Microbial Contaminants on Inanimate Surfaces and Non-critical Instruments at a Major Reference Hospital in Makurdi, Nigeria

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Authors’ contributions

This work was carried out in collaboration between both authors. Author AKA performed the study, wrote the protocol and managed the literature searches. Author IWN designed the study, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: There have been links between healthcare-acquired infections, contaminated surfaces, and medical instruments. The aim of this study was to: (i) investigate the variety of microorganisms that persist on inanimate/noncritical devices at Benue State University Teaching Hospital Makurdi, Nigeria, as a possible source of healthcare-acquired bacterial and fungal infections, and (ii) determine the prevalence of microorganisms on the instruments sampled.

Study design: This study was a hospital-based cross-sectional study.

Place and duration of study: Microbiology Laboratory (Bacteriology, Media/Washroom, Serology, and Phlebotomy Units), Benue State University Teaching Hospital Makurdi, Nigeria, between January 2021 and May 2021.

Methodology: Swab specimens were collected from tables (14), sinks (8), hand jars (7), scissors (5), inoculating loops (7), refrigerators (6), and autoclaves (3) using sterile stick swabs. The bacterial and fungal investigation was performed using standard culture tests-gram stain, colony morphology, and biochemical tests.

Results: All the samples tested positive for either bacteria or fungi, indicating a contamination rate of 100%. Tables were the most contaminated (28%), sinks (16%), inoculating loops (14%), hand
1. INTRODUCTION

The impact of healthcare surfaces contaminated with microorganisms on the risk of transmitting healthcare-associated infections to patients has long been contested, but it is now widely acknowledged that the environment can play a role in the spread of many healthcare-associated microorganisms [1]. Undeniably, non-critical medical devices employed in hospitals can serve as fomites harboring microbes, where they might live for days, escalating the risk of transmission from person to person whilst also contributing to nosocomial infections and outbreaks in hospitals [2,3]. Microbes such as Vancomycin-resistant enterococcus (VRE), Methicillin-resistant Staphylococcus aureus (MRSA), and gram-negative bacteria can persist on non-living objects such as hospital equipment for many months [2,4]. Thus, by touching contaminated surfaces and noncritical equipment, hands may acquire and transfer microorganisms to other inanimate objects or patients [3,5]. According to Spaulding, noncritical equipment is defined as those "items that come into contact with intact skin but not with mucous membranes" [6]. Since healthy skin is a barrier to most microbes, objects that come in contact with the skin need not be sterile and cleansed when used [7]. Medical equipment used in the non-critical care setting is less likely to have standard disinfection and cleaning protocols than equipment in the critical care setting. Thus, non-critical healthcare equipment shows a higher likelihood of harboring significant pathogenic bacteria species [8]. Despite progress in hospital infection prevention and control, nosocomial infections are still a massive public health challenge globally, but especially in developing countries where resources and awareness of infection prevention and control are limited [9]. In Low and Middle-Income countries, the rates of nosocomial infections exceed 20% [10]. However, obtainable data is insufficient and more studies are urgently required in these countries. World Health Organization data indicates that currently, of every 100 hospitalized patients, 7 to 10 are anticipated to fall ill with at least one healthcare-associated infection [11]. Nosocomial infections are an important threat to hospitalized patients, visitors, and healthcare workers, especially in developing countries like Nigeria. They bring with them significant death, patient morbidity, and cost to the healthcare system [12,13].

Various authors have published studies on bacteria contaminating various surfaces and non-critical hospital devices globally [14-17]. As regards to the Nigerian situation, data has been recorded in many states [18-23]. These studies discovered gram-positive, gram-negative, and resistant bacteria, but no fungal diversity was reported. However, there are few such studies in Makurdi. The focus of one study in Makurdi was on gram-negative bacteria, with the majority of the samples being human specimens and environmental samples from hospitals [24]. Studies by others sampled different surfaces in Makurdi hospitals, and the majority of the surfaces assessed were not similar to the current study [25,26]. Due to the number and type of patients who visit each hospital (private, secondary, or tertiary), the bacteria sampled are likely to differ greatly. The present work therefore aims to evaluate the bacteria and fungi diversity that persists on inanimate surfaces and non-critical instruments at Benue State University Teaching Hospital (BSUTH), Makurdi, Nigeria, and also investigate their prevalence.

