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Bacteriological and Mycological surveillance of Bed Linens at a Major Tertiary Hospital in North-Central Nigeria

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ABSTRACT

Knowledge of the microbial diversity on hospital bed linens has implicit significance for infection control because bed linens are likely reservoir and vehicles for healthcare-associated Infections (HAIs). In this study, the diversity of microorganisms that persist on bed linens at Benue State University Teaching Hospital (BSUTH), Makurdi, Nigeria was evaluated. Patients' bed and surface areas on bed linens were chosen at random from ten different wards and the swab sampling method employed. Of the 30-bed linens sampled, bacteria were more prevalent at 19 (63.33%), fungi at 10(33.33%). We identified both bacteria and fungi from eight wards, only fungi from the antenatal ward and no contamination from the amenity ward, 1(3.33%). *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, and coagulase-negative *Staphylococcus* dominated the bacterial profile. Similarly, *Aspergillus niger*, *Candida albicans*, and *Microsporium ferrugineum* fungi were also recovered from the bed linens. Amongst the bacteria identified, *Escherichia coli* gave the highest prevalence at 7(36.84%), whereas, *Candida albicans* revealed the highest among the fungi isolates with 5(50%). Chi-square analysis showed a significant relationship ($\chi^2 = 68.48$, $df=8$, $P<0.05$) between the microbial contamination and the wards sampled. The mean bacterial count (cfu/ml) was checked for each ward and the female surgical ward showed the highest count of 31.3×10^3 while the paediatric ward had the least count of 2.3×10^3 . Control beddings were also contaminated with 1.3×10^3 . These results suggest that the bed linens from the hospital wards were contaminated with pathogenic microbes which can contribute to HAIs.

Keywords: Healthcare associated infections (HAIs), Hospital bed linen, Infection control, Microbes

INTRODUCTION

Healthcare-associated infections (HAIs) are absent in patients at the point of admission; yet occur while receiving medical care in a hospital or healthcare facility. They usually occur within the first 24-hours following

admission or 30-days after receiving healthcare.^{1,2,3} HAIs affect several patients from developing and developed countries alike, resulting in extended hospital stay, long-term disability, heightened antimicrobial resistance, increased financial burden and preventable death.⁴ In developing countries, the burden of HAIs is often

undetermined due to multiplex diagnosis, the dearth of expertise and capital for monitoring, to promote health.⁵ In 2016 and 2017, the European Centre for Disease Prevention and Control coordinated point prevalence studies of HAIs. Data obtained revealed that approximately 8.9 million HAIs occurred in both hospitals and long-term health facilities in the European Union (EU) and European Economic Area (EEA) countries.⁶ In a point prevalence study conducted in US hospitals by CDC and the Emerging Infections Program (EIP), a 3.2% prevalence of hospitalized patients had HAIs in 2015, by comparison to 4.0% in 2011⁷, and one out of 25 hospitalized patients had at least one HAIs.

According to the AWMF Working Group for Hygiene in Hospital and Practice⁸ and Ayliffe *et al.*⁹ the hospital bed, consists of bed rail, bed linen, mattress, and pillows. These components are in direct contact and are routinely touched by patients, health care workers or visitors.^{10,11} The propensity of textiles to foster, sequester and convey microorganisms, from humans to the environment may occur at any time, before, in the course of, and following laundry.^{12,13} With good environmental conditions, these microbes could result in significant public health threats of hospital-acquired infections, if not well disinfected. The sources of microbes on contaminated textile are numerous, including body fluids and tissues for instance skin, vomit, faecal matter, urine, and blood.¹⁴ Thus, effective laundering is required; first, to remove blemishes, restore appearance, and malodours and to reduce infection risk by lessening the microbial load to safe levels.^{12,15}

The hypothesis investigated in this study is that the bed linens at Benue State University Teaching Hospital (BSUTH) are contaminated with several nosocomial pathogens that can cause HAIs. The aim of this study is therefore to; assess the microbial diversity from bed linens from ten wards at the BSUTH, Makurdi.

