Unveiling the Microbial Burden on Hostel Bed Linens: A Threat to Student Health

ABSTRACT

Background and Aim: Bed linens, often overlooked as potential reservoirs of pathogens, may harbor a diverse array of microorganisms with implications for human health.This study investigated the microbial contamination of bed linens in female student hostels at the University of Port Harcourt, Nigeria.

Material and Method: Twenty-four bed linens (bedspreads and pillowcases) from six randomly selected hostels were swabbed and cultured for bacterial and fungal isolates. The samples were collected aseptically using sterile swap sticks and taken to the research laboratory where a serial dilution test was carried out to isolate and enumerate microorganisms present on the bed linen swaps taken. Furthermore, Biochemical tests of which some included; the indole test, catalase test, gram staining, motility test were conducted to ascertain the specific bacterial strains and comparing with literature, a morphological test was conducted to also determine the specific fungi strain.Disk Diffusion method was used following the McFarland standard to carry out an Antibiotic susceptibility testto determine the bacteria strains susceptible, intermediate or resistant to the different antibiotics on the disk.

Results: The research led us to these findings; *Staphylococcus epidermidis* was the most prevalent bacterium (38%), followed by *Bacillus* spp. (29%) and *Pseudomonas aeruginosa* (19%). Isolated fungi included *Aspergillus flavus*, *Penicillium* spp., *Cladosporium* spp., *Aspergillus fumigatus*, *Fusarium* spp., *Trichoderma* spp., *Aspergillus niger*, and *Mucor* spp. Antibiotic susceptibility testing revealed high susceptibility of Gram-positive isolates to ciprofloxacin, gentamicin, chloramphenicol, and erythromycin. Gram-negative isolates showed high susceptibility to pefloxacin, sulfamethoxazole, and spiramycin but resistance to chloramphenicol (67%), amoxicillin (33%), and gentamicin (33%).

Conclusion: The findings highlight the potential health risks associated with contaminated bed linens and underscore the need for improved hygiene practices in hostel environments.

Keywords: bed linens, microorganisms, bacterial isolates, fungal isolates, antibiotic susceptibility, student hostels.

INTRODUCTION

Bed linens encompass a variety of textile products, including sheets, pillowcases, blankets, comforters, and duvet covers, designed to be placed atop a mattress. These items serve multiple

functions within the sleep environment, providing warmth, protection, hygiene, and aesthetic appeal (Pyrek, 2015). The importance of clean and safe bed linen cannot be overemphasized. Due to stress encountered during the school hours, university students tend to jump on their beds to relax. In the process, microorganisms encountered from different contacts are being transferred to their beds.(Olowomofe, 2020).A hostel is a form of housing for students, which is an essential aspect of institutions of learning in all cultures and climates (Adebisi et al., 2017).One of the major challenges in managing tertiary education in Nigeria is the inability of government to adequately provide and maintain accommodation for a teeming population of students who successfully gain admission into various programs. Consequently, rooms designed for four (4) persons now houses ten (10). The poor sanitary condition of the existing few hostel facilities has thus become a vehicle for the transmission of pathogenic microorganisms (Dike et al., 2020).Hence, school hostels are known as 'underrated reservoirs' for the transmission of pathogens.Creamer et al. (2018) found Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and Vancomycin-resistant Enterococci (VRE) 10 to have been associated with the spread of pathogens by bed linen as one of the possible environmental routes. Also, there have been reports on bacteria such as *Salmonella* and *Bacillus cereus*, viruses, fungi, and parasites being transmitted by contaminated linen through direct contact and aerosol droplets of lint generated from laying on and handling bed linens (Christopher and Bruno, 2013). Fungal spores released by soil fungi into the wind can be a natural mechanism of transportation. When clothing comes in contact with these spores, it can serve as a source of fungal infections (Fijan and Turk, 2012).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 (Gopal and Solabannavar, 2020).

