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Bacteriological Analysis/Bioburden Evaluation of Bedsheets of Students in Ambrose Alli University Ekpoma

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Abstract- ***This study investigates the bacterial contamination present on bedsheets in a university residential hostel, shedding light on potential health risks associated with this overlooked aspect of communal living. The prevalence of microbial transfer to bedsheets from various sources, including direct contact, is examined. The research also delves into the presence of pathogenic and non-pathogenic bacterial floras on bedsheets and their implications for student health.***

The study, conducted at Ambrose Alli University's main campus in Ekpoma, Edo State, Nigeria, involved the collection of samples from 18 students residing in the university hostels. The data obtained from both microbial analysis and questionnaires administered to the students provide a comprehensive overview of bedsheet hygiene practices and potential risks. Results revealed a high prevalence of bacterial contamination, with identified species including Escherichia coli, Bacillus mycoides, Serratia marcescens, and Enterobacter cloacae. These findings underscore the need for improved hygiene practices, such as regular bedsheet changes and thorough laundering, to mitigate potential health risks associated with prolonged microbial exposure. Furthermore, the study highlights the importance of awareness campaigns aimed at educating students about the significance of maintaining clean bedsheets. Recommendations for enhancing hygiene in residential hostels are provided, including strategies to reduce bacterial contamination and improve overall cleanliness. This research contributes to the growing body of knowledge on bioburden assessment and hygiene practices in shared accommodations, emphasizing the critical role of environmental cleanliness in safeguarding student health. The findings offer practical insights for implementing interventions to create a healthier living environment within residential hostels, ultimately promoting the well-being of university students.

I. INTRODUCTION

The majority of university students have demanding academic schedules throughout the day and typically seek solace in their beds in the evening until morning. As a result of the stress experienced during school hours, many students simply flop onto their beds to unwind. Consequently, microorganisms encountered through various contacts are being transferred to their bedding. Moreover, these microbes can also infiltrate the beddings during the manufacturing process through airborne contamination, as Shiomori et al. (2002) provided evidence showcasing the potential for transmission from linens.

The numbers of surface and airborne Methicillin-resistant Staphylococcus aureus (MRSA) were assessed by a research team in a Japanese hospital. The study examined the levels of MRSA before, during, and after bed-making procedures for 13 patients who were either infected or colonized with MRSA. Additionally, the researchers investigated the potential role of soil fungi in releasing fungal spores into the air as a natural means of transportation. They also explored the possibility of clothing acting as a source of fungal infections when it comes into contact with these spores (Fijan et al., 2012).

Certain individuals have such a strong affection for their pets that they are willing to let them onto their beds, disregarding the potential presence of harmful microorganisms on these animals. This behavior can create a significant threat to public health as these pets may carry microorganisms that can cause illness in humans and are resistant to multiple types of antibiotics (El-Tras et al., 2015).

Because you inadvertently transfer your own natural skin bacteria and any other germs you may be carrying onto bedsheets each time you use one, bedsheets make excellent bacteria traps. The surrounds of the home are full of microorganisms; in nature, they are ubiquitous and can be found wherever. The environment carries a

significant danger of microbiological contamination and disease (Larson et al., 2003).

Microbes on skin can be classified as either pathogenic or non-pathogenic floras (Smith, 2009). Transient floras take over the skin's external look and are readily eliminated by washing. They can be disseminated by direct contact with individuals and their surroundings. These microorganisms include those linked to nosocomial infections, including Enterococci, Pseudomonas species, Klebsiella species, Acinetobacter species, and Staphylococcus aureus (Smith, 2009). These organisms can cause illnesses of different intensities, ranging from minor to deadly. Another connection between damp and humid conditions and public health is that bacteria spread more easily in these types of settings than in dry ones. Weaknesses on linens and towels may originate from within us. Everybody has germs in their noses, stomachs, and on the surface of their skin. While most of these are safe, some can lead to infection, especially in those who have wounds or skin conditions. Germs are more likely to be found on towels and underwear than on outerwear like pants or sweaters. Underwear may harbor bacteria from genital illnesses like thrush and from traces of feces (poo).

Microorganisms can be found on used bedsheets in our houses; a warm, humid environment is ideal for the growth and survival of bacteria. These microorganisms can spread through direct contact with our hands and other inanimate objects in the surroundings. The skin harbors a wide range of microorganisms that are typically found in skin cells, sweat, sputum, vaginal and anal secretions (Creamer and Humphrey, 2008), and some of these microbes play a role in promoting immunity and combating harmful invaders. Most of the bacteria found in bedsheet can cause a number of ailments, such as ringworm, acne, and pimples. Because they are moist, old bedsheets that haven't been cleaned provide the perfect environment for both pathogenic and non-pathogenic germs to proliferate. Because most individuals don't wash their hands often, they can transfer germs from the environment to their towels. One important factor in the transmission of germs is contaminated hands (Curtis and Cairncross, 2003). These beneficial microbes contribute to protecting humans from pathogens and assist the immune system in maintaining a delicate balance between protection and harmful inflammation. However, alongside these health-promoting microorganisms, there are also pathogenic organisms that can cause diseases and even death when they accumulate on the body.

Environmental contamination is a major factor in the spread of multiple infections that are significant to epidemiology, including Clostridium difficile, VRE (Vancomycin-resistant Enterococcus), and MRSA (Methicillin-resistant Staphylococcus aureus). After disinfection, surfaces and equipment should be hygienically clean—that is, devoid of microorganisms in sufficient quantities to cause disease in humans. Improved environmental decontamination aids in the control of outbreaks, and there is growing evidence that contaminated surfaces play a significant role in the epidemic and endemic transmission of Clostridium difficile, methicillin-resistant Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, and norovirus (Otter et al., 2011).

Reducing pathogen shedding and increasing the effectiveness of cleaning and disinfection are two ways to improve environmental hygiene. In order to ascertain the role surfaces play in nosocomial transmission and the efficacy of various strategies in lowering related infection rates, more excellent research is required.

1.1 AIMS

The aim of the project is to assess the bacterial contamination present on the bedsheets of students residing in the residential hostels of Ambrose Alli University, Ekpoma.

1.2 OBJECTIVE

The objectives of the project "Bacteriological Assessment/Bioburden Evaluation of Bedsheets of Students in Residential Hostels" include the following:

1. Bacterial Identification: Determine the types of bacteria present on the bedsheets.
2. Quantification of Bioburden: Measure the level of bacterial contamination on the bedsheets.
3. Health Risk Assessment: Evaluate the potential health risks associated with the identified bacteria.

4. Hygiene Evaluation: Assess the cleanliness and hygiene practices of students in relation to their bedsheets.
5. Comparative Analysis: Compare bacterial contamination levels across different hostels or groups of students.
6. Recommendations: Suggest strategies to improve hygiene, reduce bacterial contamination, and enhance the overall cleanliness of the residential hostels.
7. Awareness Creation: Raise awareness among students about the importance of maintaining clean bedsheets for personal and public health.
8. Educational Component: Contribute to understanding the significance of regular cleaning and hygiene practices in communal living spaces.
9. Contribution to Literature: Add to the existing body of knowledge on bioburden assessment and hygiene practices in shared accommodations.
10. Practical Application: Provide insights that can lead to practical interventions for maintaining a healthier living environment within residential hostels.

These objectives collectively aim to enhance the living conditions and well-being of students by addressing potential health concerns related to bacterial contamination on bedsheets in residential hostels.

II. LITERATURE REVIEW

The skin microbiota is made up of millions of bacteria, fungi, and viruses that live in our skin. Skin microorganisms play crucial functions in the defense against invasive infections, immune system education, and degradation of natural products, much as the bacteria found in our gut (Scharschmidt and Fischbach, 2013). The skin, the biggest organ in the human body, acts as a physical barrier to keep infections out and is colonized with helpful bacteria. Skin conditions or even systemic diseases may arise in situations when the barrier is breached or when the balance between commensals and pathogens is upset. The physiological traits of human skin sites, such as whether they are moist, dry, or sebaceous (oily), can be used to categorize them. It was discovered that the physiology of the skin site largely determined the makeup of microbial communities in sequencing surveys of healthy persons. Lipophilic Propionibacterium species dominated sebaceous locales, whereas Staphylococcus and Corynebacterium species, which are tolerant of high humidity, were more prevalent in wet places like the bends of the elbows and the foot. Regardless of physiology, the composition of fungal communities was comparable across core body regions, in contrast to bacterial communities (Findley et al., 2013). At the core body and arm sites, Malassezia spp. dominated, whereas a more varied combination of Aspergillus spp., Cryptococcus spp., Rhodotorula spp., Epicoccum spp., and other fungi colonized the foot sites (Findley et al., 2013). According to Oh et al. (2016), fungi were the least common kingdom across all sites, while bacteria were the most common. Invading germs are physically eliminated by the sloughing of dead keratinocytes. Skin is somewhat acidic and has a lower temperature than the rest of the body; most bacteria thrive at 37°C and a neutral pH. The immune system, or skin-associated lymphoid tissue, provides the next line of defense against organisms that manage to elude cutaneous host defenses (SALT).

The quantity of bacteria present on an unsterilized surface is known as bioburden. The total number of live bacteria in or on a medical equipment is monitored by bioburden analysis. A crucial process in many industries, but particularly in healthcare, pharmaceuticals, and biotechnology is bioburden evaluation. Measurement and analysis of the level of microbial contamination on or in a product, substance, or environment are required. Finding the number and types of microorganisms (including bacteria, fungus, and viruses) in a certain sample or location is the aim of bioburden assessment.

2.1 MICROORGANISMS FOUND ON SURFACES

While certain infections can exist in a dormant condition without a living host, many pathogens need a living host to survive. All diseases, however, need ways to spread from one host to another. For this reason, understanding how bacteria, fungi, viruses, and protozoa survive on surfaces—and, in a larger sense, in the human environment—is crucial for putting strategies in place to prevent Healthcare-acquired Infections (HAI). If inanimate environmental sources match the requirements for transmitted

pathogens to survive and reproduce, they may turn into secondary reservoirs for microorganisms that have been transmitted from animated sources.