MATERIALS AND METHODS

Study Area

The present study was carried out from May - July 2019 at the BSUTH. Sampling was carried out in ten (10) different wards of the hospital namely; Accident and Emergency (A&E) male, A&E female, Male Surgical, Female Surgical, Male Medical, Female Medical, Postnatal, Paediatrics', Antenatal and Amenity wards. Ethical

clearance for this study was obtained from the Health Research Ethics Committee, BSUTH.

Culture media

Sarbouraud dextrose agar (SDA) (Oxoid) and Nutrient agar (HiMedia Laboratories, India) were used in isolating microbes. The media was prepared according to the manufacturers recommended procedure and supplemented with antifungal and antibiotics.

Collection of samples

A total of 30-bed linens were evaluated for bacterial and fungal contamination. The sampling points include; edge/middle of bed linen, outside and inside bed sheets. Two designated bed linen were used; dirty bed linens (stained with blood, urine or faeces) and clean (unused) bed linens, which served as the control. Samples were collected with the aid of swab sticks. This was achieved by rubbing a swab stick already moistened with normal saline on the surface of the bed linen as described.¹⁶ The swabs were put into sterile test tubes, closed tightly and labelled appropriately. Samples were immediately transported to the Evidence medical and research laboratory, Makurdi for analysis.

Isolation and identification of microorganism from bed linens

The contaminated swab sticks were used to inoculate nutrient agar plates for bacteria growth. Incubation was at 37°C for 24 hours in a DNP-9052A, Easy way Medical England, incubator. For the identification of fungi, swab sticks were used to inoculate SDA agar plates and incubated at 25°C for 3-4 days. Identification of fungi was achieved by comparing the morphological characteristics as described¹⁷, whereas bacteria identification was done using Bergey's Manual of Determinative Bacteriology as reported.¹⁸

laboratory tests

The laboratory procedures were performed on the bacterial isolates:

Motility test

A semisolid motility test was used. The medium was stabbed with a small amount of bacteria inoculum using a sterile needle and incubated overnight at room temperature. If the bacteria species is motile, the medium

growths spread out from the line of inoculum but if not motile, only the stab line has visible bacterial growth.¹⁸

Catalase test

A 24-hour old culture was used to make a smear on a clean slide, and 3% hydrogen peroxide was added in drops. Production of gas indicates a positive reaction while a negative reaction shows no gas or bubbles. This test was done to determine the presence of enzyme catalase possibly produced by the isolates.¹⁹

Gram's staining

A heat-fixed smear from 24-hour old bacterial isolates was prepared, stained with crystal violet, flooded with Gram's iodine, and decolourized with 70% alcohol solution. This was rinsed under running water and flooded with Safranin O stain. The slides were then washed under clean water and examined under the microscope using the oil immersion objective (x100). The staining technique also shows the shapes and arrangement of bacterial isolates.¹⁹

Coagulase test

A drop of distilled water was placed on each end of a clean grease-free slide with the aid of a sterile inoculating loop; a colony of the test organism was picked using a sterile inoculating loop and the colony was emulsified on the drop of distilled water. An inoculation loop was used to add a loopful of plasma suspension and was checked for the clumping of organisms. No plasma was added to the second suspension; thus, this is used to differentiate any granular appearance of the organism from true coagulase clumping. Clumping of the organism indicates a positive result, while no clumping indicates a negative result.¹⁸

Indole test

About 10 ml of peptone water was aliquoted into a test tube; with the aid of a sterile loop, a small colony of the isolate was inoculated into the peptone water. The test tube was incubated at 37°C for 24 hours, and a drop of Kovac's reagent was added whilst observing for any reaction. Red colouration of the lower part of the strip confirms the organism as being positive to the indole test.¹⁸

Germ tube test for the confirmation of Yeast

About 0.5-1ml of human serum was pipette into a test tube

inoculated with a small colony of the yeast and incubated for 2-3 hours. Microscopic examination was done by placing a small colony onto a glass slide stained with Lactophenol in cotton blue and viewing under the ×40 of the compound microscope. A tube-like outgrowth from sprouting cells was used to confirm the identification of *Candida albicans*.¹⁸

Mean bacterial count of bacteria isolates

The total viable count was estimated using the pour plate method as reported.¹⁶ Swab samples were initially soaked properly in 1 ml of 0.1% peptone water, and 10-fold dilution made. A 1 ml aliquot was inoculated on prepared nutrient agar plates and incubated at 37°C for 24 hours. The average counts of the colonies of 30 to 300 were used to obtain the total viable bacterial count of the sample.

