
BM8	10.0±0.11^a	7.90±0.01^a	10.5±0.45^a	11.0±0.77^a	7.72±1.20^a	7.91±0.30^a
BM9	12.0±0.80^a	12.0±0.00^a	12.0±1.00^a	11.0±0.55^a	7.58±0.55^a	16.94±2.52^a
BM10	11.0±0.73^a	10.5±1.05^a	14.5±1.0^a	13.5±1.05^a	12.6±0.51^a	13.6±0.85^a
BM11	9.51±0.65^a	13.0±0.88^a	14.5±0.95^a	11.5±0.95^a	5.71±0.22^a	10.03±0.24^a
BM12	9.09±0.00^a	12.0±0.66^a	11.0±0.88^a	13.0±1.05^a	16.2±0.94^a	9.71±0.55^a
BM13	8.03±0.24^a	5.26±0.62^a	13.5±0.72^a	11.9±0.73^a	8.77±0.71^a	10.53±1.57^a
BM14	9.71±0.55^a	14.3±0.90^b	6.76±0.76^a	8.23±0.67^a	8.03±0.40^a	10.39±1.22^a
BM15	10.5±1.57^a	8.00±0.23^b	9.72±1.20^a	10.9±0.90^a	14.3±0.48^a	12.5±0.28^a

BM16	8.39±1.22[a]	6.84±0.27[b]	7.58±0.55[a]	6.94±0.52[a]	12.3±0.62[a]	14.7±0.76[a]

Values are expressed as Mean ± Standard Error of triplicate experiments. Mean values with similar superscript within row are not significantly different from each other ($P>0.05$). Mean values with different superscript within row are significantly different from each other ($P<0.05$).

Table 4.5: Cultural,cellular and biochemical characteristics of bacteria obtained from bed linen and mattresses

Morphological characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Elevation	Flat	Flat	Flat	Raised	Raised
Margin	Undulate	Undulate	Coarse	Entire	Smooth
Color	Cream	Cream	milk white	Cream	Cream
Shape	Irregular	Irregular	concave	Circular	Irregular
Size	Large	Large	Large	Medium	Small
Gr. diff. agar	EMB	EMB	BCA	PCA	MSA
Colour	Green	Purple	Straw	Green	Yellow
Staining					
Gram stain	-	-	+	-	+
Cell type	Rod	Rod	Rod	Rod	Cocci
Arrangement	Disperse	Disperse	Disperse	Pair/chains	Clusters
Color	Pink	Pink	Purple	Pink	Purple
Spore staining	-	-	+	-	-
Biochemical characteristics					
KOH String Test	+	+	-	+	-
Catalase	+	+	+	+	+
Indole	+	-	-	-	-
Citrate	-	+	-	+	+
Oxidase	-	-	-	+	-
Motility	+	+	+	+	-
Urease	-	-	-	+	+
Glucose	+	+	+	-	+
Sucrose	-	+	-	-	+
Lactose	+	+	-	-	+
Mannitol	-	-	-	-	-
Gas formation	+	+	-	-	-
H_2S formation	-	-	-	-	-
TSI (Slant/Butt)	A/AG	A/AG	K/A	K/K	A/A*

reaction					
Esculin Hydrolysis	-	(+/-)	+	-	-
Probable isolates	*Escherichia coli*	*Enterobacter cloacae*	*Bacillus mycoides*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*

Table 4.6:Percentage occurrence of bacterial isolates from Bed linensamples.

	Keystone		Hall 7		Akinbola			
Isolates	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	Total	Occurrence (%)
E. coli	4(26.7)	8(28.6)	6(60.0)	12(40.0)	2(22.2)	8(44.4)	40	36.4
E. cloacae	2(13.3)	4(14.3)	1(10.0)	3(10.0)	0(0.00)	3(16.7)	13	11.8
B. mycoides	2(13.3)	0(0.00)	0(0.00)	3(10.0)	1(11.1)	0(0.00)	6	5.45
P. aeruginosa	2(13.3)	5(17.9)	0(0.00)	3(10.0)	3(33.3)	1(5.56)	14	12.7
S. aureus	5(33.3)	11(39.3)	3(30.0)	9(30.0)	3(33.3)	6(33.3)	37	33.6
Total	**15**	**28**	**10**	**30**	**9**	**18**	**110**	**100**