2.1.1 BACTERIAL

The majority of Gram-positive bacteria, including Enterococcus species, including VRE, S. aureus species, including MRSA, and Streptococcus pyogenes, can last on dry surfaces for months. Numerous Gram-negative organisms, including Acinetobacter spp., Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Serratia marcescens, and Shigella spp., can endure on inert surfaces for weeks or even months. However, several other Gram-negative bacteria, like Vibrio cholera, Haemophilus influenzae, Proteus vulgaris, and Bordetella pertussis, only survive for a few days. Spore-forming bacteria like C. difficile and mycobacteria like Mycobacterium tuberculosis can live for months on surfaces (Alex and Ojan, 2014). Additionally, all bacteria strains may be shown to be transmissible from hands to paper and back. Similar studies revealed that paper currency notes could contain and spread infections (Uneke and Ogbu, 2007; Vriesekoop et al., 2010).

2.1.2 VIRUSES

Most respiratory tract viruses, such as the corona, coxsackie, or influenza virus, SARS, or rhinovirus, can only survive on surfaces for a short period of time (Casanova et al., 2010). Cytomegalovirus and Herpes simplex types 1 and 2 are two examples of herpes viruses that have been demonstrated to last for only a few hours to seven days. Viruses from the digestive tract, such as Rotavirus, Hepatitis A virus, Poliovirus, and Astrovirus, survive for roughly two months. Blood-borne pathogens like the Human Immunodeficiency Virus (HIV) and Hepatitis B virus can survive for longer than a week (Alex and Ojan, 2014).

2.1.3 FUNGI

The most significant nosocomial yeast, Candida albicans, can endure on surfaces for up to 4 months. Other yeasts were said to have similar (Torulopsis glabrata: 5 months) or shorter (Candida parapsilosis: 14 days) periods of persistence. However, physical aspects of nature, such as temperature and relative humidity, have a significant impact on the survival of fungi in the environment. Molds are common in nature, resistant to heat, and long-lived in household dust. Measurements of indoor airborne mold highlight the survival for several months (Baudisch et al., 2009; Baudisch et al., 2009).

2.1.4 PROTOZOA

Water-born infections can be caused by Cryptosporidium species. Their oocysts can endure up to 120 days in soil and months in surface water (Carey, 2004). One of the most prevalent protozoa in soil, as well as regularly occurring in fresh water and other natural settings, is the amoeba. Hydrogel contact lenses constitute a significant habitat and vector for infection, leading to contact lens-associated keratitis brought on by Acanthamoeba and Fusarium (Patel and Hammersmith, 2008), especially given that the wet environment of the lenses promotes protozoal survival.

The majority of respiratory viruses, including the influenza, para-influenza, corona, respiratory syncytial, herpes simplex, measles, rubella, and varicella zoster viruses, will typically survive longer at lower relative humidity (20-30% RH) (Tang, 2009). A notable example is the Cytomegalovirus, which was most likely isolated from wet surfaces (Stowell et al., 2012). Adenovirus, Enterovirus, and rhinoviruses are non-lipid enclosed viruses that, in contrast to enveloped viruses, have a tendency to persist longer at higher relative humidity levels (70–90% RH) (Tang, 2009). The ability to survive of microbes on surfaces may be influenced by a variety of additional factors. It is obvious that the surface's material makeup may be crucial. On the other hand, conflicting findings on the impact of material type on microbial survival are documented.

A pathogen may be transferred to the hand from a contaminated surface to another hand in varying degrees. E. coli, Salmonella spp., S. aureus, C. albicans, Rhinovirus, Hepatitis A virus, and Rotavirus were the most successful in spreading from surfaces to hands (all 100%), followed by Hepatitis A virus (22-33%), and Rotavirus (16%). Other transfer rates were determined for Salmonella enteritidis, Shigella spp., and E. coli O157:H7 with 33% and 50% transmissibility, respectively, for echovirus, poliovirus, and rotavirus (Tanner, 2009). Viral transmission from infected hands can reach 14

additional patients or 5 additional surfaces. As the Hepatitis A virus has shown (Kramer et al., 2006; Kramer et al., 2006), contaminated hands can potentially be the source of surface contamination.

2.1.5 OCCURRENCE OF PATHOGEN FOUND IN BEDSHEETS

The majority of germs connected to bedsheets come from human skin. Other sources include food, aerosols, and body excreta. Several recent papers have investigated the prevalence of infections in laundry (Bloomfield et al., 2011). Due to their increased infectivity (fewer numbers having a larger likelihood of generating an illness) than bacterial and fungal species, viruses generally provide the greatest risk in contaminated fabrics. Textiles representing blood, pulmonary, gastrointestinal, and cutaneous contamination sources have all been found to contain a variety of viruses and other diseases (Bockmühl, 2017).

2.2 SURVIVAL OF MICROORGANISMS IN BEDSHEETS

Relative humidity (RH), air temperature, and material type are all elements that affect the survival of infections in/on laundry items (Yeargin et al., 2016). The majority of microbial inactivation happens when a bodily fluid carrying a pathogen is dried (such as saliva or mucus), followed by a slowing of the inactivation rate. The vitality titre of respiratory viruses often decreases 10- to 100-fold when dried at room temperature (Harbourt et al., 2020; Kratzel et al., 2020). At 22 and 37°C, SARS CoV-2 survived less than 8h on clothing, and at 4°C, it survived for at least 96h (Harbourt et al., 2020). In a further investigation, SARS CoV-2 viability on clothing was reduced by 99% within 2 hours and by more than 99.99% within 48 hours (Chin et al., 2020). At some RHs, some bacteria are more resilient than others. Because some dyes may be anti-microbial, the kind of material and the presence of dyes or coloring agents may also have an impact on persistence. In extensively dirty fabrics, the presence of organic matter may also prolong survival, or, in the case of some bacteria that can use the organic soils as a food source, growth may take place (Kampf, 2020). The majority of respiratory viruses, including SARS CoV-2, cannot survive in garments at room temperature for more than a day or two (Ikeda et al., 2015). Some enteric viruses, such as the rotavirus and the hepatitis A virus, can, nevertheless, persist for a number of weeks (Boone & Gerba, 2007; Yeargin et al., 2017). It was discovered that the rate of water loss while drying was connected to the survival of influenza virus in garments (Ikeda et al., 2015). Survival was correlated with the fabric's thickness and color, with black material inactivating more quickly. Salmonella and MRSA, for example, can persist for weeks in garments (Bloomfield et al., 2011). Acinetobacter sp. and Pseudomonas aeruginosa can thrive naturally in garments after cleaning the workers' uniforms for wastewater treatment (Maal-Bared, 2019). Evidently, naturally occurring bacteria have adapted to the conditions of laundry, and even after laundering, there is still enough organic matter for their renewal during storage.

2.3 SOURCES OF MICROORGANISMS ON BEDSHEETS

Human skin, bodily secretions, and excretions are the main sources of germs in clothing. The distribution of the microbial flora found on the skin and in bodily excretions can be influenced by activities including eating and cooking, being outside, and work. The microflora on linen (bedsheets) cleaning supplies (sponges, kitchen towels, and dishcloth), and towels might vary. The fabric kinds, applications, and filth load that each category of textiles possesses are distinctive. This affects the growth of bacteria that cause odors and infections in laundry.

2.3.1 BACTERIA

Textiles have been linked to pathogenic bacteria that can cause skin infections and gastrointestinal diseases like Salmonella and MRSA (methicillin-resistant Staphylococcus aureus). According to one study (Enriquez et al., 1997), 3% of hand/face towels and 15% of household sponges in the United States contained Salmonella. Other intestinal bacteria, such as Escherichia coli, were also prevalent. Reusable shopping bags have also been found to contain E. coli (William et al., 2011). Both children's and adults' underwear frequently contains feces (Gerba, 2001). A heat-stable toxin is produced by Staphylococcus species, which can also result in superficial skin lesions. Before or after laundry, depending on the

storage conditions (closet or hamper), germs can multiply (Hammer et al., 2011).

2.3.1 FUNGI

According to Ossowski and Duchmann (1997), it has been hypothesized that fungus found in garments may also contribute to the spread of dermatitis and onychomycosis (nail infection). Tinea pedis patients have had fungus infections identified from them (Amichai et al., 2013). Microsporum canis transmission has been connected to clothing (Bloomerfield et al., 2011). The keratinized tissues produced from the stratum corneum of the epidermis, such as the skin, nails, and hair of humans and animals, are susceptible to invasion by the dermatophyte fungus, a group of closely related microorganisms. M. canis is one of these microorganisms. These fungi cause dermatophytosis, sometimes known as ringworm or tinea, which is an infection. 79% of the 70 home washing machines that were tested in one investigation for fungi tested positive (Babic et al., 2015). The opportunistic fungus Candida and Fusarium were found among the species. Infections caused by fungi can be fatal in people with impaired immune systems. According to Roden et al. (2005), mucormycosis, an infection of the Mucorales, can result in fatality rates as high as 50%. In the healthcare industry, outbreaks have been linked to linens (Sundermann et al., 2019). According to research by Sundermann et al. (2019), 33% to 73% of newly washed linens contained Aspergillus.

2.3.1 VIRUSES

Rotavirus, hepatitis A and B, herpes simplex, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza virus, HIV, and papillomavirus are only a few of the enteric and respiratory viruses that have been found in textiles. Hepatitis B virus is a blood-borne infection that has been spread through exchanging towels in the restroom. It has been demonstrated that textiles can spread the hepatitis A virus and the vaccinia virus (smallpox virus) as well (Bloomerfield et al., 2011).