2. METHODOLOGY

2.1 Sample Collection

This is a cross-sectional study of the indoor surfaces and tools at BSUTH in Makurdi, Nigeria. The research was carried out at the microbiology laboratory between January and May 2021, to identify and determine which microorganisms
were the most prevalent. Because of the high volume of patients who come in for various medical examinations, the microbiology laboratory was chosen. For approximately 10 seconds, a single sterile stick swab with a polystyrene shaft and a viscose tip 45 mm from the tip was utilized to sample a 25 cm$^2$ surface. Each swab stick was inserted into its own tube. A total of 14 tables (top), 7 inoculating loops (handle), 3 autoclaves, 8 sinks (lid and handles), 7 hand jars (lid and sides), 5 scissors (finger rest, thumb ring, and blades), and 6 refrigerators (top), totaling fifty were sampled at random. Each sample was assigned a unique identification number and processed within one hour [27]. Approval for this study was obtained from the Ethics Committee of Benue State University Teaching Hospital, Makurdi.

2.2 Isolation and Identification of Bacteria and Fungi Isolates

Aseptically collected samples were immediately inoculated by swabbing on the surfaces of Salmonella Shigella agar (SSA) (Oxoid, UK), Blood agar (BA) (Oxoid, UK), Chocolate agar (CA) (Thermo Scientific™ Ltd., UK), MacConkey (Oxoid, UK) agar, and Cystine-Lactose-Electrolyte-Deficient agar (CLED) (Becton Dickinson™, UK) plates as described previously [27]. Triple Sugar Iron agar (Oxoid, UK) was also utilized in this study to differentiate between members of the genus Enterobacteriaceae. All of the media were prepared as directed by the manufacturer. To prevent the growth of broad-spectrum gram-positive and gram-negative bacteria, the samples were cultured on Sabouraud Dextrose agar (Thermo Scientific™, UK) and 50 mg of chloramphenicol and tetracycline were added to the plates. The plates were then incubated for 2–7 days at 30°C. Inoculated bacteria plates were incubated aerobically at 37°C for 24 – 48 h. Bacteria recovered from the plates were classified as gram-positive or gram-negative bacteria using gram staining. Colony morphology and biochemical assays were also used to help identify isolates [28,29].

3. RESULTS

After the bacteria isolates were gram stained, they were examined for colony shape, motility, opacity, and pigmentation. To aid in fungal identification, the pigmentation, type of hyphae, conidiophore, and surface type of fungi were all evaluated. Specific microorganisms have been found to characterize specific hospitals in terms of their hospital microbiota. Some bacteria, however, are more prevalent and/or harmful than others [1]. The results are presented for bacterial and fungal colony morphology, isolating agar, and surface. Findings from this study identified the following bacteria: *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. On autoclaves and inoculating loops, two fungi, *Aspergillus niger* and *Candida albicans*, were found. At least one bacterium or fungus was identified from each of the surfaces/instruments investigated (Table 1).

| Colony Morphology                                      | Presumptive Organism | Isolating Agar | Isolating Surface |
|--------------------------------------------------------|----------------------|----------------|------------------|
| Shape: Straight rods                                   | *Salmonella* spp.    | Salmonella-Shigella agar | Tables          |
| Motility: Motile                                       |                      |                 |                  |
| Opacity: Transparent                                   |                      |                 |                  |
| Pigmentation: Colourless colonies with black Centre     |                      |                 |                  |
| Shape: Cocci                                           | *S. aureus*          | Blood agar     | Tables           |
| Motility: Non-motile                                    |                      |                 | Hand jars        |
| Opacity: Opaque                                        |                      |                 |                  |
| Pigmentation: golden appearance with zones of clear β hemolysis |                      |                 |                  |
| Shape: Cocci                                           | *Streptococcus* spp. | Chocolate agar | Tables           |
| Motility: Non-motile                                    |                      |                 |                  |
| Opacity: Translucent                                   |                      |                 |                  |
| Pigmentation: whitish-greyish colonies with zones of β-hemolytic colonies |                      |                 |                  |