Table 4.7 shows the percentage occurrence of bacterial isolates from mattressessamples. *S. aureus* was the highest occurring bacterial isolate (40.9%) from Keystone (female) hostel, followed by *P. aeruginosa* (36.4%) from male hostel while *B. mycoides* (4.5%) was the least occurring bacterial isolate. The occurrence of bacterial isolates in mattresses from Hall 7 shows that *S. aureus* (50%) was the most occurring bacterial isolates while *B. mycoides* (13.6%) was the least occurring bacterial isolates. Bacteria frequency of occurrence in mattresses from Akinbolahostel shows that *S. aureus* (30.8%) was the highest occurring bacterial isolate while *P. aeruginosa* (7.7%) was least occurring bacterial isolate. Out of the 96 bacteria isolated, *S. aureus* (32.3%) was the most occurring bacterial isolate while *B. mycoides* (10.8%) was the least occurring bacterial isolate.

Table 4.8 shows the molecular identification of bacterial isolates from bed linen and mattresses from postgraduate hostels. Bacteria identified in bed linen include*Escherichia coli* and *Pseudomonas aeruginosa* while that from mattresses include *Enterobacter cloacae, Bacillus mycoides* and *Staphylococcus aureus.*

The phenotypic virulence properties of the bacterial isolates from bed linen and mattresses is shown in Table 4.9. *Bacillus mycoides* and *Pseudomonas aeruginosa* showed positive Dnase and lipase characteristics, *Staphylococcus aureus*showed positive Dnase, lipase and hemolysin characteristics, *E. coli* and *Enterobacter cloacae* exhibited positive DNase and Lipase characteristics while all bacterial isolates with the exemption of *Staphylococcus aureus* did not exhibit hemolysin characteristics.

Table 4.10 shows the antibacterial sensitivity of bacterial isolates obtained from bed linen surfaces. Gentamycin, Cefuroxime, Ciprofloxacin and Clindamycin had highest zone of inhibition against (100mm) against *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter*

cloacae, Bacillus aerius and *Staphylococcus aureus* while Metronidazole, Amoxycillin and Tetracycline had least zone of inhibition (20mm) against *E. coli, Enterobacter cloacae* and *Bacillus mycoides* respectively. *Staphylococcus aureus* had the highest multiple antibiotic resistance (0.36%), followed by *Enterobacter cloacae* (0.33%) while *E. coli* had least multiple antibiotic resistance (0.29%).

Table 4.7:Percentage occurrence of bacterial isolates from mattresssamples.

	Keystone		Hall 7		Akinbola			
Isolates	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Total**	**Occurrence (%)**
E. coli	2(18.3)	5(22.7)	2(16.7)	6(27.3)	5(38.5)	3(23.1)	23	24.7
E. cloacae	1(9.09)	2(9.1)	0(0.00)	4(18.2)	3(23.1)	3(23.1)	13	14
B. mycoides	1(9.09)	1(4.5)	3(25.0)	3(13.6)	0(0.00)	2(15.4)	10	10.8
P. aeruginosa	4(36.4)	5(22.7)	1(8.33)	4(18.2)	2(15.4)	1(7.7)	17	18.3
S. aureus	3(27.3)	9(40.9)	6(50.0)	5(22.7)	3(23.1)	4(30.8)	30	32.3
Total	**11**	**22**	**12**	**22**	**13**	**13**	**93**	**100**

Table 4.8: Molecular Identification of Bacterial Isolates from Bed linen

SAMPLE ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
BB2(A)Ba	*Escherichia coli*	2106	2106	99%	0	99.91%
BM11(7)	*Enterobacter cloacae*	2126	14714	100%	0	100.00%
BM3(7)6	*Bacillus mycoides*	2113	14624	99%	0	99.91%
BB7(7)A	*Pseudomonas aeruginosa*	2126	14714	100%	0	100.00%
BM1a	*Staphylococcus aureus*	2104	2104	99%	0	99.91%

Table 4.9: Phenotypic virulence properties of the bacterial isolates from bed linen

Bacterial Isolates	Phenotypic virulence properties		
	DNase	**Lipase**	**Hemolysin**
E. coli	+	-	-
Enterobacter cloacae	-	+	-
Bacillus mycoides	+	+	-
Pseudomonas	+	+	-
Staphylococcus aureus	+	+	+

