# AMERICAN UNIVERSITY OF NIGERIA DEPARTMENT OF NATURAL AND ENVIRONMENTAL SCIENCES

Thesis

# ASSESMENT OF BACTERIA FOUND IN THE AMERICAN UNIVERSITY OF NIGERIA (AUN) FACILITIES AND POTENTIAL

**HEALTH IMPACTS**

by

aun.edu.ng

# Fadila Mohammed Kabir

Submitted in partial fulfillment of the Requirements for the degree

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# AMERICAN UNIVERSITY OF NIGERIA DEPARTMENT OF NATURAL AND ENVIRONMENTAL SCIENCES

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# HEALTH IMPACTS

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**HEALTH IMPACTS**

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# DEDICATION

This project is dedicated to my family.

# ACKNOWLEDGMENTS

I give thanks to almighty Allah for giving me the strength to successfully complete this project. I would also like to thank my beloved family for the support and encouragement they provided during my academic career. Their words of encouragement were very helpful and kept me going. Also, my deep appreciation goes out to my friends that were there for me emotionally and mentally. I am really grateful to them all because they made all this possible.

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# ASSESMENT OF BACTERIA FOUND IN THE AMERICAN UNIVERSITY OF NIGERIA (AUN) FACILITIES AND POTENTIAL

**HEALTH IMPACTS**

# Fadila Mohammed Kabir

American University of Nigeria, 2017

Major Professor: **Hayatu Raji, Ph.D.**,

Assistant Professor of Natural and Environmental Sciences

# ABSTRACT

Globally, human health is under threat due to the presence of diseases caused by microorganisms such as bacteria and viruses. Bacteria are found on almost all surfaces with which people come into contact and therefore pose a threat to human health. Antimicrobial resistance is a major health concern as these microorganisms can cause untreatable diseases. I tested the surfaces found in three buildings on the campus of the American University of Nigeria in Yola, Adamawa State, for the presence of harmful bacteria. Swabs collected were streaked on lysogeny broth agar to determine if bacteria were present. Selective and differential media were used to identify the type of bacteria found. Also, the samples were subjected to an antimicrobial susceptibility test using replica plating. The result showed the surfaces were contaminated with *Escherichia coli*, *Enterobacter aerogenes*, and *Enterococcus faecalis*, with *E. coli* and

*E. aerogenes* being resistant to ampicillin. This study showed that AUN is not only contaminated with pathogens, but also with bacteria resistant to ampicillin. The institution should provide hand sanitizers around campus and also create awareness

programs on the importance of hand washing. Also, the efficiency of cleaning can be increased by making use of stronger agents and increasing the frequency of cleaning.

# Keywords

American University of Nigeria, bacteria, antimicrobial resistance, microbial contamination, surfaces, students and staff

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# CHAPTER 1 INTRODUCTION

Human health is under serious threat globally by microorganisms, especially viral and bacterial diseases. Bacteria and viruses can be found on surfaces and could potentially cause harm. Infectious diseases are emerging at an alarming rate and have contributed to a good number of deaths globally. Although over the past decade the mortality rates of these diseases have declined, the impact these diseases have on the world remains substantial. Infectious diseases are the second leading cause of death worldwide, with 57 million deaths occurring each year, as reported by the Center for Disease Control and Prevention (CDC) (Fauci, Touchette, & Folkers, 2005).

There are increasing global concerns about certain issues such as antibiotic-resistant bacteria, Zika virus, HIV/AIDS, Ebola virus, and SARS. These diseases have a high mortality rate and have been identified by the World Health Organization as diseases likely to cause epidemics (WHO, 2015). In Africa, emerging diseases are a public health concern not only due to their high mortality rate, but also due to the fact that developing countries lack good health facilities. Despite countermeasures that have been developed due to advanced technology (therapeutics and vaccines), infectious diseases that affect both human health and the economic stability of societies have not been contained (Morens & Fauci, 2013). Bacteria and viruses that have developed resistance to antimicrobial agents are more life threatening and are now a serious public health concern.