2.3.1 HELMINTHS

Helminths (worms) and their eggs are also likely to be present in clothing and materials that have been exposed to feces. These include Ascaris, pinworms, and tapeworms, among others. Most intestinal worm infestations are rare in the industrialized world as a result of good hygiene practices and access to clean water and food. The most prevalent helminth in North America and Western Europe is the pinworm, with prevalence rates in some populations reaching 30% to 50% (Burkhart and Burkhart, 2005). It has been proposed that their transmission may be aided by bedsheet and garment contamination with pinworm (Enterobius vermicularis). On garments and linens, pinworm and ascaris eggs can live for two to three weeks (Maguire, 2017). There is no information on the prevalence of helminths in garments and materials that could be found.

2.4 REMOVAL OF MICROORGANISMS BY LAUNDERING

Laundry has a number of different effects on the elimination of microorganisms. Numerous variables may affect both the possibility for contamination and the removal (detachment and/or inactivation) of microorganisms (e.g., the occupation of a wastewater worker vs a schoolteacher) (Abney et al., 2021). It is probable that these factors also lead to the development of a local microflora that is adapted to mixtures of these components. It is crucial to keep in mind that techniques meant to separate and inactivate microorganisms may also introduce new microbial communities (such as the resident microflora from washers and dryers) and affect scents. The methods used for washing and drying clothes have a big impact on how many pathogens are removed from the laundry. Pathogen reduction-release and/or inactivation are affected by detergent choice, other chemicals (such bleach), water temperature, and drying. Washing with cold water, obtained from a cold water tap, is a popular habit in North America. The ability of various detergents to remove or inactivate enveloped viruses, such as SARS-CoV-2 and influenza virus, can cause the elimination of these viruses even in cold-water washes (Heinzel et al., 2010; Gerhardts et al., 2016). In the US, the average cold water wash temperature is 14.4°C (57.9°F). For more hardy enteric and cutaneous diseases, it has been suggested that temperatures of 40°C to 60°C (104°F to 140°F) and/or the use of bleach are required (Gerhardts et al., 2016). A further defense against transmission and survival is provided by drying, with disinfection influenced by both the temperature and

the length of time. Longer drying times and higher temperature settings can considerably lower microbial populations. The non-spore-forming bacteria found in laundry that are most resistant to drying and heat include Acinetobacter baumannii and Staphylococcus aureus (Gerhardts et al., 2016). To obtain a 99.9% decrease, washing at temperatures over 60°C is necessary (Shin et al., 2020). Higher washing and drying temperatures are also necessary for considerable decreases in mycobacterium, fungus, and enteric viruses (including the hepatitis A virus, adenovirus, and rotavirus). The quantities of bacteria and viruses will be greatly reduced but may not be completely eliminated when activated-oxygen bleach (AOB) is used in cold-water washes (40°C) (Shin et al., 2020).

III. METHODOLOGY

3.1 Study Area

This study was conducted at Ambrose Alli University's main campus in Ekpoma, Edo State, Nigeria. The campus offers housing to students in its ten university hostels, including Emotan, Igbinedion, Onyearegbulem, Rev. Martins, Jereton Mariere, Big Joe, Iyayi, Kudirat Abiola, Basic Medical Science, and Dangote hostels and randomly selected off campus residents. Samples for this study were collected from Ambrose Alli University campus hostel facilities that house numerous students.

3.2 Study design and sample collection

Students' permission was requested prior to the start of the study and the collection of the sample. For the investigation, a total of 18 samples (18 bedsheets) were gathered from students. Students whose bedsheets were taken for bacterial analysis were given questionnaires. This was carried out to gather data on their towel handling procedures. In 2023, samples were taken in August. Samples were collected from 18 students who were staying in the hostels at Ambrose Alli University, Ekpoma. The random sampling method was used to sample the student population. The bedsheets were swabbed with the use of a sterile cotton swab stick immersed in 9ml of normal saline over an area of 15cm by 10cm and the collected samples were stored back in their original containers and then taken to the laboratory for analysis.

3.3 Preparation of Samples, Inoculation, and Incubation

Swabbing a known region with a sterile swab that has been moistened with sterile tryptone soya broth is the preferred technique for inspecting towel surfaces. The bacteria per cm2 can be counted using this semi-quantitative methodology, and the method outlined by Public Health England (2014) can make it easier to interpret the findings. The upper and bottom sides of the bedsheets that come into direct touch with the body were cleaned using sterile swab sticks that had been aseptically soaked in 10ml of tryptic soy broth. Following that, the swab sticks were immediately placed in 10 ml of tryptone soya broth. This is equivalent to 100, and if 1 mL is plated, it provides a lower limit of detection of 10 colony forming units (cfu) per swab, according to Public Health England (2014). The total heterotrophic bacterial count was then determined using tryptone soya agar (Oxoid) and a subsequent 10-fold serial dilution.

Serial dilution

From each of the bedsheet samples, 10ml was measured using a volumetric flask to prepare a stock solution. Ten test tubes were filled with 9ml of sterile water to make a dilution from 10^{-1}–10^{-10}. Using a micropipette, 1ml was taken from the stock solution and added to 9ml of distilled water to make the first (10^{-1}) dilution, then 1ml was taken from the first dilution and mixed with 9ml of distilled water to make a second dilution (10^{-}Z). The procedure was repeated until a dilution of (10^{-10}) was achieved.

Preparation of Nutrient Agar

Nutrient Agar was used as the medium for the antibacterial assay. The medium was prepared according to the manufacturer's specification. 15.1g of nutrient agar powder was dissolved completely in 540ml of distilled water to prepare 36 plates of petri dishes. The resulting mixture was then poured into a conical flask with an aluminum foil paper and sterilized in an autoclave at 121°C for 15 minutes.

Inoculation

The samples were inoculated using the pour plate technique. 0.1ml was transferred from each test tube into a petri dish using a micropipette and molten agar (nutrient agar) was poured and mixed with the inoculant and the medium was allowed to cool and

solidify. The plates were incubated at room temperature then in an inverted position to prevent water of condensation from trickling back into the medium.

Viable Colony Count

Colony forming units per milliliter (CFU/m2) were calculated from the overall bacterial count to estimate the total viable count for the samples. To calculate the number of viable bacterium isolates, Public Health England (2014) utilized the appropriate equation, where C = the total number of colonies on the plates, V is the inoculum volume, n1 is the number of plates counted during the initial dilution, n2 is the quantity of plates counted during the second dilution, n3 equals the initial volume of a tidy suspension, and d is the dilution at which the initial count was made.

To determine the count per cm2, the result obtained will be divided by the area swabbed. This value is the count per swab. The enumeration approach used the pour plate method, and the plates were incubated for 24 hours. For the bedsheet samples examined in the study, coliform levels were also assessed.

Gram Staining Test

To identify the presence of Gram-positive and Gram-negative isolates, a Gram staining test was performed. A sterilized loop was used to smear the organism onto clean, grease-free, and sterile-dried microscope slides with labels. The organism was then air-dried and heated to fixation over a burning Bunsen burner. Crystal violet was applied to the fixed smear, allowed to react for one minute, and then wiped off with distilled water. The mordant Lugol's iodine was applied, allowed to sit for one minute, then removed with distilled water. The smear was decolored for 30 seconds with 95% ethyl alcohol, then removed with distilled water. Following a minute of counter staining with safranin solution, distilled water was used to rinse the streak. Finally, immersion oil was added for a microscopic look using an immersion objective lens light microscope after allowing the smear to air dry. The identification of the organisms was done using colors, shapes, and arrangements. Gram-positive organisms kept the crystal's purple hue, while Gram-negative ones kept safranin's pink hue.

Potassium Hydroxide (KOH) Test

In addition to Gram staining, the potassium hydroxide (KOH) test was used to identify or confirm Gram-negative bacteria and to easily distinguish between Gram-negative and Gram-positive bacteria. Gram-negative bacteria's thin peptidoglycan cell walls are broken down by KOH, but the thick layer of Gram-positive bacteria's cell walls is unaffected. Bacterial cell walls being damaged causes the cell to lyse and release its contents, which includes the genetic material. On a labeled, clean microscope slide, a drop of 3% KOH solution was put, and using a loop, pure, isolated culture was spread across it. With careful stirring, it was noticed that the liquid transformed into a viscous or dense suspension, which within 60 seconds formed a slimy or mucoid string. The appearance of that signaled a good result as the existence of Gram-negative isolates. While the outcomes for non-slimy viscous suspensions remained negative.

3.4 Bacterial Isolation and Identification

Bacteria from the corresponding samples were isolated, characterized, and identified using conventional bacteriological methods. Biochemical testing and colonial and morphological features were also considered as criteria (Cheesbrough, 2000). Catalase, oxidase, indole, urease, citrate, KOH tests, TSI test, and lactose fermentation are a few examples of biochemical testing.

Biochemical Tests

Biochemical assays, including the following, were carried out to more fully define these isolates:

Catalase test

These two types of bacteria—those that produce the catalase enzyme, like Staphylococci, and those that do not, like Streptococci—were distinguished using this test. Hydrogen peroxide (H2O2) is broken down by catalase into oxygen (O2) and water (H2O). In this experiment, 2 mL of hydrogen peroxide solution was placed into a test tube. Using a sterile glass rod, several colonies of the test organism were selected and submerged into the H2O2 solution. Gas bubbles (oxygen) were formed by the bacteria that produced catalase (positive result), but none were produced by the bacteria that did not (negative result).

Oxidase test

The oxidase test was used to find out whether there was an enzyme called cytochrome oxidase or indophenol oxidase in the bacteria that would catalyze the transfer of electrons between the bacterium's electron donors and a redox dye called tetramethyl-p-phenylene-diamine. If given to favorable reactions, the dye would be reduced to a deep purple color. For this work, 1% tetra-methyl-p-phenylenediamine dihydrochloride in water was employed as the Kovacs oxidase reagent. The pure culture was smeared on the filter paper using a platinum loop after it had been saturated with a Kovacs oxidase reagent solution. Within 10 to 60 seconds, color development was permitted and monitored. The presence of a deep purple-blue or blue color showed the presence of oxidase, whereas the absence of color change represented a negative outcome.