Key:
- = Absent

+ = Present

Table 4.10: Antibacterial Sensitivity of Bacterial Isolates Obtained from Bed linen Surfaces

Isolates	No.	GEN	CS	CB	M	AG	E	CIP	TE	CD
E. coli	5	100	100	100	20	40	40	100	40	100
Enterobacter cloacae	5	100	60	100	20	60	20	100	40	100
Bacillus mycoides	10	100	30	100	30	100	30	100	20	100
Pseudomonas aeruginosa	5	100	40	100	40	60	40	100	40	100
Staphylococcus aureus	10	100	40	100	30	40	40	100	30	100

Key: AG - Amoxycillin (20+10mcg), CIP - Ciprofloxacin (5mcg), TE - Tetracycline (30mcg), GEN - Gentamycin (10mcg), CB - Cefuroxime (30mcg), E - Erythromycin (15mcg), CD - Clindamycin (2mcg), M - Metronidazole (5mcg), CS - Colistin (10mcg), S = Sensitive, R = Resistant

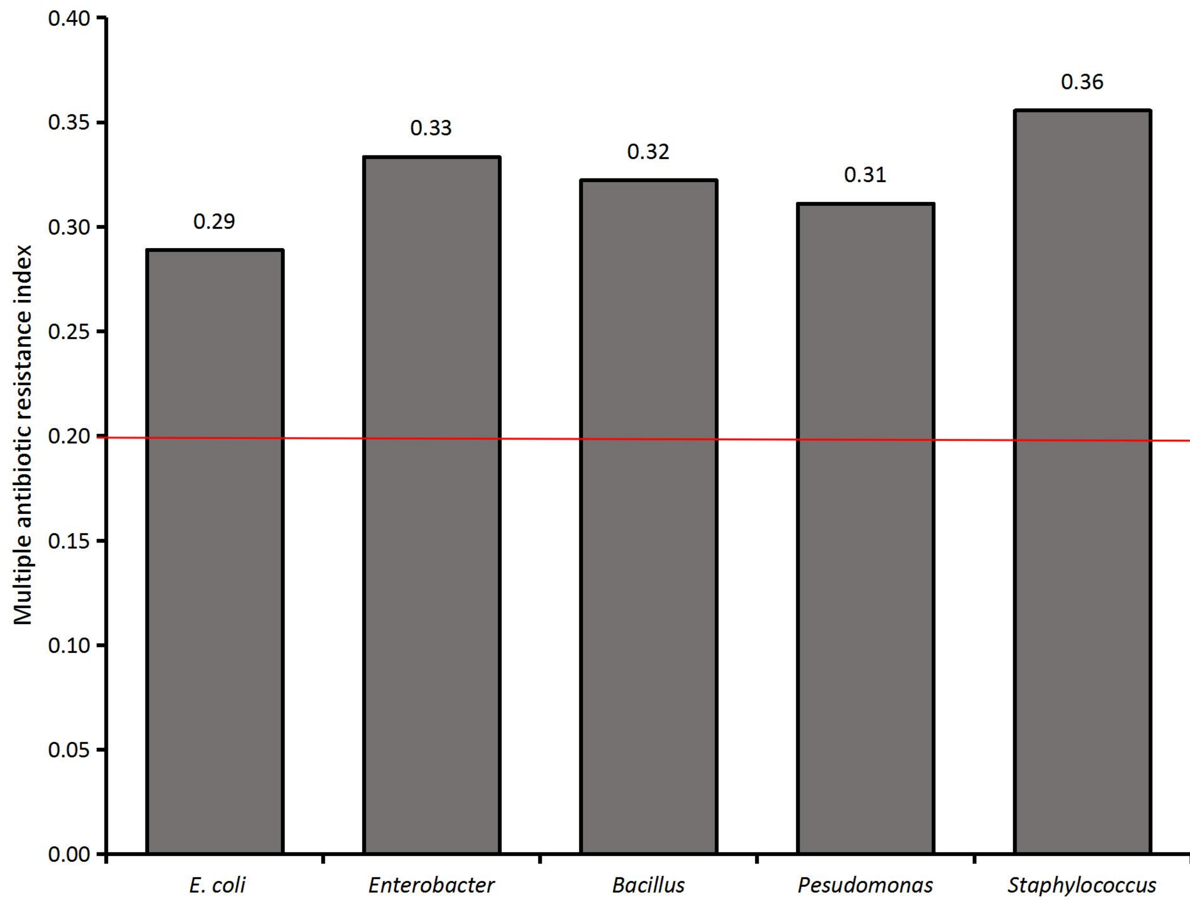

Figure 4.1: Multiple Antibiotic Resistant (MAR) index of bacterial isolates

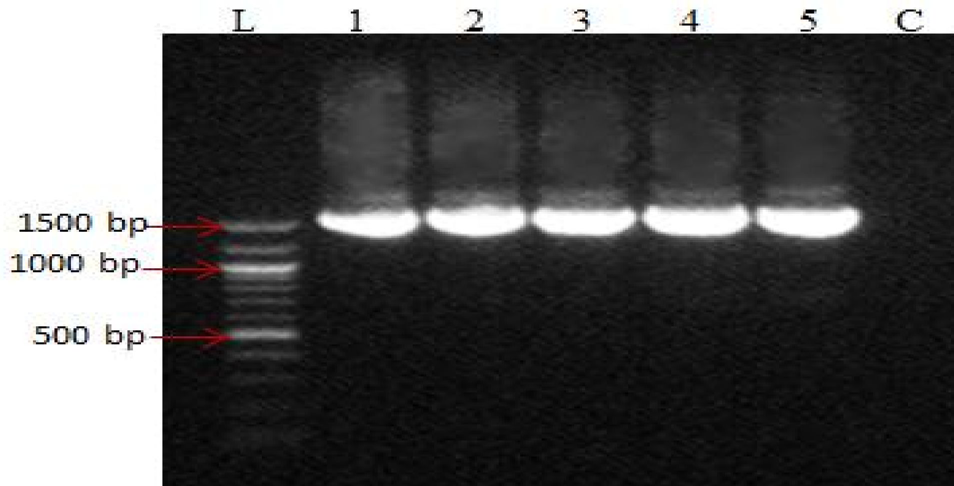

Plate 4.1: Plasmid DNA of multidrug resistance bacterial isolates analysed with 0.8% agarose gel electrophoresis stained with ethidium bromide. L is 0.5kb – 48.5kb DNA ladder (molecular marker). Isolates 1, 2, 3, 4 and 5 are positive for plasmid genes with band ranging from 500 bp–1500bp.

Key**:**

1 = *Escherichia coli*

2 = *Enterobacter cloacae*

3 = *Bacillus mycoides*

4 = *Pseudomonas aeruginosa*

5 = S*taphylococcus aureus*

CHAPTER FIVE

DISCUSSION

The results of the bacterial profile of bed linen and mattress from the various postgraduate hostels in University of Benin shows high bacterial counts. This is similar to findings Olowomofe*et al.*(2020) who reported high bacterial counts in hospital linen. This is also in agreement with the report of Giroti*et al.