*Antibiotic resistance*

Antimicrobial resistance has become a major global concern. Aside from bacteria and viruses, other microorganisms are rapidly developing a resistance to antimicrobial agents being used to kill them. This evolution makes the treatment of infectious diseases less effective and, in the long run, may cause death. Though sometimes viewed as an apocalyptic fantasy, antibiotic resistance, which might allow even minor injuries to kill, is indeed a reality in the 21st Century (WHO, 2014). Antimicrobial resistance is a global concern because it will make the treatment of infectious diseases less effective and prolong illnesses (WHO, 2016). Additionally, organ transplantation, C-section delivery, and other medical procedures may become more risky due to the lack of effective antimicrobials.

The increasing global concern for antimicrobial resistance has urged scientists to further research this issue. Projections made by scientists showed deaths that could be attributable to antimicrobial resistance yearly by 2050 will be high (Fig.1). Most deaths will most likely occur in Asia and Africa. Although antimicrobial resistance is increasing, less researches have been carried out on new drugs to curtail these resistant pathogens.



Figure 1. Projections of deaths that could be attributable to antimicrobial resistance yearly by 2050

*Resistance in bacteria*

Various bacteria have developed a resistance to particular antibiotics. To name a few, bacteria such as *Mycobacterium tuberculosis* and *Staphylococcus aureus* have developed a resistance to certain antibiotics (Shanks & Peteroy-Kelly, 2009). *Klebsiella pneumoiae*, a bacterium known for causing intestinal infections, is resistant to carbapenem antibiotics and has spread globally, leading to several deaths (WHO, 2016). Additionally, *Escherichia coli* which causes intestinal infections, is resistant to fluoroquinolone antibiotics. Currently, many countries around the world have no effective treatment for these bacteria. Other bacteria, such as *S. aureus* (Fig.2) and members of the family Enterobacteriaceae, which are resistant to methicillin and carbapenem, have also contributed to many untreatable conditions in different countries (WHO, 2016). The more resistant a strain of bacteria is, the greater the threat to human health.

*Increasing health risks due to microorganisms*

Pathogens can be found on almost all surfaces. Bacteria have the ability to grow anywhere, even in narrow surfaces, and have the ability to move (Männik et al., 2009). They can also survive on surfaces for an extended period. Some diseases caused by bacteria and viruses are life threatening and require immediate attention (Morris, 2016). Prophylaxis such as vaccinations may help lower the probability of getting infected. Also, good hygiene behaviors and good cleaning practices could lower the risk of getting infected. Globally, pandemics are rapidly spreading and have left people concerned about their health. People are more likely to catch diseases caused by bacteria and viruses in places where people congregate and where they make frequent contact with non-living objects.

Before assessing the level of microbial contamination on surfaces, hygiene standards must be considered which are set by regulatory bodies (White, Dancer, Robertson, & McDonald, 2008). Such standards for pathogens were set by the World Health Organization (WHO) at <1cfu/cm2, which means colony forming unit should not exceed 1 per cm2.

People in congregated areas are increasingly exposed to health risks daily. Health risks associated with public places vary; however, bacteria and viruses are the most easily contracted microorganisms. Microorganisms have several routes through which they can infect humans, including the mouth and from the hand to the mouth (Butz, Fosarelli, Dick, Cusack, & Yolken, 1993). Exposure routes of bacteria could be from shopping carts, offices, restrooms (especially public restrooms), and even on university campuses.

*Public places and bacteria*

The human skin is the main organ of contact with microbes. The skin on its own houses many bacteria which could be beneficial or harmless. Even though most bacteria found on the human skin are harmless, *S. aureus* (Kloos & Musselwhite, 1975), *Corynebacterium minutissimum*, and *Pseudomonas aeruginosa* could cause skin diseases, such as atopic dermatitis and erythrasma (Ross & Neufeld, 2015).