Citrate Utilization Test

The ability of organisms to use citrate as their main source of energy is determined by a test called the citrate utilization test. To distinguish between Enterobacteriaceae species that can ferment citrate in the presence of the citrate enzyme, a citrate test was carried out. Simon's citrate agar, which was made for inoculation on Petri dishes, had citrate as a substantial energy source. By streaking the surface with a sterilized loop, pure isolated culture was inoculated onto well-prepared, sterilized citrate agar plates. After that, the plates were incubated for 24 hours at 37°C. A positive citrate test resulted from color changes brought on by bacteria growing on the medium as a result of citrate metabolism. The bromothymol blue indicator in the medium changes from green to blue as a result of the pH change (good outcome). A negative test was shown to occur when there was no growth, no color change, or when the medium's color remained green.

Indole test

The capacity of specific bacteria to convert the amino acid tryptophane to indole was demonstrated using an indole test. The indole synthesis test is crucial for locating the Enterobacteriaceae family, which degrades the amino acid tryptophan by producing indole in the presence of intracellular enzymes referred to as "tryptophanase." On a piece of filter paper, several drops of Kovac's indole reagent were applied. With an inoculating loop, a part of a pure isolated colony was taken from the TSA pure culture and spread onto the filter paper that had been saturated with reagent. Within two to three minutes, it was permitted and studied to watch for color development. In this spot test, indole reacted with the chemicals in the matrix of the filter paper to change the color of the bacterial smear from blue to blue-green, while unfavorable reactions remained colorless or light pink.

Urease test

The urease test is used to determine which bacteria are able to produce urease. Urease-secreting organisms have the ability to hydrolyze urea into ammonia and carbon dioxide. The urease-positive bacteria were distinguished from other Enterobacteriaceae using this assay. The Christensen-modified urea broth was thoroughly prepared and autoclaved before the isolated pure bacteria were added, and the mixture was then incubated for 24 hours at 37°C. Because the indicator's color changed when ammonia was present, urease-positive cultures generated a pink color, whereas the negative result was either no color change or a yellow-orange color.

Mannitol Test

The mannitol test is a selective (high salt concentration is present; sodium chloride inhibits the majority of Gram-negative and Gram-positive bacteria) and differential (the organism's capacity to ferment or not the mannitol) test. Mannitol can be fermented, which causes acidity and changes the medium's color from red to yellow. Mannitol salt agar was properly prepared, autoclaved at 121°C for 15 minutes, chilled, and then plated. Pure isolates were used as the inoculum, which was then incubated for 24 hours at 37°C. Positive growth outcomes included yellow zones and yellow colonies. The unfavorable outcome continued to be red-pink with growths.

Triple Sugar Test

An organism's capacity to ferment sugars and create hydrogen sulfide (H2S), gas (O2), or both is tested using the Triple Sugar Iron (TSI) test. The test was primarily used to distinguish Enterobacteriaceae family members from other Gram-negative rods based on their patterns of sugar fermentation. This test was performed in a sterile test tube at a slanted angle using an agar slant made of TSI agar. Using a straight

inoculation needle, TSA pure culture was streaked around the surface of the agar slant before being injected into the slanted medium through the tube's center. Following inoculation, the test tubes were wrapped in foil paper and incubated for 24 hours at a temperature of 36°C. When test tube reactions were observed, sugar fermentation was revealed by the release of H2S gas and a switch in color from red to yellow (acid). The only indication of dextrose (glucose) fermentation was the appearance of an alkaline/acid (red top/yellow bottom) slant response. Acid/acid (yellow top/yellow bottom) slant reactions indicated the fermentation of dextrose, lactose, and/or sucrose as they manifested themselves. In the absence of sugar fermentation, an alkaline/alkaline (red top/red bottom) slant reaction appeared. H2S production was shown by the blackening of the medium in the slant. Gas production (creation of CO2 and H2) was detected in the slanted agar by bubbles, fissures, or bottom-raised space.

3.7 Growth on Differential Media

3.7.1 Base of Bacillus Cereus Agar

Bacillus species and pathogenic Staphylococci species were identified and isolated using Bacillus cereus agar. Gram-negative bacteria can grow more slowly on Bacillus cereus agar, and this differentiating medium enables the differentiation of Gram-positive Bacillus species. Bacillus cereus agar that had been dissolved was autoclaved at 121°C for 15 minutes before being allowed to cool and being placed onto Petri plates. Pure isolated cultures were streaked into the medium as an inoculum, and the plates were then incubated for 24 hours at 37°C. The morphology of the spores, colony shapes, and color patterns were used to identify typical growths on the plates.

3.7.2 Mannitol Salt Agar (MSA)

The majority of the Staphylococcus species, which were produced and autoclaved at 121°C for 15 minutes, were selected and differentiated using MSA before being cooled and placed into Petri dishes. After streaking the isolated pure cultures onto the medium, the plates underwent a 24-hour incubation period at 37°C. Plates were examined, and usual growth was noted.

3.7.3 Pseudomonas Agar

Fluorescein is a pigment that is frequently generated by Pseudomonas species. The synthesis of pigment by Pseudomonas species was examined using Pseudomonas agar. In addition to isolating Pseudomonas species, Pseudomonas agar is a selective and differential medium that prevents both Gram-positive and Gram-negative bacteria from growing. After the medium had been autoclaved at 121°C for 15 minutes, cooled, and poured into plates, petri dishes were ready for inoculation. The isolated pure inoculums were used to contaminate the plates, which were then incubated for 24 hours at 37°C. It was then studied and checked for growths; the discovery of growths was confirmed by the presence of cream to greenish-yellow coloration in the agar, which can glow when exposed to UV light.

3.7.4 Eosin Methylene Blue (EMB) agar

By discriminating between organisms that digest lactose and those that cannot handle a color signal, Eosin Methylene Blue (EMB) agar is a differential medium that prevents the growth of Gram-positive bacteria. It is used to show Gram-negative pathogenic enteric bacteria. EMB was used to produce a sterile petri plate, which was then autoclaved at 121°C for 15 minutes, allowed to cool, and streaked with pure inoculums. After 24 hours of incubation at 37°C, inoculated plates were checked for changes in colonial morphology. In contrast to lactose non-fermenters, which were colorless, lactose-fermenting bacteria generated dark colonies with a green metallic sheen or pink mucoid colonies (positive result).

3.8 Molecular Characterization and Genomic Extraction of Deoxyribonucleic Acid

In order to extract deoxyribonucleic acid (DNA), the boiling technique described by Chakravorty et al. (2007) was used. In conclusion, a nutrient broth culture of the bacteria in 2 mL was transferred to an Eppendorf tube and centrifuged for 5 minutes at 10,000 xg (high speed). The pellets were combined with 200 µL of sterile distilled water (SDW) by vortexing for 1 minute and heating at 100°C for 15 minutes after the supernatant was discarded. The combination was then centrifuged at high speed for 2 minutes, and the resulting supernatant was regarded as pure DNA. From there, 10 µL of DNA were used for PCR gene amplification. Using the 1540R primer (5'-

TACGGYTACCTTGTTACGACT-3') and the 27F primer (5'-AGAGTTTGATCMTGGCTCAG), the genomic bacterial DNA was successfully amplified.

3.8.1 Polymerase Chain Reaction (PCR)

Following usual protocol, a reaction mixture (cocktail) made up of different ingredients was used in the produced PCR. These contained 1 µL of each primer (1540R-5'-TACGGYTACCTTGTTAACT-3' and 27F-5'-AGAGTTTGATCMTGGCTCAG-3'), 10 µL of 5X GoTaq®, 3 µL of MgCl2 (25 mM), 1 µL of dNTPs mix (10 mM), and 0.3 u/µL of Taq DNA polymerase. The amount of sterile distilled water (SDW) and DNA template added to the reaction mixture was adjusted to a total volume of 42 µL (Taq DNA polymerase from Promega, United States). A GeneAmp 9700 thermal cycler (produced in the United States by Applied Biosystem Inc.) was used to conduct the experiment (PCR). An initial denaturation phase at 94°C for 5 minutes made up the PCR profile. This was followed by 30 cycles of annealing for 1 minute at 50°C, extension for 1 minute and 30 seconds at 72°C, and denaturation for 30 seconds at 94°C. Extension termination was performed during the final 10 minutes at 72°C. Following the completion of the aforementioned stages, the PCR result was kept refrigerated at four degrees Celsius until it was ready for use.

3.8.2 Amplified DNA Integrity

The generated bacterial DNA fragments were purified using ethanol to get rid of any PCR chemicals that might have been present in the samples, ensuring the integrity of the gel. Initially, 240 µL of 95% ethanol and 7.6 µL of 3M sodium acetate were put into a fresh, sterile 1.5 mL Eppendorf tube. 40 µL of the amplified PCR product were added to this mixture, vigorously mixed with a vortexer, and then kept at -20°C for at least 30 minutes. The tube was then properly discarded of the supernatant by flipping the tubes once over a trash can after being centrifuged at 13,000 x g for 10 minutes at 4°C. The pellets were then thoroughly mixed after which 150 µL of 70% ethanol was added to wash them. A second cycle of centrifugation took place for 15 minutes at 7500 x g and 4°C. Once more, the supernatant was eliminated by inverting the tubes over tissue paper, and the pellets were then dried for about 10-15 minutes at room temperature in a fume closet or hood. 20 µL of sterile distilled water (SDW) was used to resuspend the mixture, and the cleaned sample was then kept at -20°C until further sequencing. The integrity of the purified fragment was examined using a 1.5% agarose gel. For one hour, the gel was run at 110V. With the use of a Thermo Scientific Nano-Drop model 2000 UV-Vis spectrophotometer, the integrity was verified as previously stated.