* (2023) who reported that heterotrophic bacterial count of mattresses surfaces. Total bacterial populations were found to be higher in bed linen and mattresses from female hostels compared to male hostels. This may be due poor hygienic nature of residence in the hostels and also mattresses and bed linenmay contain many growth factors that could be easily utilized by the organisms which are less available in the other locations used in this study.

There were significant differences in bacterial counts of bed linen and mattresses from all the postgraduate hostels ($P<0.05$). This indicates that some of the locations practice better sanitation when compared to others. The variations in microbial counts according to bed linen and mattresses samples may be as a result of difference in temperature, humidity, moisture contents of the locations at different time of collection (Brady *et al*., 2009). This agrees with the report of Saka *et al.* (2017) who reported that increase in period of isolation of microorganisms from hospital surfaces results to increase in microbial count. The difference in microbial count might be attributed to the frequency of bed linen and mattresses decontamination, types of decontaminants used, improper health professional practice, nature of the hand disk surfaces to harbor bacteria (Gurjeet *et al*., 2013; Gabriele *et al*., 2013). This might be through bacterial cross-contamination of surfaces (Abrar *et al*., 2016). Heavy microbial contamination of bed linen and mattresses may be caused by dominant skin of users, skin contamination after bed linen and

mattresses use occurs rapidly (Ulger *et al*., 2009). Surfaces may act as reservoirs of microbes which could in-turn leads to the spread of infection upon being touched by humans.