Studies have shown that bacteria, such as *Acinetobacter calcoaceticus* and *S. aureus,* commonly occur on hospital surfaces. “Hospital” bacteria lead to nosocomial infections which are infections acquired in hospital settings. In this research, the life span of the bacteria found on dry hospital surfaces was found to be 13 days, which could be an additional factor in transmission of nosocomial infections (Getchell- White, Donowitz, & Groschel, 1989). In the past years, much attention has been paid to nosocomial infections as the pathogens causing these infections became resistant to antibiotics. In comparison, there are few studies focusing on infections originating on public surfaces, such as public telephones, shopping carts, buses, shopping carts, office furniture, and even on surfaces in university campuses.

Public telephones, which are commonly used in some parts of the world, have been investigated and are found to harbor pathogens. A particular study carried out in Melbourne, Australia, identified certain potentially pathogenic bacteria on 20 telephones, such as *Acinetobacter anitratus*, *Enterobacter cloacae*, *Pantoea agglomerans*, and *S. aureus* (Ferdinandus, Hensckhe, & Palombo, 2001). From this study, it was ascertained that telephones could be labelled as potential reservoirs for pathogens.

Similarly, transportation systems such as buses and trains harbor pathogens. Transport systems in Portland, Oregon, USA, were investigated for the presence of pathogens on seats, floors, and railings (Yeh, Simon, Millar, Alexander, & Franklin, 2011). The research further analyzed the pathogens for any resistance in certain antibiotics. Bacteria were found to be more abundant on floors than railings, handles, and seats. Various species of *Staphylococcu*s, such as *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus, S. warneri*, *and S. xylosus*, were found in the buses. Even though there was no high level of multi-drug resistance, some of the species such as *S. xylosus* and

*S. haemolytics* were found to be resistant to gentamycin and erythromycin and could engage in horizontal transfer of resistance to other species (Yeh et al., 2011).

Subsequently, pathogens are also commonly found on shopping carts, which may be touched by a large number of people each day. A study carried out across the United States in 5 grocery stores found *E. coli*, *Yersinia pseudotuberculosis*, *Klebsiella oxytoca*, and *E. cloacae* on shopping carts (Gerber & Maxwell, 2012). In offices, pathogens may be present on surfaces and passed around from one surface to another. Office equipment and furniture, such as printers and computers, carry these pathogens, and since they may be not be regularly cleaned, people may have a high chance of getting infected (Reynolds, Watt, Boone, & Gerba, 2005).

Another “public” place, university campuses, may have high population densities, which makes it easier for bacteria to spread. Students may be exposed to many health risks on campuses, which can be contracted from unclean surfaces, cafeteria food, or bathrooms. Communicable diseases spread easily in places with many people as is the case with universities. The presence of harmful bacteria in a university can pose a

threat to the health of students, staff, and faculty (Morris, 2016). These bacteria can be easily passed around because as people interact in congregated areas, they may pick up bacteria which could potentially infect them as well as people around them (Shanks & Peteroy-Kelly, 2009). Students in a university normally make use of computers, library study tables, cafeteria tables, and restrooms. All these surfaces may harbor bacteria and could potentially harm people. Unless students are frequently washing their hands or making use of hand sanitizers, they are more likely to get infected.

*Bacteria in universities*

Previous studies on bacteria in university campuses have confirmed the presence of bacteria on surfaces. For example, pathogens such as *E. coli* have been found on university tables in clinics, laboratories, and libraries (Burnham, Peterson, Vavrek, & Haas, 2009). Disinfection protocols may have an impact on the level of contamination. However, pathogens could still be present regardless of the cleaning being done on surfaces.

Health risks to students on university campuses could result from the use of public computers, ingestion of cafeteria food, use of bathrooms, or contact with railings, and door handles. Cafeterias may be cleaned frequently, but may still harbor bacteria. Food particles could fall off on the tables in a cafeteria, and if the tables are not probably cleaned, could be the cause of microbes being present. Contact between students and these tables could transfer pathogens to people. Bathrooms are generally known to harbor bacteria. Public restrooms are visited by people with different hygiene behaviors, which can influence the type of bacteria found on restroom surfaces (Flores et al., 2011). Urinary Tract Infections (UTIs) may be contracted from the use of public

toilets, and these are often caused by *E. coli,* which is commonly found on restroom surfaces. Depending on the cleaning schedule of a facility, the level of contamination can be minimal.