Data Analysis

Data obtained was analysed using Microsoft Excel version 16. The data was first subjected to descriptive statistics (frequency and percentages), and then Chi-square analysis was applied to test for the relationship in microbial rate and the sampled wards.

RESULTS

We tested three-bed linens per ward to ascertain the bacterial and fungal contamination if any, and the load (cfu/ml). Of the 30 sampled bed linens, 19 were positive for bacteria, 10 for fungi and 1 had no microbe. The distribution of bacteria and fungi in the ten wards is as

Table 1: Percentage contamination frequency of bacteria and fungi on bed linen at BSUTH

Wards	Bacteria (%)	Fungi (%)
A&E male	3 (15.78)	2 (20)
A&E female	1 (5.26)	2 (20)
Male surgical	3 (15.78)	2 (20)
Female surgical	1 (5.26)	1 (10)
Male medical	3 (15.78)	1 (10)
Female medical	4 (21.05)	1 (10)
Postnatal	2 (10.53)	0
Pediatrics	2 (10.53)	0
Antenatal	0	1 (10)
Amenity	0	0
Total	19 (100)	10 (100)

shown (Table 1).

The results from our study show that all the four bacteria were isolated from the female medical ward, resulting in a percentage occurrence of 4(21.05). Data in Table 1 reveal that the A&E male ward, male surgical, and male medical all showed three bacterial contaminants 3(15.78). Correspondingly, the A&E male ward, A&E female ward, and male surgical had the highest fungal occurrence at 2(20). With one bacterium identified, the A&E female and female surgical wards showed the least percentage occurrence, 1(5.26). While only one fungus was isolated in the female surgical, male medical, female medical, and antenatal wards with a percentage occurrence of 1(10). We

recovered only *C. Albicans* on the bed linen 1(10) from the antenatal ward and no bacteria were isolated. Amenity wards showed no microbial contamination.

The bacteriological profile of BSUTH bed linens was further differentiated based on morphological characteristics, and biochemical tests were also performed, using the procedure described in the Materials and Methods section. The results are displayed in Table 2. As depicted in Table 2 and Figure 1, the identified bacteria from our study were divided equally between Gram-positive (*S. aureus* and Coagulase-negative *Staphylococcus*) and Gram-negative bacteria (*E. coli* and

Table 2. Bacterial diversity in the sampled wards as characterized using cultural, morphological and biochemical characteristics of isolated bacteria

Cultural characteristics	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella</i>	Coagulase -negative <i>Staphylococcus</i>
Shape	Circular	Rod	Circular	Circular
Colour	White	Milky	Cream	White
Size	Large	Small	Large	Large
Elevation	Flat	Raised	Raised	Flat
Transparency	Opaque	Translucent	Translucent	Opaque
Morphological characteristics				
Gram stain	+	-	-	+
Cell types	Cocci	rods	Rod	Cocci
Cell arrangement	Chains	clusters	Single	Chains
Spore staining	-	-	-	-
Biochemical test				
Catalase	+	+	+	+
Motility	-	+	+	-
Indole	-	+	+	-
Coagulase	+	-	-	-

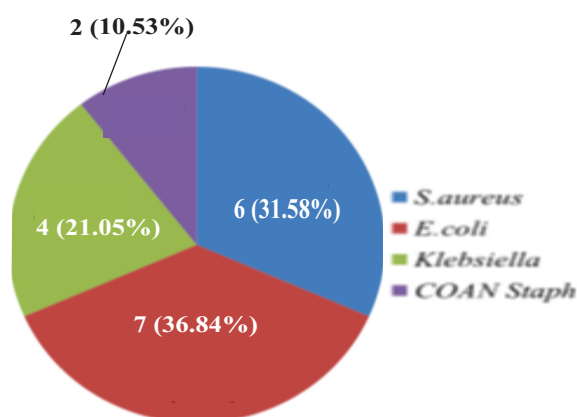


Figure 1: Percentage occurrence of bacterial isolates on used hospital bed linen

Klebsiella sp.).