All the samples of bed linen and mattresses examined in this study showed varying degree of bacterial contamination. The results of this study showed that bacteria such as *Escherichia coli, Enterobacter cloacae, Bacillus mycoides, Pseudomonas aeruginosa* and *Staphylococcus aureus*were present on the bed linen and mattresses. This is in agreement with the study of Nazmunnahar*et al*. (2022) who isolated seven bacterial species *Citrobacter freundii, Escherichia coli, Enterobacter cloacae, Proteus vulgaris*, *Bacillus mycoides, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The level of usage of the bed linen and mattresses by students makes it a matter of public health importance. The organisms isolated have been incriminated in one disease condition or the order.

Staphylococcus aureus(32.3%) was the most commonly isolated bacterium from the examined bed linen and mattresses samples. This confirm the study of Pinon*et al.* (2013) who reported 17.9% of *Staphylococcus aureus* occurrence in hospital bed linen. This organism has also been isolated from previous studies by Enaam and Nevine(2016), Openshaw*et al.* (2016). *S. aureus* has been incriminated largely in nosocomial infections with multiple drug resistant strains, staphylococcal food poising and skin infections such as boils, styes and furuncules (Eggimann *et al*., 2003). This organism is also associated with pneumonia , Urinary tract infections, bacteremia and endocarditis (Medrano *et al.*, 2011). *S. aureus* isolation from these cloth samples no doubt is of public health concern.

The presence of *Pseudomonas aeruginosa* in this study is in agreement with the studies of Okareh (2018) who reported 19.7% cases of *Pseudomonas aeruginosa* from bed linen. Misgana *et al*. (2015) reported the role played by contaminated fomites in the transmission of

pathogens. Crowded conditions within the environment, frequent transfer of inanimate objects from one unit to another may contribute to proliferation of microorganisms in bed linen(Giroti *et al*., 2023).

Escherichia coli was the highest occurring bacterial isolate. The presence of *Escherichia coli* in this study may be due to sweat secretion on the bed linen that have been used for a long time, closeness of these environment to dumpsites and toilet. This agrees with the report of Russell and Jarvis (2001), stating that *E. coli* is expelled into the faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russell and Jarvis, 2001). *E. coli* has also been reported to be harmless and part of normal flora (Vogt and Dippold, 2005). *E. coli* isolated in this study are reported to cause infections such bacterial meningitis (Mola *et al.,* 2008*).* Their presence in this study may be as a result coughing and sneezing of car disk users and population of environment (Hall *et al.,* 2010).

Bacillus sp was the principal bacterium isolated by Anthony*et al.*(2013) from bed linen which was also isolated from this study. *Bacillus* sp is not known as human pathogen but can cause food poising. Its isolation might be related to the fact that *Bacillus* sp generally can withstand harsh environmental conditions like heat, desiccation, toxic chemicals and ultraviolet irradiation because of its ability to form endospores by which it can remain dormant for years.

Staphylococcus aureus is known to be a permanent and ubiquitous colonizer of human skin, *S. aureus* is not usually pathogenic but patients with compromised immune systems are often at risk for developing an infection. *Staphylococcus aureus* is one of the most common causes of both community and hospital acquired infections (Ekhaise *et al*., 2008). *Staphylococcus aureus* is known to be the causative pathogen in a range of diseases: impetigo, folliculitis, septic arthritis, osteomyelitis, septicaemia, pneumonia and meningitis (Kozitskaya *et al*., 2005). Bed linen and

mattresses may have got contaminated with *Staphylococcus aureus* by human contact with bed surfaces.

The presence of *Pseudomonas aeruginosa* in this study is in agreement with the studies of Okareh(2018) who reported 46.1% cases of *Pseudomonas aeruginosa* from bed linen. Ekhaise and Omoregie (2010) also in their work have reported the role played by hospital surfaces in the transmission of emerging healthcare-associated pathogens. Crowded conditions within the hostels, regular visitors and poor hygiene practices may contribute to proliferation of microorganisms on bed linen. Microbial flora may contaminate surfaces of objects, devices and materials which subsequently contact susceptible body sites (Kramer *et al*., 2006).