In addition to restrooms, computer keyboards in computer labs could also be infected with bacteria. Campus computers are often accessed by many people daily, and frequent contact could contribute to high microbial activity. Keyboards may be not routinely cleaned, as well (Anderson & Palombo, 2008). Microorganisms, such as *S. aureus*, Enterobacteriaceae, *Enterococcus faecalis*, and *Bacillus cereus*, were found on computer keyboards in a university computer lab with *S. aureus* having the highest colony growth on computers used by multiple people.

Another study conducted in a university in New York City sampled different surfaces in lecture halls, restrooms, libraries, and cafeterias (Shanks & Peteroy-Kelly, 2009). The findings revealed that the bacteria found on these surfaces, including *E. coli*, *Salmonella typhi*, *S. aureus*, were resistant strains. This poses a greater risk to people on campuses as the diseases caused by these pathogens will be harder to treat (Shanks & Peteroy-Kelly, 2009). This shows that students may potentially become infected and that these infections might be hard to curtail due to the resistant strains.

The health risk to students may be even higher if bacteria found on campuses have developed a resistance. Such resistance is often due to a mutation or frequent use of an antibacterial agent to clean the surfaces. The bacteria could adapt to the substance being used to clean and natural selection will take place. The only way to curtail diseases caused by bacteria resistant to antibiotics is by prescribing the right dosage of

antibiotics. This is a pressing public health issue.

In addition, the rise in antimicrobial resistance has also been attributed to the frequent use of antimicrobial agents while cleaning. Some bacteria found on public surfaces that have resistant strains include *E. coli*, *Salmonella*, and *Enterococcus* species (Conly, 2002). The bacteria mentioned are often found on campuses. The different types of bacteria found on surfaces depends on seasons and the different uses of the buildings (Dunn, Fierer, Henley, Leff, & Menninger, 2013).

The level of microbial contamination in a university depends on several factors. These include the cleaning schedule practiced by the Facilities Management and, cleaning materials and products being used (e.g., types of chemicals). The surfaces of a university campus can be seen as non-critical since the surfaces come in contact mostly with human skin and is not necessarily contaminated by blood and bodily fluids (Rutala & Weber, 2001). The U.S.-based Center for Disease Control and Prevention (CDC) has emphasized the need to disinfect surfaces even though they have not been contaminated with blood or body fluids (Rutala & Weber, 2001). If the surfaces get cleaned frequently with a strong disinfectant, the bacterial load will be minimal or negligible.



Figure 2. Structure of *Staphylococcus aureus*

Pathogens could be the cause of the high prevalence of bacterial infections on campuses. Numerous infections such as typhoid and flu recorded by clinics on university campuses are most likely transmitted in the dormitories or academic buildings due to the crowded lifestyle on campuses and contact with surfaces (Pechter, 2011). Inadequate personal hygiene increases the level of contamination and also puts students at risk of contracting diseases. Some of these diseases are life threatening and require immediate attention.

The literature on health risks to university students on campuses appears sparse, particularly for universities in developing countries where standards may be lower. Therefore, I investigated facilities on the American University of Nigeria (AUN) campus in Yola, north-eastern Nigeria, for the presence of bacteria on surfaces commonly touched by students. The aim of my study was to determine which strains of bacteria occur in these public places and whether any strains were resistance to antibiotics. The outcome of this work helps raise awareness regarding potential

harmful pathogens on campus. Findings will be shared with Facilities Management of the university.

# RESEARCH QUESTION

Are students and staff exposed to harmful bacteria when they use the AUN facilities?

# HYPOTHESES

**Null hypothesis (H0):**

Bacteria found on surfaces in AUN facilities pose no health risks to students and staff.

# Research Hypothesis (H1):

Bacteria found on surfaces in AUN facilities pose health risks to students and staff.

# AIMS & OBJECTIVES

**Aim:**

* To determine potential health risks to students and staff who use the AUN facilities.