Human beings shed 500 million skin dry cells a day; all these dead cells pile up on the sheets in between washings. Dead skin cells, sweat, saliva, and more can turn a student's bed into a petri dish for germs to grow. For instance, laboratory tests found that swabs from pillowcases unwashed for a week harbored 17,000 times more colonies of bacteria than samples taken from a toilet seat. After a week, pillowcases and sheets contained between 3 million and 5 million CFUs (Maggie Seaver, 2023). There are microbes that are beneficial to our health as well as ones that are detrimental; those ones are known as pathogenic organisms. Pathogenic organisms can result in diseases that can further lead to death, especially in immune-compromised individuals or persons with an ailment that makes them prone to infections(Maggie Seaver, 2023).
The main cause of microbial contamination of bed linens is poor hygiene. Regular washing of bed linens and spreading them under the sun are means of eradicating these microbes especially if the students sleep in the nude, eats on the bed or sweats a lot at night. Unfortunately, many of these students do not practice such hence exposed to the risks posed by these microbes and also possibility of transfer of different infections among other students (Olowomofe, 2020).

The purpose of this study was to assess the microbial load of bed linens in students' hostels and also determine the antibiotic resistance of their organisms. This way, disease prevalence amongst the university students will be reduced or even completely eliminated.

MATERIALS AND METHODS

Collection of samples

Samples were aseptically collected from female hostel bedspreads and pillowcases using sterile swab sticks dipped in sterile normal saline, following the methods as described by Erica et al. (2014). Two rooms were randomly selected from six female hostels in the University of Port Harcourt, Rivers State, Nigeria. Samples were transported to the laboratory for immediate microbiological analysis. Nutrient agar, Potato Dextrose agar, and Mannitol salt agar were used for the isolation and enumeration of microorganisms, and they were prepared according to the manufacturer's guidelines.

Isolation and Enumeration of Bacteria and Fungi from Bed Linens

The swab sticks were dipped into normal saline to dislodge the sample, and it is used as the stock solution for preparing a 10-fold serial dilution. Serial dilution was carried out by taking 1 ml from the stock into the first test tube (10^{-1}). Exactly 1 ml was transformed from the first tube to the second; this process was repeated. Dilutions 10^{-5}, 10^{-6}, and 10^{-7} were plated out on sterile nutrient agar, Mannitol salt agar, and potato dextrose agar plates in duplicate using the pour plate method. The Mannitol salt agar and nutrient agar plates were incubated for 24 hours at 37^0 C, while potato dextrose agar plates were incubated for 3-5 days at 27^0C. In selected plates, between 30-300 colonies were observed and recorded.

Single colonies of bacteria were randomly selected from the media plates based on their morphology and these bacteria cultures were isolated by streaking to get pure cultures. The plates were incubated at 37^0C for 24 hours in preparation for identification using biochemical test.

Biochemical and Morphological Identification of Bacteria Isolates from Bed Linens

The biochemical tests carried out on the isolates were: Indole test, sugar fermentation test, oxidase test, citrate test, coagulase test, methyl red-VoguesProskauer test, and triple sugar iron test. The fungi isolates were identified by comparing their morphological characteristics with literature.

Anti-microbial Susceptibility Test

Anti-microbial susceptibility tests are used to determine which specific antibiotics a particular bacterium is sensitive to. The disk diffusion method was used. Ten different well-isolated colonies were used to prepare different suspensions standardized using the McFarland standard. Mueller Hinton agar was prepared and sterilized. Antibiotic disks containing antibiotics specific to Gram-positive bacteria (Streptomycin (30 g), Ciprofloxacin (10 g), Neomycin (30 g), Amoxicillin (10 g), Chloramphenicol (30 g), Ampiclox (30 g), Gentamycin (10 g), Levofloxacin (30 g), Erythromycin (15 g), and Gram-negative bacteria (Amoxacillin (30 g), Augmentin (30 g), Gentamycin (30 g), Pefloxacin (30 g), Ofloxacin (5 g), Streptomycin (30 g), Ciprofloxacin (10 g), Sulfamethoxazole (30 g), Chloramphenicol (10 g), Spiramycin (10 g) were placed on Mueller Hinton agar plates. The plates were then incubated for 24 hours at 37^oc.

The zones of inhibition were observed, measured with a transparent ruler, and recorded as intermediate, susceptible, or resistant according to the National Committee for Clinical Laboratory Standard Guidelines (CLSI) Wayne, 2017.

RESULTS

The total bacteria count (CFU/g) on the bedspreads from randomly selected rooms in six female hostels ranged from 8.5 x 10^5 CFU/g to 0.5 x 10^4CFU/g, while on the pillowcases, the total bacteria count ranged from 0.1 x 10^5 to 7.4 x 10^4.