This study provides data about pathogenic potential contaminants in bed linen and mattresses. The ability of pathogenic bacteria to cause disease in a susceptible host is influenced by their ability to produce virulence factors. Virulence factors acting individually or together may induce infection depending on the host resistance. These factors compromise the host's defense mechanisms resulting in successful colonization and establishment of infection (Bushen *et al.,* 2021). All the bacteria isolated from bed linen and mattresses samples in this study exhibited variable forms of virulence with *Bacillus mycoides* and *Pseudomonas aeruginosa* showed positive Dnase and lipase characteristics. *Staphylococcus aureus*also possess virulent traits by showing positive Dnase, lipase and hemolysin activities. Bacteria possessing DNase can escape from neutrophil extracellular traps (NETs), thereby helping them survive (Haas *et al*., 2014). The bacteria isolated in this study are therefore capable of inducing infections due to the possession of virulent traits. This is in agreement with the study of Akinjogunla and Enabulele (2010). Although *E. coli* did not haemolysed red blood cells in this study (β-haemolytic activity) but is

arguably the most prevalent pathogen of humans which may cause up to one third of all bacterial infections ranging from boils and pimples to food poisoning. The ability to produce haemolysin by an organism is a measure of its pathogenicity (Sora and da Silva, 2021). Organisms with alpha or beta hemolytic ability are able to lyse red blood cells or other nucleated cells in the blood thereby enabling it to invade and cause disease. The serum resistant attribute of an organism is the ability of the organism to evade serum killing (Orole *et al*., 2021).

Concerning the production DNase enzyme, *E. coli*, *Bacillus mycoides, Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates were positive to this test. This is in agreement with the study of Kanemitsu *et al*. (2001) who reported that *Pseudomonas aeruginosa, E. coli* and *Staphylococcus aureus* were positive to DNase test. Panus *et al*. (2020) reported that *Bacillus* species was positive to DNase test which is accordance with this study. Ismail (2006) reported that the production of DNase are depending on the site of infection. Virulent traits such as hemolysin production, Dnase and lipase activity of bacterial isolates observed in this study also agree with the reports of various authors such as Retamal *et al*. (2022) who detected virulent factors in *Staphylococcus aureus* and *Escherichia coli*isolated frominanimate objects.Sarowska *et al*. (2022) detected hemolysin production in *E. coli* isolated inanimate objects

Negative hemolysin production by *E. coli, Enterobacter cloacae, Bacillus mycoides* and *Pseudomonas aeruginosa* observed in this study differs from the report of Nyinoh*et al*. (2018) who reported a hemolysin production in *E. coli, Enterobacter cloacae, Bacillus mycoides* and *Pseudomonas aeruginosa* isolated from bed linen. The production of hemolysin by *Staphylococcus aureus*detected in this study agrees with the report of Okareh (2018).

The antibiotic susceptibility profile of bacterial isolates from bed linen and mattresses showed that Gentamycin, Cefuroxime, Ciprofloxacin and Clindamycin had highest zone of inhibition

against (100mm) against *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter cloacae, Bacillus aerius* and *Staphylococcus aureus*respectively. This shows that most of the antibiotics were effective in the inhibiting the growth of the bacterial isolates. This result is in corroboration with the studies of Mohammed *et al*. (2019) which reported that *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter cloacae, Corynebacterium* species and *Bacillus aerius*were susceptible to amoxicillin, ciprofloxacin, augumentin and erythromycin. This study is also in agreement with the studies of Sahile *et al.* (2016) who revealed that *S. epidermidis, E. coli*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Corynebacterium* species were susceptible to ofloxacin, pefloxacin, ampicillin, ciprofloxacin, augumentin and erythromycin.

In this study, some of the tested isolates showed multiple antibiotic resistance to the tested antibiotics, especially *Staphylococcus aureus* which had the highest multiple antibiotic resistance (0.36%), followed by *Enterobacter cloacae* (0.33%) while *E. coli* had least multiple antibiotic resistance (0.29%). This is similar with the studies of Abebaw *et al.* (2018) who reported *P. aeruginosa, S. aureus* and *E. coli* to be multidrug resistant to gentamycin, pefloxacin, ampiclox, zinnacef and augmetin. This is also in agreement with the study of Tigist *et al*. (2018) who reported that *Staphylococcus* spp*, Enterobacter cloacae, Citrobacter* spp., *Klebsiella* spp. and *E. coli* were the leading multidrug resistance bacterial isolates. The resistance of the bacterial isolates to some of the antibiotics could be due to chromosomally or plasmid mediated resistant genes in the bacteria genetic make-up. This is associated with indiscriminate usage of antibiotics (Manisha *et al*., 2017). The development of high resistance gene pool may increase antibacterial resistance. Taken together, these findings clearly show how resistance strains are expanding at an alarming rate in the area. With this trend, an antibiotic that was effective a year ago might no

longer be used. This creates great burden, especially to people living in resource-poor countries, where they could not ensure their daily bread let alone for medication.