3.9 Phenotypic Virulence Test

Based on their phenotypic virulence characteristics, such as gelatinase, deoxyribonuclease (DNase), lipase, and hemolysin, the isolates' pure cultures were identified and described.

3.9.1 Nutrient Gelatin Test

Based on their capacity to hydrolyze gelatin, nutrient gelatin was used to classify and separate gelatinase-producing bacteria into several groups. The ability of an organism to manufacture gelatinase and its capacity to liquefy gelatin in the presence of extracellularly secreted gelatinase enzyme were both tested using the gelatin liquefaction test. After dissolving nutrient gelatin, 5 mL of a medium were added to a test tube, which was then foil-wrapped. The test tubes and the medium were autoclaved at 121°C for 15 minutes. Before injection, the tubed medium was allowed to cool in an upright position. The sterile tubes containing nutritional gelatin were stab-inoculated with the isolated pure inoculum. For up to 48 hours, the infected tubes were incubated at 37°C. To establish that the gelatin liquefaction was caused by gelatinase activities, the tubes were later submerged in an ice block and bathed for 30 minutes. If any gelatinase-positive bacteria were present in the stabbed-inoculated tubes, the produced gelatinase would hydrolyze the gelatin, causing the medium to liquefy. After being subjected to a cold treatment, gelatin medium that had been inoculated with a gelatinase-negative bacterium remained firm.

3.9.2 Dnase Test Agar

The DNase test was used to distinguish non-pigmented strains from the majority of other Enterobacteriaceae and to assess an organism's capacity to produce the extracellular DNase enzyme. DNases are DNA hydrolyzing enzymes that discharge phosphate and

free nucleotides, which are smaller monomers that are easily absorbed by bacterial cells. The DNase agar was properly prepared, autoclaved at 121°C for 15 minutes, allowed to cool, and then put into sterile petri plates. Pure isolated inoculums were used to inoculate agar plates, which were then incubated for 24 hours at 37°C. DNase-positive strains were identified based on the DNA-digested zone that was clear or transparent and surrounded the colonies. The absence of a cleared zone represented a failure of the test.

3.9.3 Spirit Blue Agar

The identification of lipolytic bacteria that can produce the lipase enzyme was done using spirit blue agar. Triglycerides are hydrolyzed by the secretion of lipase into glycerol and three long-chain fatty acids. Because of their size, these monomers can get through the bacterial cell wall. After dissolving the spirit blue medium powder, it was autoclaved at 121°C for 15 minutes. The lipoidal substrate was cooled and 5% sterile olive oil was well blended. Using isolated, pure inoculums, the mixture was placed into Petri dishes where it was incubated for 24 hours at 37°C. When colonies of the lipolytic organisms were observed on plates, a cleared zone or a deep blue hue appeared surrounding and beneath each colony.

3.9.4 Hemolysis of Blood Agar Base

The different types of hemolytic reactions were studied using sheep blood agar base or Columbia agar blood base for the isolation, identification, and antimicrobial susceptibility testing of fastidious and non-fastidious microorganisms. Haemolysin is an exotoxin produced by bacterial extracellular enzymes that lyse red blood cells. To make the blood agar plates for the medium, an enrichment culture, sheep blood is preferred over other blood types, such as horse, rabbit, or goat blood. Sheep blood agar was made, sterilized by autoclaving at 121°C for 15 minutes, chilled, and properly enriched with 1% sterile sheep blood. The following cultural traits were seen when it was incubated at 37°C for 24 hours using isolated pure inoculums that were dispersed into Petri dishes. On the colonies of the sheep blood agar plate, various types of hemolysis were reported as follows: a greenish discolouration zone appeared surrounding the colonies as a result of partial hemolysis (hemoglobin to methemoglobin); red blood cells are completely lysed in beta hemolysis, leaving a clear or translucent area around the colony; and gamma-hemolysis showed no hemolysis, leaving the medium unchanged.

3.10 Antimicrobial Susceptibility Test

The method used to test for antibiotic susceptibility was the Kirby-Bauer disk diffusion test. On Muller Hinton agar, eight (8) antibiotic sensitivity discs were tested against bacterial isolates according to the Clinical and Laboratory Standards Institute's (CLSI, 2020) recommendations. These discs match medications frequently used to treat human and animal infections. Using the disc diffusion technique on Mueller-Hinton agar, it was determined that the test isolates were sensitive to standard antibiotics. Using sterile swabs, 0.1 ml of standardized bacterial solution was dispersed onto a plate using standardized bacterial cells (0.5 McFarland turbidity standard). Discs containing antibiotics were aseptically placed on the plates after they had dried. The antibiotic disks contain the following medications: CIP, Ciprofloxacin (5 mg), CRO, Ceftriaxone (30 mg), RL, Sulfamethoxazole (1.25/23.75 mg), E, Erythromycin (15 mg), TET, tetracycline (30 mg), MEM, Meropenem (10 mg), AMC, Amoxicillin/clavulanic acid (20 mg), and CN, Gentamicin (10 mg). After 24 hours of incubation at 37°C, the growth inhibition zones around the antibiotic discs on the plates were measured. According to the CLSI standard guidelines (CLSI, 2020), zones of inhibition were measured, analyzed, and growths were detected. A phenotypic drug resistance pattern was developed among the isolated bacteria using the data acquired.

3.11 Evaluation of Multiple Antibiotics Resistance (MAR) Index

For the discovered isolates, the multiple antibiotic resistance (MAR) index was calculated using the standard formula, where y = number of resistances assessed, n = number of isolates, and x = number of antibiotics. The typical allowable limit for MAR index is 0.2. As a result, bacteria isolated were declared resistant when their evaluations were over the permissible limits, which could have negative effects on health (Olannye et al., 2022).

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3.12 Statistical Analysis

One-way ANOVA and the Tukey HSD test were used to evaluate the data from this study in R Studio, and PhyloT was utilized to examine sequencing data to determine the degree of relatedness between bacterial isolates included in the study.

IV. RESULTS

The range of the bedsheets' total bacterial mean heterotrophic count, which was examined, was 8.5 to 7.25. Figures 1 through 21 depict how each student handled the bedsheets during the study and how the participants responded to the questionnaire. Males made up 41.5% of the respondents to this study, while females made up 58.6%. The percentage of respondents who had bedsheets and used them were 98.9% while those that preferred not to say was 1.1%. Their frequencies of changing their bedsheets are as follows; once a week 38.9%, twice a week 9.5%, once in two weeks 28.4%, once a month 9.5% and others 12.6% respectively. The percentage of those that changed their bedsheets routinely were 54.7%, those that changed it when it smells were 11.6%, those that changed it when it stained were 5.3%, those that changed it when they felt like were 26.3% and those who preferred not to say were 2.1%. The last time they washed their current bedsheet had the following percentages; two weeks ago 15.8%, last week 38.9%, this week 25.3% and those that could not remember were 20% respectively. The number of bedsheets each respondent had are as follows; people with no bedsheets 11.6%, those with only one bedsheet 17.9%, those with two bedsheets 37.9% and those with three bedsheets 32.6%; the number of people using a bedsheet together had the following percentage: 2-3 persons 32.7%, 3-4 persons 4.2%, above 4 persons 4.2% and just the respondent 58.9%.

Figure 23 shows the multiple antibiotics index of the isolates. Escherichia coli showed the highest Multi Resistance Antibiotics Index (MARI) at 35.72. Followed by Enterobacter cloacae which had a MARI of 28.57. Bacillus mycoides had a MARI of 21.43. The lowest MARI was recorded by Serratia marcescens at 0.55.

Table 4.2 shows the biochemical results of the bacterial isolates. Organisms identified include Escherichia coli, Enterobacter cloacae, Bacillus mycoides, and Serratia marcescens.

Table 4.4 shows the antibiotics sensitivity pattern of the bacteria isolates. Enterobacter cloacae showed the highest resistance to antibiotics, resisting four of the antibiotics it was tested against. Bacillus mycoides showed the highest susceptibility to almost all the antibiotics it was tested against, followed by Serratia marcescens. Only Escherichia coli showed a varying resistance pattern to the test antibiotics.

Table 4.3 shows the pathogenicity results of all isolates obtained in this study. All organisms showed β hemolysis, which shows its ability to lyse red blood cells.

The chart below compares the proportion of respondents who lived in university hostels on campus with those who lived off campus.

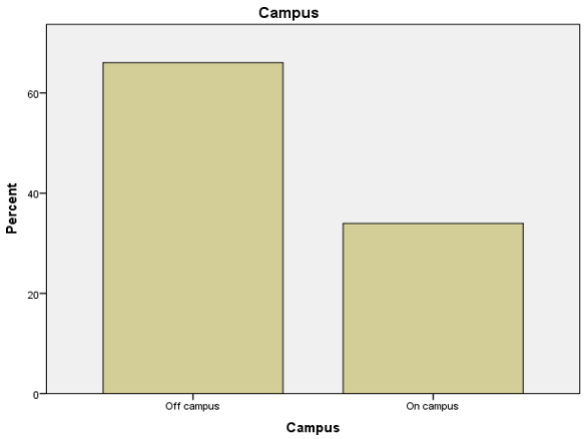

Fig 1: showing Data of students both on campus and off campus.

The chart below breaks down off-campus respondents by their specific residential area around the university.

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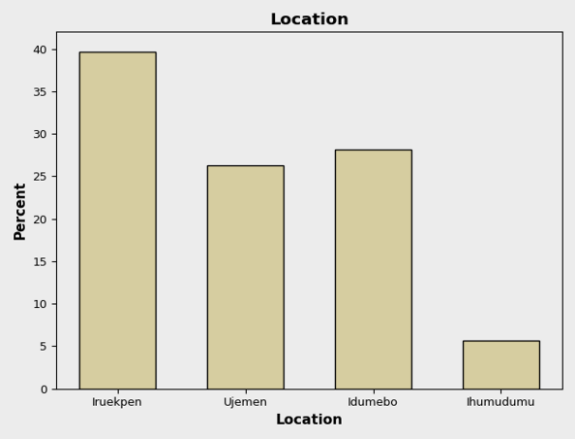

Fig 2: showing Data of students residential Location (off campus)

The chart below presents the gender distribution of the respondents.