The research and study showed that Bacillus spp., *Pseudomonas aeruginosa*, and *Staphylococcus epidermis* were the prevalent bacterial isolates on the bed linens, while *Proteus mirabilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* were scanty bacterial isolates. The highest on the frequency and percentage of occurrence of bacterial isolates obtained from the different hostel beddings were *Staphylococcus epidermis* (38%), *Bacillus* spp. (29%), *Pseudomonas aeruginosa* (19%), *Pseudomonas aeruginosa* (5%), *Proteus mirabilis* (4%), *Staphylococcus aureus (4%), and Escherichia coli* (1%) (Table 1).

Table 2 shows the fungi isolates identified by using their morphological characteristics. The identified fungi isolate included *Aspergillus flavus, Penicillum* spp., *Cladosporium* spp., *Aspergillus fumigates, Fusarium* spp., *Trichoderma* spp., *Aspergillus niger, and Mucor* spp.

From the different biochemical tests represented in Table 3, isolates were identified based on their morphological and biochemical reactions.

Tables 4.a and 4.b show the antibiotic resistance pattern of gram-positive and gram-negative bacteria, respectively, isolated from bed linens in different rooms of female students at the University of Port Harcourt, Rivers State. The gram-positive isolates were highly susceptible to chloramphenicol, gentamycin, erythromycin, and ciprofloxacin, while the gram-negative isolates were highly susceptible to pefloxacin, sulfamethoxazole, and spiramycin.

Staphylococcus aureus was strictly resistant to amoxicillin and streptomycin, while the gram-negative isolates were resistant to chloramphenicol (67%), amoxacillin (33%), and Gentamycin (33%).

Table 1. Frequency of occurrence of bacteria isolates from bed linens in University of Port Harcourt Female Hostels

Organisms	Number of Isolates	Frequency of occurrences (%)

***Bacillus* spp.**	**23**	**29**
***Pseudomonas aeruginosa*15**		**19**
***Staphylococcus epidermis*30**		**38**
***Proteus mirabilis*3**	**4**	
***Escherichia coli*1**	**1**	
***Staphylococcus aureus*3**		**4**
***Klebsiella aerogenes*4**	**5**	
Total	**79**	**100**

Table 2. Characteristics of fungal organisms obtained from hostel bedding sample

Macroscopic characteristics	**Microscopic characteristics**	**Probable organism**
Colonies are granular, flat, with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Conidial heads are typically radiate, Conidiophore stipes are hyaline and coarsely roughened.	*Aspergillus flavus*
Grey-blue, dense flat conidiophores with white edge, ovoid and rough with yellow reverse, 2.3 mm in diameter.	Brown hyphae with brush-like conidial head, septate conidiophores.	*Penicillium* spp
Colonies are circular with a cracked surface, fluffy mycelia and a dark-colored cracked reverse.	Brown hyphae, septate, erect and pigmented conidiophores and conidia.	*Cladosporium* spp
Colonies show typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores.	Conidial heads are typically columnar and uniseriate.	*Aspergillus fumigates*
Colonies are circular, entire and flat mycelia with whitish to cream color, colorless reverse.	Hyaline septate hyphae, conidiophores, phialides, macroconidia, and microconidia are present.	*Fusarium* spp
Colonies are circular, entire	Septate hyaline hyphae,	*Trichoderma* spp

with fluffy mycelia. Presence of conidia, scattered yellow-green patches.	conidiophores, phialides, and conidia.	
Colonies contain spores, black surface, dense mycelia and a yellow cracked reverse.	Smooth colored conidiophores and conidia. The conidiophores are protrusions from aseptate and hyaline hyphae.	*Aspergillus niger*
Colonies covered the surface of agar containing fluffy mycelium with greyish-brown color and a white reverse.	Apophysis, rhizoid and stolon are absent. Sporangiophores are short, erect, and taper towards their apices.	Mucor spp

Table 3 : The biochemical characteristics of sub-culture isolates of sample from different female Hostels.