Variation of multiple antibiotic resistances of bacteria isolates from bed linen and mattresses from the different locations observed in this study may be due to multi-drug resistance to strains of the bacterial isolates. The plasmid profiles of the bacterial isolates from bed linen and mattresses before curing revealed that the bacteria showed high level of multi-drug resistance. Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance (Yah *et al*., 2007). In this study, plasmid profiles were detected which indicates that plasmid profiling can also be used as an epidemiological tool for typing *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus mycoides* and *Enterobacter cloacae*as described by Adekunle *et al*. (2011) stated that generally epidemiologically unrelated isolates contain different plasmid profiles whereas related isolates could also display variation in plasmid profiles. Bacterial antibiotics resistance patterns are sometimes associated with the presence of large plasmids and ability of plasmids for conjugation process (Zhang *et al*., 2014) which was observed in this study. According to Yah *et al.* (2007), the antibiotic resistance in isolates that possess plasmids was associated with chromosome and/or transposons instead of being plasmid-mediated.

5.2 Contribution to Knowledge

This study has contributed to knowledge in the following ways;

1) Potential pathogenic microorganisms are present in bed linen and mattresses.

2) The population of bed linen and mattresses microorganisms is an important source for the transfer of antibiotic resistance genes to humans.

3) All the tested bacterial isolates exceeded the multiple antibiotic resistance index of ≥ 0.2 which is of public health importance.

5.2 Conclusion

Bed linen and mattresses have been found to harbour disease-causing microorganisms and are easily transmitted through contact from one person to another. Mutations and other various factors have aided these organisms to mount resistance towards commonly used antibiotics which has rendered treatment of infectious diseases inefficient. There is indeed a dire need to scale up and strengthen existing infection control strategies through reassessment of the strengths and types of the decontaminants used to clean Bed linen and mattresses, frequency of decontamination or sterilization and good personal hygiene.

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APPENDIX 1

QUESTIONNAIRE

A POST-GRADUATE QUESTIONNAIRE DESIGNED FOR THE BACTERIOLOGICAL EVALUATION OF MATTRESS AND BED LINEN IN POSTGRADUATE HOSTELS, UNIVERSITY OF BENIN

(Please kindly fill in the required details by ticking right in the appropriate box as this will enable us carry an effective research)

AGE GROUP OF STUDENT

- **20 - 25 years (Yes / No)**
- **25 – 30 years (Yes / No)**
- **30 years and above (Yes / No)**

GENDER OF STUDENT

- **Male ( ), Female ( )**

1. How often do you have a visitor? Never ( ), Occasionally ( ), Sometimes ( ), Often ( )
2. How many occupants are in your room? Just me ( ), 2-3 ( ), 4-6 ( ), 7 and above ( ),
3. Do you have a bed-linen? Yes ( ), No ( ),
4. Do you use a bed-linen? Yes ( ), No ( ),
5. How many bed-linen do you have? 1 ( ), 2 ( ), 3 ( ), 4 and above ( )
6. How many times do you change your bed linen per week? once ( ), twice, thrice and above ( ) no-time ( )
7. How many times do you wash your bed linen per month? once ( ), twice, thrice and above ( ) non-time ( )

8. Why do you wash your bed linen? To prevent rash and itching ( ), to prevent infection ( ), to prevent dour ( ), a way of life ( )
9. What do you wash your bed linen with? Detergent and water ( ) bar-soup and water ( ), water only
10. Do you use disinfectant on your bed linen? Yes ( ) , No ( )
11. When do you wash your bed linen? When is its dirty ( ), I have my specific duration of washing my bed linen dirty or not ( ).
12. How many persons lay on your bed? Just me ( ), 2-3 ( ), 3-4 ( ) 4 and above
13. When was the last time you purchase a bed linen? Less than one year ( ), one year ( ), two years ( ), three years and above ( )
14. Do you lay on the mattress without bed linen at any point? Yes ( ), No ( )
15. Where do you place your mattress? Mattress stand ( ), one the floor ( )
16. Do you use disinfectant on your mattress? Yes ( ), No ( )
17. How many times do you sweep your floor per day? once ( ), twice, thrice and above ( ) no-time ( )
18. Are you aware that your mattress and bed linen can be a reservoir of bacterial infection? Yes ( ), No ( )
19. Do you take your bath before you sleep on your mattress? Yes ( ), No ( )
20. Do you take your bath before you sleep on your mattress without a bed linen? Yes ( ), No ( )