Table 1. Colony morphology of the isolated bacteria and fungi in this study
Colony Morphology | Presumptive Organism | Isolating Agar | Isolating Surface
---|---|---|---
Shape: Rods | E. coli | MacConkey agar | Tables
Motility: Motile | | | Sinks
Opacity: Opaque | | | Refrigerators
Pigmentation: Dark pink | | | 

Shape: Rods | P. aeruginosa | Cystine-Lactose-Electrolyte-Deficient agar | Scissors
Motility: Motile: | | | 
Opacity: Opaque | | | 
Pigmentation: Green colonies | | | 

Shape: Rods | K. pneumoniae | MacConkey agar | Sinks
Motility: Non-motile | | | 
Opacity: Opaque | | | 
Pigmentation: shiny and dark pink | | | 

Pigmentation: Initially white but change to black after a few days, and edges of the colonies appear pale yellow | A. niger | Sabourad Dextrose Agar | Autoclaves
Hyphae: Septate hyphae | | | 
Type of conidiophore formed: Smooth coloured | | | 
Conidiophores | | | 

Pigmentation: Creamy white | C. albicans | Sabourad Dextrose Agar | Inoculating loops
Hyphae: Pseudohyphae | | | 
Surface: Smooth | | | 

| Surfaces/tools sampled | GR | CAT | COA | MT | TSIA H₂S | Gas | Presumptive organism |
|---|---|---|---|---|---|---|---|
| Tables | - | + | - | + | - | + | Salmonella spp |
| Tables | + | + | + | - | - | - | S. aureus |
| Tables | + | - | + | + | - | + | Streptococcus |
| Tables | - | + | - | + | - | + | E. coli |
| Sinks | - | + | - | + | - | + | E. coli |
| Sinks | - | + | - | - | - | - | K. pneumoniae |
| Hand jars | + | + | + | - | - | - | S. aureus |
| Refrigerator door handles | - | + | - | + | - | + | E. coli |
| Sinks | + | - | + | - | - | + | Streptococcus spp |
| Hand jars | + | + | + | - | - | - | S. aureus |
| Scissors | - | + | + | + | - | + | P. aeruginosa |

*Key: GR- Gram reaction; CAT – Catalase; COA- Coagulase; MT- Motility test; TSIA- Triple Sugar Iron Test; H₂S- Hydrogen sulphide*

Additional assays, such as gram reaction, catalase test, coagulate test, motility test, Triple Sugar Ion test, and gas, were performed on the bacterial isolates in addition to colony morphology evaluation. From the results obtained, gram-positive and gram-negative bacteria were found on tables (Salmonella spp, S. aureus, Streptococcus spp, and E. coli), while only gram-negative bacteria were identified on sinks (E. coli, K. pneumonia), refrigerator door handles (E. coli), and scissors (P. aeruginosa) predominantly had gram-negative organisms, only the gram-positive S. aureus was found on hand jars. The results are shown in Table 2.

The prevalence of microbial contamination on the instruments sampled was determined to evaluate which surface and/or instrument was more contaminated. From the results obtained, tables (28%), sinks (16%), and hand jars (14%) were more contaminated, while autoclaves were the least contaminated (Fig. 1).
4. DISCUSSION

Bacteria on medical devices have been reported to come from the environment, patients, and healthcare staff [30]. The fungi A. niger and C. albicans, as well as the bacteria Salmonella spp., P. aeruginosa, E. coli, Staphylococcus aureus, and Streptococcus aureus, were found on selected surfaces, instruments, and devices at BSUTH, Makurdi’s largest referral hospital. The findings of this study are consistent with those of others [13,15,16,19,31,32]. During clinical sample collection, healthcare professionals are exposed to body fluids, blood, mucous membranes, and other bodily fluids daily. Even though most of them wear gloves when obtaining samples in BSUTH’s microbiology lab, contamination is still a possibility. According to Moore et al. [33], gloves can become contaminated with microorganisms during the use process. These can subsequently be transmitted to other surfaces that the gloves come into contact with.