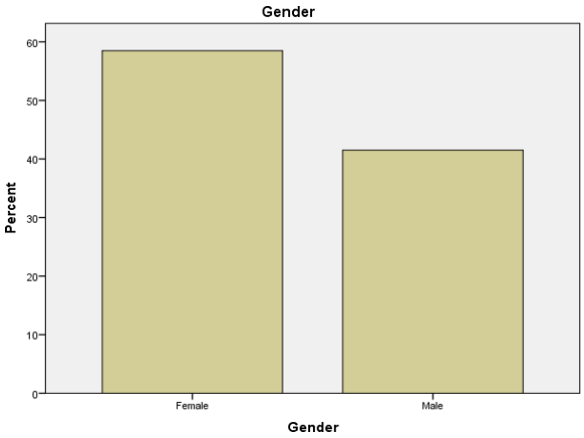

Fig 3: showing the gender of the respondents.

The chart below shows the proportion of respondents who were students compared with those who were not.

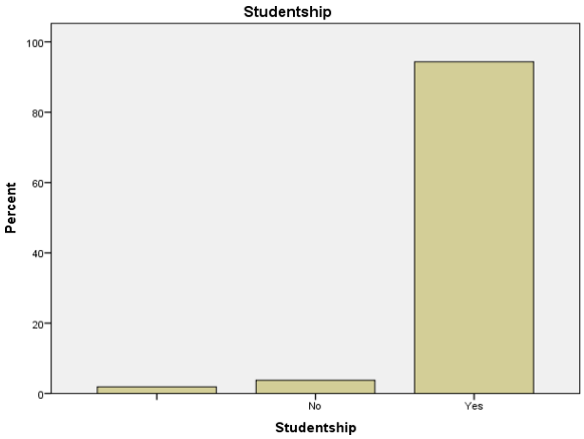

Fig 4: showing the studentship of the respondents.

The chart below shows the academic level distribution of the student respondents.

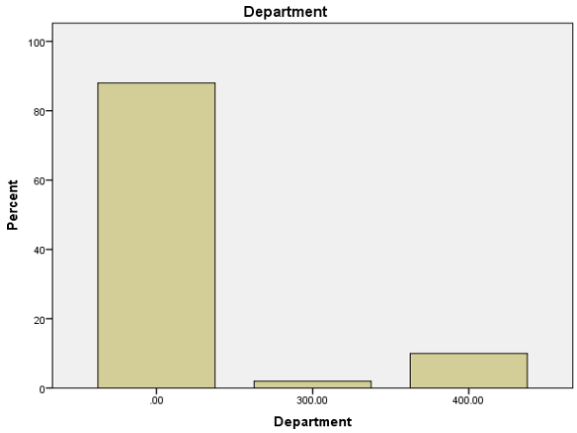

Fig 5: showing the Level of the respondents.

The chart below shows the distribution of bed/bedsheet sizes used by respondents.

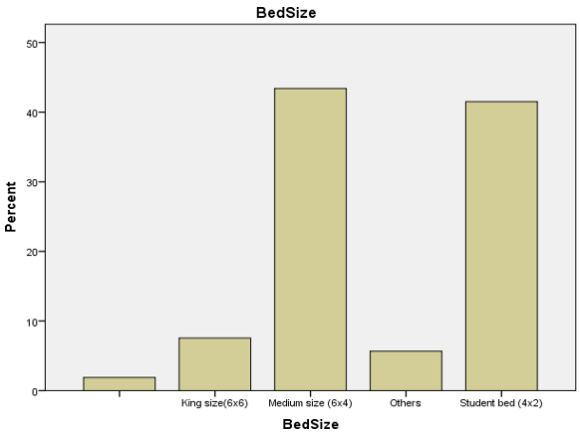

Fig 6: showing the Bed Size of the respondents.

The chart below shows the number of occupants per room reported by respondents.

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Fig 7: showing the Occupants of the respondents.

The chart below shows the proportion of respondents who owned and used a bedsheet.

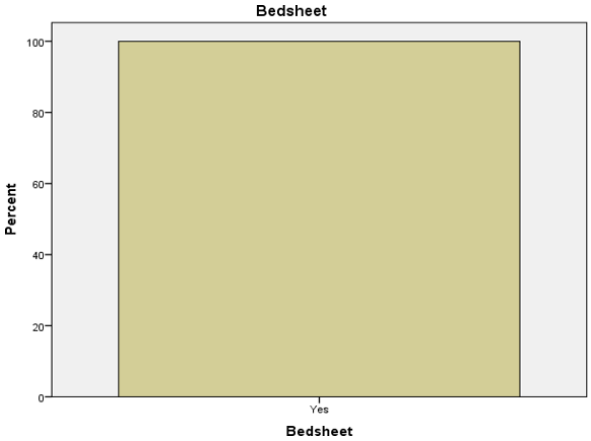

Fig 8: showing the Data of respondents with Bed sheet.

The chart below shows how respondents reported using their bedsheets.

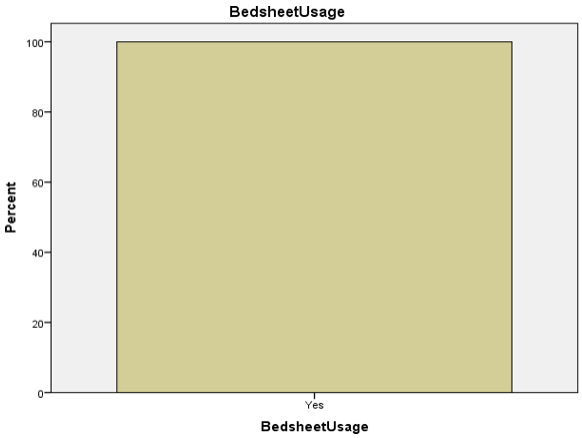

Fig 9: showing the Data of respondents with Bed sheet usage.

The chart below shows the number of bedsheets owned per respondent.

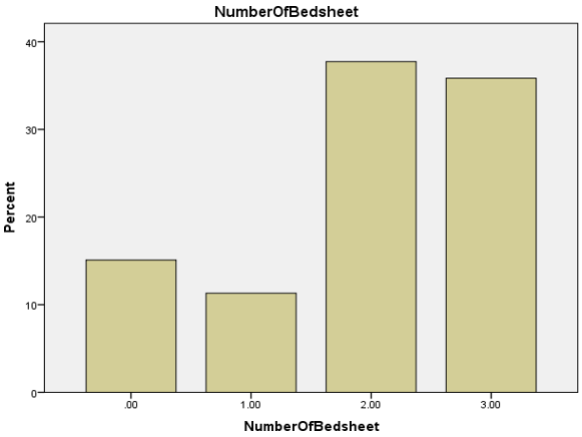

Fig 10: showing the Data of respondents with Number of bed sheet.

The chart below shows how often respondents reported changing their bedsheets.

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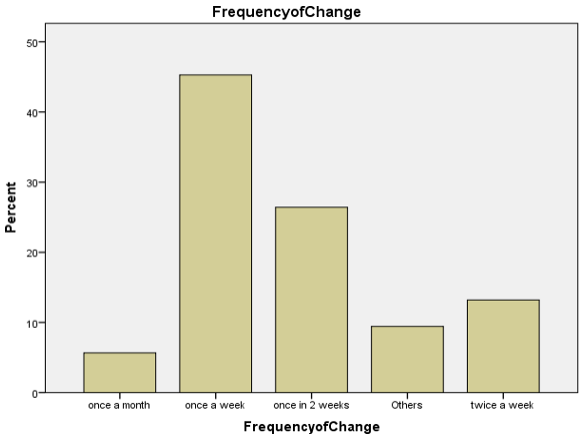

Fig 11: showing the Data of respondents with Frequency of change.

The chart below shows the circumstances under which respondents reported washing their bedsheets.

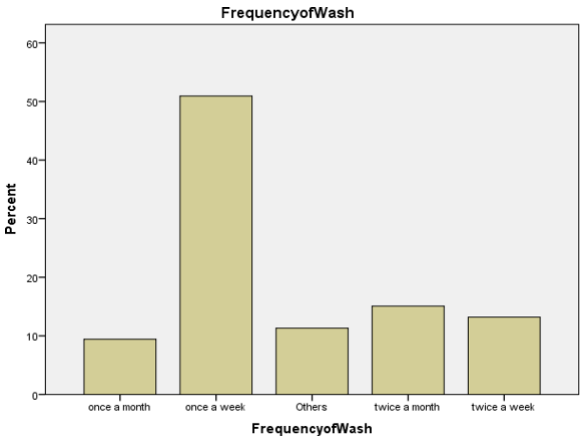

Fig 12: showing the Data of respondents with Frequency of wash.

The chart below shows the number of persons sharing a bedsheet among respondents.

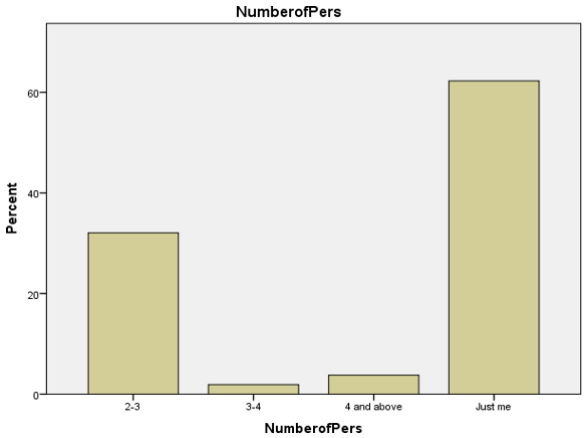

Fig 13: showing the Data of respondents with Number of pers.