IN	GS	SH	SP	MOT	CAT	CIT	IND	MR	VR	H_2S	SLT	BUTT	GAS	GLU	LAC	ORGANISM

1	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
2	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
3	-	rod	+	+	+	+	-	+	-	+	A	A	+	+	-	*Proteus mirabilis*
4	-	rod	-	-	+	+	-	-	-	-	A	A	-	-	-	*Pseudomonas aeruginosa*
5	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
6	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
7	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
8	-	rod	+	+	+	+	-	+	-	+	A	A	+	+	-	*Proteus mirabilis*
9	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
10	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
11	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
12	-	rod	+	+	+	+	-	+	-	+	A	A	+	+	-	*Proteus mirabilis*
13	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
14	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
15	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
16	-	rod	-	-	+	+	-	-	-	-	A	A	-	-	-	*Pseudomonas aeruginosa*
17	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
18	-	rod	-	-	+	+	-	-	-	-	A	A	-	-	-	*Pseudomonas aeruginosa*
19	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
20	-	rod	-	+	+	-	+	+	-	-	A	A	+	+	+	*Escherichia coli*
21	+	cocci	-	+	+	+	-	+	-	+	B	A	+	-	-	*Staphylococcus aureus*
22	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
23	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
24	-	rod	-	-	+	+	-	-	-	-	A	A	-	-	-	*Pseudomonas aeruginosa*
25	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
26	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Staphylococcus epidermis*
27	-	rod	-	-	+	+	-	-	-	-	A	A	-	-	-	*Pseudomonas aeruginosa*
28	+	cocci	-	+	+	+	-	+	-	+	B	A	+	-	-	*Staphylococcus aureus*
29	+	cocci	-	-	+	-	-	-	+	+	B	B	+	+	+	*Pseudomonas aeruginosa*
30	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.
31	-	rod	-	-	+	+	-	-	+	-	A	A	+	+	+	*Klebsiella aerogenes*
32	-	rod	-	-	+	-	-	-	+	+	B	B	+	+	+	*Pseudomonas aeruginosa*
33	+	cocci	+	+	+	+	-	+	-	-	B	A	+	+	+	*Bacillus* sp.

KEY: IN=isolate number, GS=gram-stain, SP=spore, SLT=slant, MOT=motility, CAT=catalase, IND=indole, GLU=glucose, LAC=lactose, B=Base, A=Acid.

Table 4.aThe antibiotic resistance pattern of gram-positive bacteria isolates from the hostel beddings.

TEST ORGANISMS (Gram-positive)	S	CPX	N	AMX	CN	APX	CH	LEV	E
Bacillus spp	I	S	I	S	S	S	S	S	S
Staphylococcus epidermis	S	S	S	S	S	I	S	S	S
Staphylococcus aureus	R	S	S	R	S	S	S	I	S

KEY: S-Streptomycin; CPX-Ciprofloxacin; N-Neomycin; AMX-Amoxicilln; CH-Chloramphenicol; APX-Ampiclox; CN-Gentamycin; LEV-Levofloxacin; E-Erythromycin; R-Resistance; I-Intermediate; S-Susceptibility

Table 4.b The antibiotic resistance pattern of gram-negative bacteria isolates from the hostel beddings.

TEST ORGANISMS (Gram-negative)	AM	AU	CU	PEF	OFX	S	SXT	CH	SP	CPX
Klebsiella aerogenes	S	S	S	S	S	S	S	R	S	S
Proteus mirabilis	R	I	S	S	I	S	S	I	S	I
Pseudomonas aeruginosa	S	S	R	S	S	S	S	R	S	S

Key: AM-Amoxacillin; AU-Augmentin; CN-Gentamycin; PEF-Pefloxacin; OFX-Ofloxacin; S-Streptomycin; SXT-Sulfamethoxazole; CH-Chloramphenicol; SP-Spiramycin; CPX-Ciprofloxacin; R-Resistance; I-Intermediate; S-Susceptibility

DISCUSSION

Used towels and bedding in hospitals, homes, and school hostels have the ability to retain microbes because they provide a favorable environment for their growth and survival, and they can be transmitted through direct contact with hands and other inanimate objects within the environment (Creamer et al., 2018; Olowomofe, 2020). In the present study, it was observed that the total microbial count in some hostels was higher than others, and this could be as a result of the poor quality of these hostels and their facilities. The study also revealed that the pillowcases of the different rooms ranged from 0.1×10^5 cfu/g to 7.4×10^4 cfu/g, which is higher compared to the bedspreads that ranged from 8.5×10^5 cfu/g to 0.5×10^4 cfu/g, and this could be as a result of saliva drools, nasal discharge, and sweat. This coincides with Olowomofe's (2020) observation in his study on hostel bedding. Furthermore,Teng et al. (2019) demonstrated that textiles act as reservoirs of microorganisms since pathogens may be able to survive on such surfaces for periods ranging from a few minutes to several months. From the several biochemical