In the current study, a large number of bacteria were discovered on tables (Table 2). Tables are frequently touched by patients, visitors, and medical personnel. As a result, it is not surprising that they have the greatest prevalence rate (28%). Medical autoclaves, on the other hand, were the least contaminated (6%). These devices, which are believed to be sterile, are used to steam sterilize surgical, laboratory, and other tools. In the current study, however, A. niger was found on autoclaves. Aspergillus fungus is found in the environment, in the air, soil, water, trash, and decaying plants, according to Richardson et al. [34]. Aspergillus spp. were also found in several hospital sources, such as air, food, dust from construction and/or refurbishment, and surfaces in one investigation [35]. By breathing spores via aerosols, the fungus causes aspergillosis in humans. Infected medical equipment has been connected to sporadic outbreaks of the disease [36]. Aspergillus spores can withstand a wide range of circumstances, including dryness, heat, and cold, and can proliferate after the autoclave surfaces have been cooled. Because the autoclaves in question were in the media/wash room, contamination was most likely caused by an external source, such as polluted dust from open windows. The source of contamination, however, was not investigated.

Salmonella spp. causes salmonellosis and, like P. aeruginosa, can be found in a variety of environments [37,38]. P. aeruginosa, on the other hand, has also been reported as a normal human and animal flora [39]. Given the diversified nature of the population that visits hospital environments, patients with cuts and wounds can transmit microorganisms from contaminated equipment. The presence of P.aeruginosa on scissors in this study is worrying. The pathogen is a key cause of nosocomial infections globally and can cause life-threatening diseases, e.g. in cystic fibrosis patients. It is able to achieve this by adapting to and surviving numerous environmental conditions and intrinsically resisting multiple antibiotics and disinfectants [37]. Other bacteria isolated in this study, for instance, K. pneumonia, E. coli, and S. aureus are inhabitants of the respiratory tract, gut, and skin of healthy humans respectively. Thus, if infection control protocols
are not strictly adhered to, they may result in the spread of nosocomial infections. However, this study did not link the possibility of the isolated organism causing any infections. Molecular analysis using 16s rRNA sequencing was not performed on the samples, which is one of the current study's limitations. Furthermore, there was no way to standardize the sampling pressure used in obtaining the samples, and there could have been varied contamination on the surfaces analyzed.

5. CONCLUSION

There are few published studies on the contamination of inanimate objects and non-critical medical equipment in hospitals in Makurdi. We established in this study that the non-critical instruments and surfaces at BSUTH microbiology laboratory are contaminated with both bacteria and fungi, which could serve as possible vectors for the transmission of microbes to healthcare workers, visitors, and patients. Patients and healthcare staff may have contaminated these surfaces and tools with nosocomial bacteria, which is concerning because they may offer a risk in certain conditions. It is consequently vital to detect their presence in order to provide prompt diagnosis and treatment. Similarly, the findings are significant because knowing which microbes are abundant in the hospital has consequences for nosocomial infection control. Although BSUTH has recommendations for infection prevention and control protocols, which include stringent hand hygiene before and after each patient contact, as well as cleaning and disinfecting practices for floors, non-critical, and other equipment, these are not adhered to. So, strict monitoring to ensure compliance is recommended. The data obtained from this study has provided baseline results requiring further molecular studies, but the non-adherence to infection control protocols is evidenced by the microorganisms identified in this study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Approval for this study was obtained from the Ethics Committee of Benue State University Teaching Hospital, Makurdi.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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