# Objectives

* To swab surfaces in AUN and check for bacteria.
* To identify the types of bacteria found on surfaces in AUN facilities.
* To determine if the bacteria found are potentially harmful to health.
* To examine antimicrobial resistance in the bacteria found in AUN.
* To perform molecular analysis so as to determine the cause of antimicrobial resistance
* Based on my findings, to recommend potential changes in cleaning approaches to AUN Facilities management and clinic.

# CHAPTER 2

# MATERIALS & METHODS

*Study Site*

The American University of Nigeria (AUN) is located in the north-eastern part of Nigeria, in Yola, Adamawa State. It is a rural university with a total of 16 buildings, including a cafeteria, library, school clinic, and classroom buildings. At the start of Spring semester 2017, there were about 800 students enrolled in the university. The total population of the university including students, staff, and faculty, is about 2000 people. The AUN library is a relatively large building with three floors; it contains many private study rooms, offices, the President’s office, several classrooms, as well as many study tables and desk in the common (open) areas. The cafeteria comprises of two serving points, tables, chairs, and restrooms which are frequently used by students. The Arts and Science building comprises of three different schools which are located on different floors. It is made up of three floors; the school of information technology is on the ground floor, school of arts and science on the first floor, and the school of business and entrepreneurship on the second floor. The arts and science building contains classrooms and laboratories with table surfaces as well as restrooms which are used by people daily.

*Sampling*

For my sampling method, I used complete census for the restroom faucets and door handles in the Arts and Science building (8 faucets on each floor and 4 restroom door handles on each floor, n = 36). For the AUN Library, I used stratified sampling; I sampled 20 individual desks and tables from each of the three library floors (n = 60). Finally, I made a complete census of the door handles and restroom faucets in the

cafeteria (7 doors and 6 faucets, n = 13). Total sample size used for this research was

109. Using sterile swabs, I then swabbed across tops of study tables once over a one- week period.

*Materials*

Distilled water, Lysogeny broth agar (LB), Petri-dishes, Autoclave, Sterile swabs, tape, pipette, inoculating loop, weighing balance, beakers, velvet cloth, chloramphenicol, ampicillin, tetracycline, GET Plasmid Mini Prep, Tris/Borate/EDTA (TBE buffer), 42◦C water bath, ice, competent DH5a cells, lysis buffer, Longlife RNase, neutralizing buffer, DNA wash, columns with collections tubes, 1.5ml tubes, cell suspension solution, micropipette.

*Lab analysis*

# Bacterial culture (Lysogeny Broth Agar)

Samples were taken at random from each surface. A solution of lysogeny broth agar was prepared with 1liter of distilled water to 40 g of LB. The solution was subjected to heat in an autoclave at 121◦C for about 15 minutes. The solution was poured into the petri-dishes which were used for culturing bacteria. This was done in a biosafety chamber to avoid any contamination. The agar was divided into 4 quadrants. The sterile swabs were streaked on the petri-dishes gently without tearing into it. Sections were labelled so as to know the source of each bacteria. The media were stored at 37◦C for 24 hours. Colonies formed were further streaked on LB agar to sub-culture so as to get isolated colonies.

# Sub-culture of Bacteria (Lysogeny Broth Agar)

Lysogeny broth agar was prepared by dissolving 40g of the agar in 1 liter of distilled water. The solution was autoclaved at 121◦C for 15 minutes. It was poured into petri- dishes and was aired for some minutes. The bacterial growth on the initial petri-dishes were obtained and based on the morphology of the bacteria, different colonies were inoculated and streaked on fresh LB plates. This was done for all samples obtained from all surfaces. The petri-dishes were labelled sampled 1-21 so as to know the source of the bacteria. The petri-dishes were stored upside down in an incubator for 24 hours to allow colonies to form. The colonies formed were further streaked on differential and selective media to allow identification of bacteria based on their appearance.

# Differential and Selective media for identification of bacterial species

For identifying the bacterial colonies based on appearance (Table 1), Hektoen Enteric Agar, Salmonella Shigella Agar, Eosin Methylene Blue Agar, Endo Agar, and MacConkey agar were used. The identification of the bacteria was done using replica plating and streaking method.