Next, the percentage occurrence of bacteria isolates from bed linen identified across the wards was analyzed, as shown (Figure 1).

As presented (Figure 1), *E. coli* had the highest percentage occurrence at 7(36.84), followed by *S. aureus* 6(31.58), *Klebsiella* sp. 4(21.05) and coagulase-negative *Staphylococcus* 2(10.53).

The mean bacterial count in the wards sampled was investigated and the results are presented in Table 3.

From our study, A&E male showed the mean bacterial count of 23.3×10^3 cfu/ml, A&E female had a mean bacterial count of 2.6×10^3 , the male surgical ward had a mean bacterial count of 7.0×10^3 , the female surgical ward had a bacterial count of 31.3×10^3 , the male medical ward had a bacterial count of 24.8×10^3 , the female medical ward

had a bacterial count of 6.0×10^3 , the post-natal ward had a bacterial count of 10.7×10^3 , the paediatric ward had a bacterial count of 2.3×10^3 . Both the antenatal and amenity ward had no bacteria growth. From the table, it is obvious that the female surgical ward had the highest mean bacterial count of 31.3×10^3 while paediatrics had the lowest mean bacterial count of 2.3×10^3 . The contamination of unused bed linen though very low (1.3×10^3) was unexpected. Chi-square test shows a significant difference between the bed linen across the wards and the clean bed linen, as the p -value is less than

Table 3. Mean bacterial count in the wards sampled

Wards	Bacterial count ($\times 10^3$) cfu/ml
Accident and emergency male	23.3
Accident and emergency female	2.6
Male surgical	7.0
Female surgical	31.3
Male medical	24.8
Female medical	6.0
Postnatal	10.7
Paediatrics	2.3
Antenatal	Nil
Amenity	Nil
Control	1.3

$$\chi^2 = 68.48, df = 8, P < 0.05$$

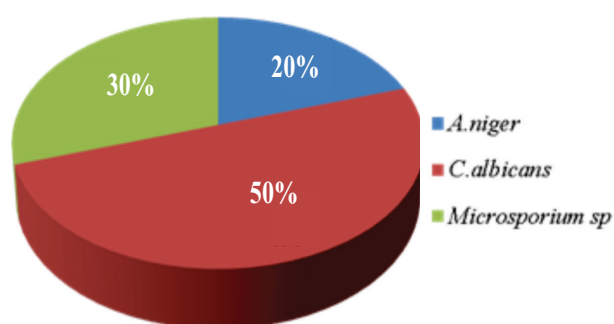


Figure 2: Mycological profile and percentage occurrence of fungal isolates on used hospital bed linen

0.05.

Data obtained from this pilot study confirm the mycological profile on bed linens at BSUTH as *A. niger*, *C. albicans*, *M. ferrugineum* (Figure 2).

As indicated, three (3) fungal species were isolated. The bed linens tested positive for *C. albicans*, the most

dominant fungi with an occurrence of 5(50). This was followed by *Microsporium ferrugineum* with 3(30) and *Aspergillus niger* with 2(20).

DISCUSSION

To our knowledge, this is the first study undertaken to survey bacterial and fungal contaminants on bed linens at the BSUTH. The reason why we choose this hospital was that; it is a busy hospital catering for large numbers of patients' inter-and-intra-state, multiple admissions occur daily resulting in the use of several reusable bed linens. Microbes are brought into hospitals from phones, shoes, clothing, and skin.¹³ This gives rise to situations where the transfer of microbes by humans to the bed linens can arise by contact with surfaces or airborne. In some hospitals, bed linens are not regularly/properly washed or changed. Thus, the continuous shedding of bacteria may contaminate them. From our study, bed linens from eight of the ten sampled wards were contaminated with both bacteria and fungi. The antenatal ward provides individualized care to women admitted during their pregnancy, and our study identified only one fungal contaminant. Amenity ward is a private ward, and medical care is provided to patients at extra costs; thus, extra hygienic practices are put in place. We found none of the bed linens were contaminated, and this may be due to reduced human traffic.