tests conducted, the identified bacteria include *Bacillus* spp., *Staphylococcus epidermis, Staphylococcus aureus, Klebsiella aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, and Escherichia coli.* These organisms are pathogenic and can result in infections, which are mostly prevalent in females. Their symptoms mostly range from fever, chills, redness, swelling, and pain as in the case of *Klebsiella* infections (Weese et al., 2016) to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, bacteremia, and sepsis as in the case of *Staphylococcusaureus* (Davis et al., 2017). *Pseudomonas aeruginosa* has been known to cause urinary tract infections, dermatitis, and gastrointestinal infections (Hanselman et al., 2019), all of which are prevalent in females.

Bacillus spp. had the highest frequency of occurrence, followed by *Staphylococcus epidermis,* which has been associated with several studies, such as the researchof A. Pinon et al. (2013) on "Microbiological Contamination of Bedlinen and Staff Uniform in a Hospital," where the used bedlinens were observed to contain 93% of *Staphylococcus* and 17% of *Bacillus.Aspergillus niger, Aspergillus fumigates, Cladosporium* spp., *Fusarium* spp., *Penicillum* spp., and *Aspergillus flavus* were the fungi identified after comparison with the literature. *Aspergillus* species are known to cause primary pulmonary infections and can potentially become a rapidly necrotizing pneumonia with the potential to disseminate (Miller and Diep, 2018). All the identified gram-positive bacteria isolates were susceptible to Ciprofloxacin, Gentamycin, Chloramphenicol, and Erythromycin, which means these antibiotics can be used in case of any infection by gram-positive bacteria identified. The identified gram-negative isolates were highly susceptible to pefloxacin, sulfamethoxazole, and spiramycin but resistant to chloramphenicol (67%), amoxacillin (33%), and gentamycin (33%).

CONCLUSION

This study suggests that bed linens are underrated reservoirs for microorganisms and that the importance of clean and safe bed linens cannot be overemphasized. The study has also shown that females are prone to infections as they create a comfortable zone for the growth of these pathogenic organisms.This finding contradicts the traditional belief that microbial contaminant levels from female hostel would be lower than in male hostel (Yanju et al., 2020).The female students most of the time come back from classes and lay on their beds with sweat and make-up and even sleep naked on the bed linens, thereby providing a good environment for the organisms to breed.According to Muthiani et al., (2020) and Singh et al (2020),these majorcontributing factors; microbe characteristics, fabric properties,andenvironmentalfactorsenhancesmicrobes'survivalonfabricssuchas bed linens, towels.Thesemicrobes(whicharecapableofsurvivingforalongtime)eveninsmall quantitiescaninfecttheirhostwhentheycomeincontact The study also points to the fact that some of the female students in the hostels do not have a good hygienic status; hence, they could be sources of transmission of disease-causing agents or bacterial pathogens to healthy female students, leading to infection among other students, and these also supports the publication of Naja'atu et al. (2021) on 'The Bacteriological Examination of Used Towels from Female and Male Hostels of the Federal University of Lafia' and that of Hannah et al.(2021) on ' Toothbrush and Towel Handling and their Microbial Quality: The Case of Students of University for Development Studies'.

These identified organisms could be highly pathogenic, especially in patients with weakened immune systems or with existing ailments that have left their system vulnerable to any infection. Students should go for regular medical checkups and be tested whenever they notice symptoms of an infection instead of self-medicating because these pathogens can easily acquire immunity or resistance against the antibiotics. The study suggests that for the treatment of infections caused by gram-positive bacteria, chloramphenicol, gentamycin, erythromycin, or ciprofloxacin should be administered, while for the treatment of infections caused by gram-negative bacteria, ciprofloxacin, sulfamethoxazole, or spiramycin can be used. In the case of a fungal infection treatment, antifungal medicines such as Itraconazole, Voriconazole, Amphotericin-B, and Posaconazole can be administered.

It is acceptable to say that poor hygiene is the main cause of the contamination of the bed linens, and as a way of eliminating the contamination, students should bathe regularly, wash and sundry their bed linens weekly, and likewise go for medical check-ups. As it is said, 'Early diagnosis allows for good prognosis.

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