*Streaking method:*

The colonies formed on the nutrient agar were streaked on the differential medium using inoculating loop. The cover of the petri-dishes were closed and stored upside down in an incubator. They were stored in an ideally warm environment which had a temperature of 37◦C. The dishes were monitored for 24 hours to see if there was any growth, and were further analyzed to identify the various strains of bacteria that could be identified.

*Identification of microorganisms using replica plating*

The velvet cloths used were sterilized so as to avoid any contamination. The original plates that contain bacteria were used for this method. The differential media were poured into petri dishes. All these were done in the fume hood chamber to rule out sources of contamination. The petri dishes were allowed to solidify. A velvet cloth was used to cover a container using a rubber ring to hold it together. The original plate was placed on the cloth and removed, after which the differential media was placed on the same spot without pressing hard. This was done for all the differential media. The petri dishes were incubated at 37◦C for 24 hours. They were analyzed the next day to note the different strains of bacteria found.

*Differential and Selective media used*

## MacConkey Agar for identification of E. coli and Enterobacter (OXOID)

I dissolved 52g of the solute in 1 liter of distilled water. The mixture was stirred and boiled till it was completely dissolved. The solution was autoclaved at 121◦C for 15 minutes. It was cooled down and poured into petri dishes. The agar was left to solidify before inoculating. The samples were inoculated and incubated for 24 hours.

## Hektoen Enteric Agar for identification of E. coli (OXOID)

I dissolved 76g of the agar in 1 liter of distilled water and allowed it to soak for 10 minutes. The solution was boiled so as to dissolve the solute completely. The solution was not autoclaved. It was allowed to cool down to 50◦C after which it was poured into plates. I allowed it to cool after which all the 21 sub-cultures were streaked on the agar.

## Eosin Methylene Blue Agar for the identification of coliforms (OXOID)

I suspended 37.5g of the agar in 1 liter of distilled water. The solution was boiled and allowed to dissolve completely. It was poured into a jar and autoclaved at 121◦C for 15 minutes. The solution was allowed to cool down to 60◦C after which it was shaken to restore the blue color. It was poured into plates and allowed to cool down. All samples streaked on the LBA were streaked on this agar and labelled accordingly.

## Mannitol Salt Agar for identification of Staphylococcus and Micrococcus species (OXOID)

I suspended 111.2g of mannitol salt agar in 1 liter of distilled water. The solution was stirred till all the solute was dissolved. It was poured into a jar and was autoclaved at 121◦C for 15 minutes. It was cooled down and poured into the petri-dishes and let to solidify before streaking. All samples were streaked and incubated for 24 hours.

## Salmonella shigella Agar for identification of Salmonella and Shigella species (OXOID)

I suspended 57g of the solute in 1 liter of distilled water. The mixture was boiled till it started shimmering so as to dissolve the solute. Afterwards, it was transferred to a sterile jar and was let to cool down. This solution was not autoclaved. It was poured into petri-dishes and all samples were streaked and labelled. After 24 hours the petri- dishes were analyzed.

## Endo Agar for identification of Klebsiella pneumoniae and E. coli (OXOID)

I suspended 36g into 1 liter of distilled water. 4ml of a 10% w/v solution of basic fuchsin and 95% ethanol was added into the jar. The solution was stirred and boiled

till completely dissolved. It was autoclaved at 121◦C for 15 minutes. After it cooled down, it was poured into petri dishes and the get was allowed to solidify. All samples were inoculated on the media and incubated for 24 hours.