It is well documented that bed linens contribute to hospital infections. As shown in our study, we identified both Gram-positive and Gram-negative bacteria. Interestingly, the bacteria obtained from these studies are consistent with the results from other studies^{20,21,22} indicating a wider problem in hospitals and medical facilities. Studies by Borkow *et al.*¹⁰ have also demonstrated that contaminated bed linens and other non-uniform textiles were implicated as potential sources for the spread of nosocomial-related pathogens, while pyjamas and bed sheets were exposed as a key source of nosocomial pathogens in indoor air.²³ We identified three fungi *A. niger*, *C. albicans* and *M. ferrugineum* (Figure 2). Our findings are in agreement with studies by Kordbacheh *et al.*²⁴ carried out at a Tertiary Hospital in Tehran, Iran. *Candida albicans*, and *Aspergillus niger*, were isolated, in addition to *Penicillium* sp., and *Cladosporium* sp. *C. albicans*, the commonest of the *Candida* species, is a member of the gut, vaginal and gastrointestinal microbiota in humans, and is an

opportunistic fungus, causing disease in individuals with a weakened immune system.^{25, 26, 27} It forms the normal microbiota of the mouth, vagina and gastrointestinal tract. *Microsporium ferrugineum*, a dermatophyte causes *Tinea capitis*, a fungal infection of the scalp and hair in children, popularly called ringworm.²⁸ Infection can be obtained from clothing and bedding, including furniture, toys, combs, telephone, soil or spread from a localized infection.^{29,30,31} Though reports of *M. ferrugineum* isolated from hospital bed linens are rare, it was not entirely surprising to have identified the fungus. The fungus is endemic in Southeast Asian and African countries like Nigeria,^{32, 33} because these countries are characterized by tropical and sub-tropical climates.³¹ *A. niger* has been reported to be the commonest mold found in the environment globally.³⁴ However, few cases of cutaneous disease and pulmonary infections can occur in immunocompromised patients.³⁵ Data obtained from Dart and Obendorf,³⁶ suggests that spores obtained from *Aspergillus* are readily transmitted by cotton clothes, worn by patients and visitors, by comparison to other fabrics. The bed linens in this study were made of cotton fabric. Diba and colleagues also reported that *A. niger* and *Candida* spp. were among the species isolated from bed and blankets.³⁷ So as studies by¹⁵ that reported *Staphylococcus* and *Candida* as organisms that contaminate textiles. Some of the organisms isolated in this study are part of the normal human microbiota and can be transferred during handling or wearing, while others such as *Aspergillus* could have been environmental isolates.

For the mean bacterial counts (CFU/ml) in the wards sampled, the paediatric ward showed the least CFU count (2.3×10^3) while the female surgical showed the greatest count (31.3×10^3). Control bed linens had 1.3×10^3 bacteria present, which was rather unexpected. Previous studies did not report any microbial contamination on unused textiles.^{23,38} At present, the level of bacterial contamination resulting in HAIs is unknown.³⁹ Thus, we cannot speculate on whether the contamination of bed linens as obtained in this study resulted in any infections.

CONCLUSION

The results obtained from the study, therefore, suggest that the bed linens from the hospital wards at BSUTH were contaminated with pathogenic microbes. These may serve as a vehicle causing nosocomial infections in patients,

especially in those with a weakened immune system, thus raising concerns for infection control. To our knowledge, there are no cleaning and changing policies or set standards for assessing bed linens at BSUTH.

Recommendation

We recommend proactive measures to ensure BSUTH is keeping nosocomial infections at bay.

Limitation

We acknowledge the small sample size of this pilot study and recommend further research and molecular testing of the pathogens. Data obtained from this study should be interpreted with caution in the context of its limitations, as such, we cannot comment on the possibility of the contamination resulting in any infections.

Acknowledgment

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Conflict of Interest

The authors declare that there is no conflict of interest'.

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