Thanks for your cooperation

APPENDIX II

CULTURE MEDIA

Nutrient agar

Beef extract	3.0 g
Agar No.2	12.0 g
Peptone	5.0 g
Sodium chloride	8.0 g
Distilled water	1000 ml

Mueller Hinton Agar

Beef Extract .... 2 g

Acid Hydrolysate of Casein .... 17.5 g

Starch .... 1.5 g

Agar .... 17 g

Final pH 7.3 ± 0.1 at 25°C

APPENDIX III

GRAM STAINING AND BIOCHEMICAL REAGENTS

STAIN AND REAGENT

GRAM STAIN

A. Gram crystal violet

Solution A

Crystal violet	2.0 g
Ethanol (95ml)	20.0 ml

Solution B

Ammonium oxalate	0.8 g
Distilled water	80.0 ml

B. Gram iodine

Iodine	1.0 g
Potassium iodide	2.0 g
Water	300.0 ml

3.0 g of medium was dissolved in 300.0 ml of distilled water.

Gram's Safranin

Safranin	0.25 g
Ethanol	10.0 ml
Distilled water	100 ml

Biochemical reagents

Indole medium

Peptone	20.0 g

Sodium chloride	5.0 g
Distilled water	1000 ml
pH	7.4

15.0 g of peptone medium was dissolved in 1000 ml of distilled water and autoclaved for 15 minutes at 121 ^{0}C and dispensed aseptically into sterile test bijou bottles.

Simmon citrate Agar

Sodium ammonium phosphate	1.50 g
Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	0.2 g
Sodium citrate	2.5 g
Bromothymol blue	0.016 g
Distilled water	1000 ml
pH	7.0

24.0 g of Simmon's citrate medium was dissolved was dissolved in 1000ml of distilled water and autoclaved at 121 ^{0}c for 15 minutes and dispensed aseptically into sterile test tube.

Urea Agar Base

This medium was primarily used for urease test.

Composition

Peptone	1g
Sodium Chloride	5g
Potassium dihydrogen- Sulphate K H_2PO_4	2g
Glucose	5g

Agar powder	20g
Distilled water	1000ml.

Preparation

The powdered urea agar (oxoid) was used and was prepared as directed by the manufacturer. Urea agar was prepared by suspending 2.1g of the powdered medium in 95 ml of distilled was dissolved by boiling. The medium was sterilized by autoclaving at 115°C for 20 minutes. The medium was cooled to about 50°C and 5ml of 40% v/v sterile urea solution was added. The medium was dispensed into culture tubes in 15ml aliquot and were allowed to solidify in slant positions.

Peptone Water

This medium was used to enrich and develop the inoculums that were used to inoculate the agar plates. It was also used to maintain the culture for some biochemical tests.

Composition

Peptone	10g
Sodium Chloride	5g
Distilled Water	1000ml.
pH	7.6

Preparation

The powdered medium was used and it was prepared as directed by the manufacturer. Fifteen grams of the powdered medium (Oxoid) was dissolved in 1000 ml of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes

Sugar utilization medium

Peptone water	2.0 g

Sodium chloride	5.0 g
Water	1000 ml
Phenol red	7.0

10% v/v of each of the following sugar was dissolved in 1000 ml of distilled water and autoclaved at 121 ^{0}C for 15 minutes. This was dispensed aseptically into sterile test tube containing Durham's tubes

Kovac's reagent

A reagent used for the Indole test, which is used to determine which organism has the ability to split Indole from tryptophan

Amul-alcohol	15.0ml
p-dimethyl-aminobenaldehye	0.5ml
Concentrated Hcl	50ml

Preparation

Dissolve the aldehyde in the alcohol slowly add the acid prepare in small quantities and store in refrigeration shake gently before use. 0.5ml kovac's reagent is added on the 48-hrs peptone 'food containing the isolates for organism.