Table 1. Appearance of microorganisms on differential media

|  |  |  |
| --- | --- | --- |
| **Media** | **Organisms** | **Color indication** |
| Hektoen Enteric Agar | *E. coli, Salmonella typhi* | Yellow, black |
| Eosin Methylene Blue Agar | *E. coli, Enterobacter faecalis* | Metallic greenand pink |
| Mannitol Salt Agar | *Staphylococcus aureus,**Micrococcus* | Yellow and pink |
| Salmonella shigella agar | *Salmonella typhi* and *shigella* | Black and |
| Endo Agar | *Klebsiella pneumoniae, E. coli, Enterobacter aerogenes* | Light pink, golden metallicsheen, pink |
| MacConkey Agar | *E. coli, Enterobacter aerogenes* | Pink and lightpink |

# Antimicrobial susceptibility test using replica plating

The initial petri-dishes that had bacterial growth on them were used for this procedure. Not all petri-dishes were used for the library, however, two samples each were picked from each floor in the library. All the samples for the arts and science building and cafeteria were tested against the antibiotics. I prepared LB agar by dissolving 40g of the LB in 1 liter of water. It was autoclaved at 121◦C for 15 minutes. Three different commonly used antibiotics were chosen which are, chloramphenicol, ampicillin, and tetracycline. After the agar had cooled down, a concentration of 100µg/ml was used for each antibiotic, poured into the agar and allowed to dissolve completely. The agar was poured into petri-dishes and allowed to solidify. A velvet cloth was used for the replica plating. A container was used as a base to support the velvet. The velvet was placed on the container without excessive contact and was held to the container with a

band. The petri-dishes with the initial bacterial growth were replicated on triplicate plates containing chloramphenicol, ampicillin, and tetracycline. This was done for all samples using all 3 different antibiotics. The samples were incubated for 24 hours. The petri-dishes were observed to see if the bacteria were growing. This was done for all the bacteria identified from the surfaces in AUN.

# Plasmid isolation for resistant bacteria

Lysogeny broth was prepared by dissolving 25g of solute in 1 liter of distilled water. The solution was stirred till it was miscible and was autoclaved at 121◦C for 15 minutes. The lysogeny broth was poured into 5 sterile 250ml conical flasks and 1 sterile 100ml conical flask with each beaker having 100ml and 50ml respectively. The 100ml conical flask was used as a control. The resistant bacteria were inoculated into the flasks using sterile inoculating loops. This was done in a biosafety chamber to avoid contamination. The flasks were covered with aluminum foil, labelled, and were incubated in a shaker at 37◦C. The solutions were shaken at 250 rpm. After 24 hours, 50ml out of each sample was poured into centrifuge tubes and was labelled accordingly. All samples were centrifuged 8500x g for 5 minutes. The supernatant was discarded while the pellet were kept in a freezer. The samples were obtained the following day and were re-suspended using 250µl of Cell Suspension Solution and LongLife RNase. 250µl of Lysis Buffer was also added to the pellet after which the tube was inverted until the lysate was clear. 350 µl of chilled Neutralizing Buffer was added into the solution and was inverted 10 times to ensure the solution was mixed properly. The tubes were centrifuged for 20 minutes at 8500x g. After the centrifugation, the supernatant were poured into the GET Plasmid Mini column and were labelled accordingly. The columns were centrifuged for 60 seconds at maximum

speed. The flow through of each column was discarded. The columns were washed using 500µl of DNA Wash and were centrifuged for 60 seconds. The flow through was discarded again and a final spin was conducted to ensure there was no residue of the DNA Wash. The columns were removed and placed in clean 1.5ml tubes. The plasmid DNA were eluted by adding 50µl of warm Tris/Borate/EDTA (TBE buffer) directly into the column membrane. The tubes were incubated at room temperature for 2 minutes and were centrifuged for 60 seconds. The eluted plasmids were measured for the presence of DNA using a nano spectrophotometer. After the readings were obtained, the plasmid DNA were further used for gel electrophoresis.

# Gel Electrophoresis

The gel was prepared by dissolving 1g of agarose in 100ml of Tris/Borate/EDTA buffer (TBE buffer). 10 µl of 10mg/ml of ethidium bromide was added to the solution after microwaving. The gel was poured into the mold which had a comb inside to create wells for the samples. After the gel cooled and was solid, the comb was gently removed. TBE buffer was poured into the chamber. The DNA sample was prepared in a 1.5ml tube by adding 2µl of the plasmid DNA, 3µl DNA loading dye, and 10µl of sterile water. The DNA sample was loaded into the wells carefully using a micropipette. The wires were connected to the power outlet and the electric current was turned on. This was done for 30 minutes. After 30 minutes, the gel was removed and viewed under ultraviolet light.