The chart below shows the proportion of respondents who slept on a mattress without a bedsheet.

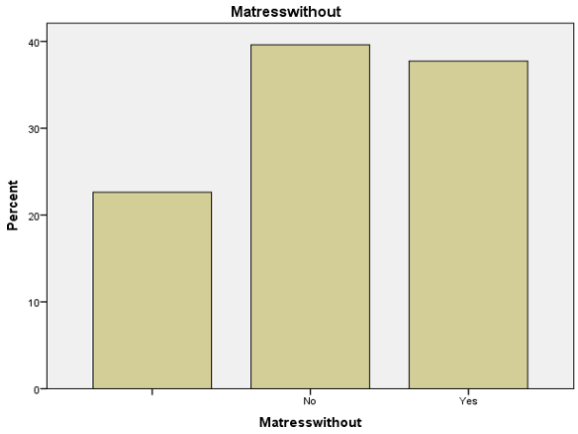

Fig 14: showing the Data of respondents with Mattress without.

The chart below shows how frequently respondents reported having visitors on their beds.

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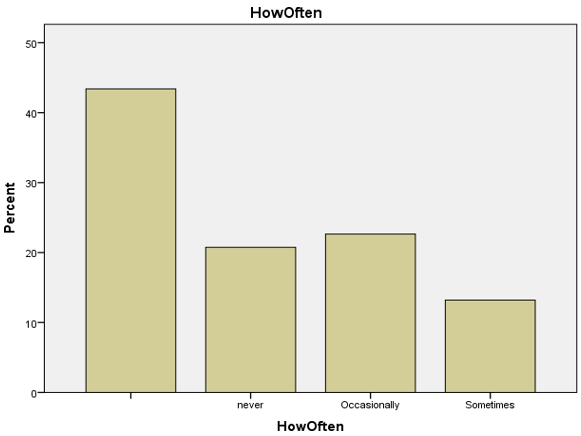

Fig 15: showing the Data of respondents with how often.

The chart below shows when respondents last changed their bedsheet.

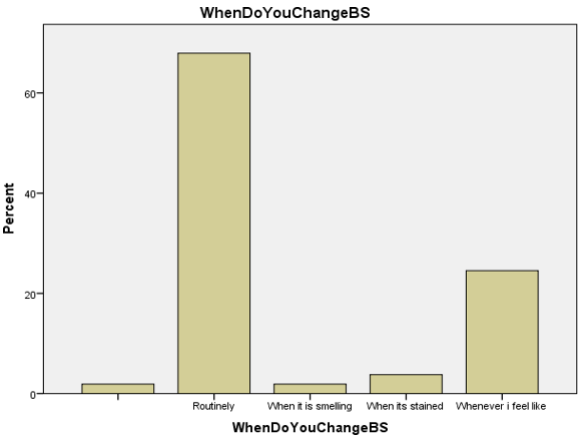

Fig 16: showing the Data of respondents with when do you change BS.

The chart below shows respondents' awareness of bedsheets as a reservoir for microorganisms.

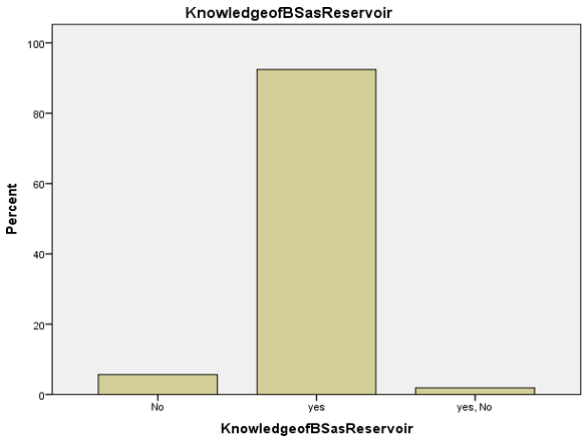

Fig 17: showing the Data of respondents with Knowledge of BS as Reservoir.

The chart below shows when respondents last washed their current bedsheet.

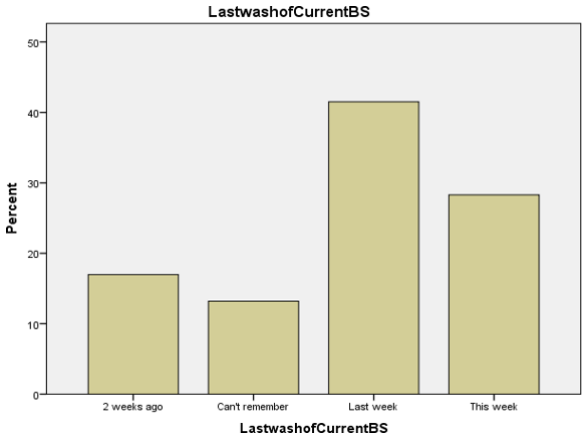

Fig 18: showing the Data of respondents with Last wash of current BS.

The chart below shows the proportion of respondents who reported sleeping unclad on their bedsheet.

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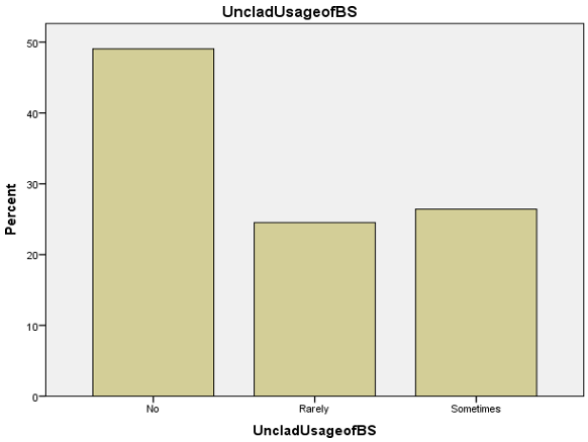

Fig 19: showing the Data of respondents with Unclad Usage of BS.

The chart below shows the proportion of respondents who habitually kept items on their bed.

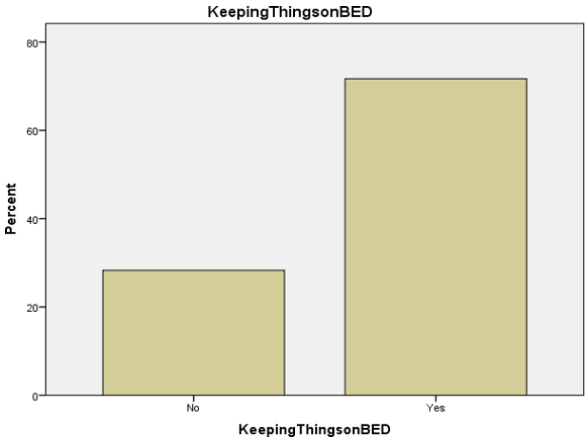

Fig 20: showing the Data of respondents with Keeping Thing.

The chart below relates the heterotrophic bacteria burden recorded on bedsheets to how often the respondents reported having visitors.

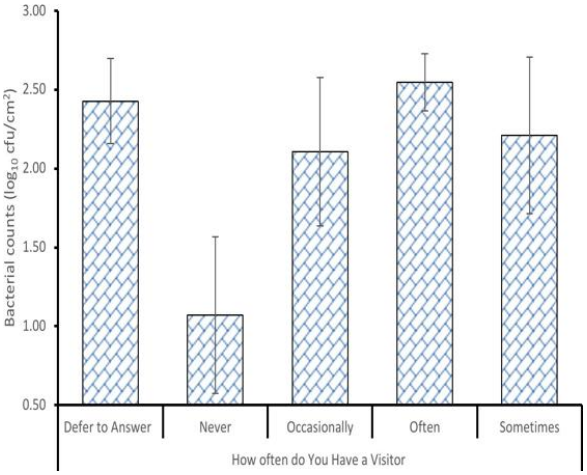

Figure 21: Heterotrophic bacterial burden of bedsheets based on how often they have visitors

Table 4.2 Cultural morphological and biochemical characteristics of bacteria obtained from bedsheets

The table below presents the cultural, morphological, and biochemical characteristics used to identify each bacterial isolate.

Characteristic	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Elevation	Flat	Flat	Flat	Raised
Margin	Undulate	Undulate	coarse	Entire
Color	Cream	Cream	milk white	Cream
Shape	Irregular	Irregular	concave	Circular
Size	Large	Large	large	Medium
Gr. diff. agar	EMB	EMB	BCA	EMB
Colour	green	purple	Straw	opaque
Gram stain	-	-	+	-
Cell type	Rod	Rod	Rod	rod
Arrangement	disperse	disperse	disperse	disperse
Color (stain)	pink	pink	purple	pink
Spore staining	-	-	+	-
KOH String Test	+	+	-	+
Catalase	+	+	+	+
Indole	+	-	-	-
Citrate	-	+	-	+
Oxidase	-	-	-	-

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Characteristic	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Motility	+	+	+	+
Urease	-	-	-	-
Glucose	+	+	+	+
Sucrose	-	+	-	+
Lactose	+	+	-	+
Mannitol	-	-	-	+
Gas formation	+	+	-	-
H2S formation	-	-	-	-
TSI (Slant/Butt) reaction	A/AG	A/AG	K/A	K/A (*A/A)
Esculin Hydrolysis	-	(+/-)	+	-
Identity	E. coli	Enterobacter cloacae	Bacillus mycoides	Serratia marcescens

The chart below shows how frequently each bacterial isolate occurred across the bedsheet samples.

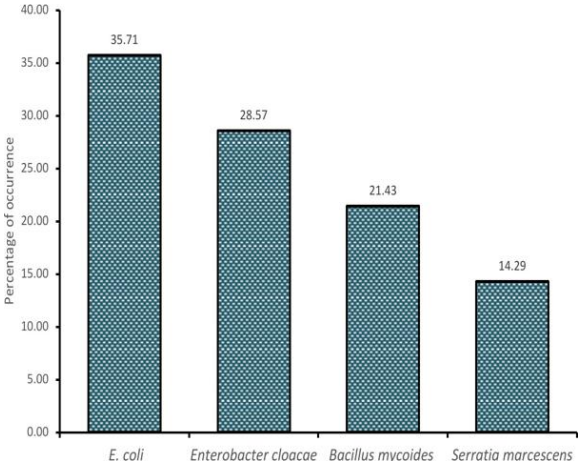

Figure 22: Frequency of occurrence of bacterial isolates from Bedsheets

Table 4.3 Phenotypic virulence properties of the bacterial isolates from bedsheet

The table below presents the phenotypic virulence properties (DNase, lipase, and haemolysin activity) recorded for each bacterial isolate.

Bacterial Isolates	DNase	Lipase	Hemolysin
E. coli	-	+	β
Enterobacter cloacae	-	+	β
Bacillus mycoides	-	+	β
Serratia marcescens	-	+	β

Key: β = Beta hemolysis, + = positive; - = negative

Table 4.4 Antibacterial sensitivity of bacterial isolates obtained from Bedsheet samples

The table below presents the antibiotic sensitivity/resistance pattern recorded for each bacterial isolate.

Isolates	CD	E	TE	GEN	M	AG	CS	CB
E. coli	S	I	I	S	R	S	R	R
Enterobacter cloacae	S	S	R	S	R	S	R	R
Bacillus mycoides	S	S	S	S	R	S	R	S
Serratia marcescens	S	S	S	S	R	S	R	R

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

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The chart below presents the Multiple Antibiotic Resistance (MAR) index calculated for each bacterial isolate.

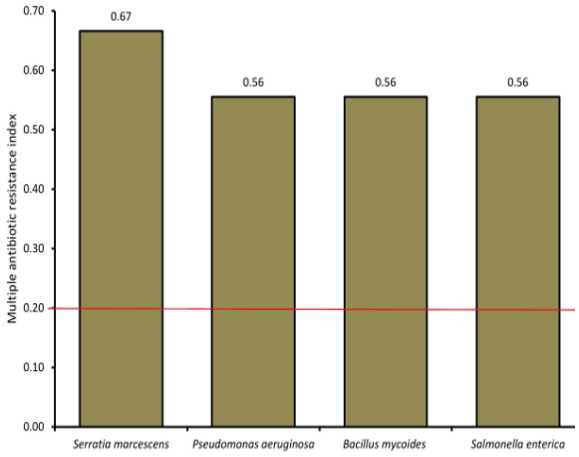

Figure 23: Multiple antibiotic resistance index of bacterial isolates

V. DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study showed that there is a high prevalence of bacterial contamination from bedsheet samples used by students at Ambrose Alli University, Ekpoma. Result of the questionnaire administered showed that 58.6% of the students were females and 41.5% were males. The percentage of people who were students were 92.6% and the percentage of non-students were 6.3%. The level of the students starting from the least levels, 200L was 1.1%, 300L 2.2%, 400L 6.6% and the ones that preferred not to say were 90.1% respectively. The percentage of students who were resident on campus was 36.8%, while those who stayed off campus were 62.1%. Among the on-campus residents, 33.7% resided within Ambrose Alli University campus hostels, while off-campus residents were drawn from Iruekpen, Ujemen, Idumebo, Ihumudumu, and Town. Number of occupants per room had the following percentage; 2-3 occupants had 43.2%, 4-6 had 12.6%, 7 and above had 23.2% and just the respondent 21.1% respectively. Their bedside/bedsheet size had the following percentage; medium sizes (6×4) 30.5%, king-sizes 6.3%, student bed (4×2) 56.8%, other 4.2% and those that preferred not to say were 2.1% respectively. The percentage of respondents who had bedsheets and used them were 98.9% while those that preferred not to say was 1.1%. Their frequencies of changing their bedsheets are as follows; once a week 38.9%, twice a week 9.5%, once in two weeks 28.4%, once a month 9.5% and others 12.6% respectively. The percentage of those that changed their bedsheets routinely were 54.7%, those that changed it when it smells were 11.6%, those that changed it when it stained were 5.3%, those that changed it when they felt like were 26.3% and those who preferred not to say were 2.1%. The last time they washed their current bedsheet had the following percentages; two weeks ago 15.8%, last week 38.9%, this week 25.3% and those that could not remember were 20% respectively. The number of bedsheets each respondent had are as follows; people with no bedsheets 11.6%, those with only one bedsheet 17.9%, those with two bedsheets 37.9% and those with three bedsheets 32.6%; the number of people using a bedsheet together had the following percentage: 2-3 persons 32.7%, 3-4 persons 4.2%, above 4 persons 4.2% and just the respondent 58.9%. Then people laying on the mattresses without bedsheets had 48.4%, those that use the bedsheets 38.9%, and those that preferred not to say 12.6% respectively. Those that had visitors occasionally had a percentage of 29.5%, those that seldomly had visitors were 15.8%, those that had visitors were 18.9%, those that had visitors often were 3.2% and those that preferred not to say were 32.6%. Those that had knowledge of bedsheet as a reservoir of microorganisms were 92.6%, those that had no idea were 6.3%, those that were not sure was 1.1%. 72.6% of people had habits of keeping things on the bed, while 27.4% do not, and finally the numbers of people that slept on bed unclad had a percentage of 30.5%, those that do not 54.7% and those that rarely sleep unclad on bed 14.7% respectively. The average number of bacteria per milliliter at the anterior and posterior ends was 8.5 and 7.25, respectively.

In this study's analysis of bedsheet samples, Escherichia coli, Bacillus mycoides, Serratia marcescens, and Enterobacter cloacae were among the isolated bacterial species. Escherichia coli is a gram-negative, non-sporulating, rod-shaped, facultatively anaerobic, and coliform bacteria that often lives in the environment, food, and the lower stomach of warm-blooded animals (Campbell and Reece, 2002). The primary niche for E. coli is the gastrointestinal (GI) tract of humans and many other warm-blooded

animals. Warm-blooded animal intestines and the environment (water, sediment, and soil) are its two main habitats, and they are both very diverse in terms of their physical characteristics, range of nutrient availability, and quantity of nutrients available. Between E. coli and its host, a symbiotic relationship develops. On the other side, E. coli strains that produce carbapenemase have genes (such as imipenem, ertapenem, and meropenem) that confer carbapenem resistance. A rapidly developing class of β-lactamases, of which E. coli are the main producers, are known as ESBL-producing E. coli (Basavaraju and Gunashree, 2022).

Serratia marcescens is an opportunistic, nosocomial, gram-negative pathogen that is a member of the Enterobacteriaceae family. Although Serratia marcescens, a motile, gram-negative bacillus that belongs to the Enterobacteriaceae family and is widely present in the environment, seldom causes human disease, it is associated with infections caused by opportunistic and nosocomial bacteria. Ambient cultures were taken from numerous surfaces, including walls, floors, corners, door handles, shelves, sinks, soap dispensers, ventilators, stethoscopes, and other medical equipment. The isolates were obtained from various samples, including blood, urine, and pus. A different investigation revealed that whereas Serratia marcescens isolates were susceptible to imipenem, meropenem resistance was high. As a result, depending on whether these antibiotics were previously utilized in the units, their susceptibilities to the antimicrobials may vary (Ashish et al., 2022). From the questionnaire administered, the population of those who changed their bedsheets when it is stained were 5.3%.

A member of the B. cereus group, Bacillus mycoides is a sporogenic, Gram-positive soil bacillus. This bacillus, which creates hyphal colonies, is made up of cells that are linked together in bundles that, depending on the strain, rotate either clockwise or counterclockwise. The member of this group that has garnered the least attention is B. mycoides since it is neither an insecticide like B. thuringiensis nor as dangerous to humans as B. anthracis and B. cereus (Luana et al., 2012). From the questionnaire administered, the number of people that slept on bed unclad had a percentage of 39.5%.

A widespread genus of rod-shaped, non-spore-forming, Gram-negative, facultatively anaerobic bacteria of the Enterobacteriaceae family is called Enterobacter. Two of its well-known species, Enterobacter aerogenes and E. cloacae, have emerged as nosocomial infections from intensive care patients and have gained clinical significance as opportunistic bacteria, especially for those who are on mechanical ventilation (Mezzatesta et al., 2012). Water, sewage, soil, and food all contain Enterobacter cloacae, which is common in both terrestrial and aquatic habitats. The species can be found as pathogens in plants and insects as well as commensal microflora in the intestines of humans and other animals. Due to the synthesis of constitutive AmpC β-lactamase, Enterobacter cloacae naturally resists ampicillin, amoxicillin, first-generation cephalosporins, and cefoxitin. It frequently displays enzymatic resistance to cephalosporins with a wide spectrum of activity (Anne and Jane, 2015).

5.2 CONCLUSION

The ability of bed linens to act as a reservoir and a means of microbial spread during an outbreak of disease has been demonstrated by the microbiological assessment of bed linens undertaken among students of Ambrose Alli University, Ekpoma. The detected species are opportunistic pathogens for humans and pose a concern to those with compromised immune systems, particularly in situations of superinfection. Due to the ease with which these bacteria might develop antibiotic resistance, it is imperative to adopt an efficient infection control policy that takes student wellbeing into account. Additionally, it advises using Cotrimoxazole for Gram-negative bacteria and Ciprofloxacin for Gram-positive bacteria because it is one of the best medicines for infections linked to bed linen produced by gram-positive bacteria. Ampicillin should not be used to treat Gram-positive bacteria because it is useless and highly resistant to all isolated bacteria. This work encourages the need to promote proper hygiene practice regarding bed linen, as some individuals can use the same linen for a month and more without washing. Also, the multi drug resistant E. coli isolated in this study harbored high molecular weight plasmids. This can pose a risk as these genetic materials can be transferred to other bacterial pathogens, therefore causing them to be resistant to antibiotics (Olowomofe et al., 2020).

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