# Bacterial transformation of plasmid DNA

The TOP10 competent cells were thawed on ice. The cells were mixed after which 50µl of the cells were put into chilled 1.5ml tubes. A sample was selected for the

procedure. Two tubes with competent cells were used as negative and positive control with the negative control containing *E. coli* not resistant to ampicillin and the positive containing a plasmid with the ampicillin resistant gene. 2µl of the plasmid DNA was added into the sample tube and mixed gently. The tubes were incubated on ice for 30 minutes. The tubes were subjected to heat at 42◦C for 30 seconds. The tubes were placed on ice again for 2 minutes. 1ml of warm LB was added to the tubes and were shaken at 200 rpm and also a temperature of 37◦C for 1 hour. LB agar was prepared and was mixed with ampicillin in a final concentration of 100µg/ml. The agar was poured into plates and allowed to solidify. For the negative and positive control, 500µl were added to two plates each and were spread across the plate. For the sample, the solution was spread on 3 petri-dishes. The dishes were left to dry and incubated at 37◦C for 24 hours. The inoculation was done in a biosafety cabinet to ensure sterile procedures.

# CHAPTER 3 RESULTS

*Surface analysis*

Three buildings frequently used by members of the university community as well as visitors were sampled for presence of microorganisms. Of the 109 samples swabbed, only 10 surfaces had no presence of bacteria while microbes were found on all the other 99 surfaces. Of all the surfaces sampled all through the university, the most microbial colonies were obtained from the restrooms in Arts and Science building.

In the Arts and Science building, more microbes were found in female restrooms than in male restrooms. In the library, the tables selected on each floor had microbial growth. Almost all the bacteria identified in the library were found to be coliform bacteria while some which could not be identified were not coliform. Coliform bacteria are gram-negative bacteria that ferment lactose. The presence of these cafeteria on surfaces indicates fecal matter contamination. In the cafeteria, all the doors and faucets sampled were observed to have microbial growth. All the bacteria found in the cafeteria restrooms and door handles were coliform. Generally most of the bacteria were found to be coliform with the exception of just a few locations. Most of the colonies had similar morphology except a few. They mostly appeared to be circular, mucoid, and convex. Just a few colonies in the restrooms in Arts and Science were rhizoid and irregular. Also, some of them appeared to be transparent. The list of the bacteria found on surfaces is shown in the table below (Table 2).

Table 2. Where bacteria were found

|  |  |
| --- | --- |
| **Sample sites** | **Microorganism found** |
| **Arts and Science Ground floor**Female restroom Male restroom | *Escherichia coli*Bacteria could not be identified |
| **Arts and Science First Floor**Female restroom Male restroom | *Enterobacter aerogenes, Escherichia coli Escherichia coli* |
| **Arts and Science Second Floor**Female restroom Male restroom | *Enterobacter aerogenes*Bacteria could not be identified |
| **Cafeteria**Entrance doors Female restroom Male restroom | *Enterobacter aerogenes* Bacteria could not be identified Bacteria could not be identified |
| **Library**Ground floor First floorSecond floor | *E. coli, Enterobacter aerogenes**E. coli, Enterobacter aerogenes, Enterococcus faecalis**E. coli, Enterobacter aerogenes* |

*Microbial Identification*

The differential and selective media used for the identification of the microbes showed the presence of coliforms. The coliform bacteria were found in most restrooms. Coliforms were also found in the library and restrooms in the cafeteria. The microbes that were identified are found to be *E. coli, E. faecalis,* and *E. aerogenes*. Most of the microbes could not be identified with the differential and selective media available.

Table 3. Differential and selective media used and the microorganisms detected

|  |  |
| --- | --- |
|  | **Selective and Differential Media Used** |
| **Sampling site** | **Endo Agar** | **Mannitol Salt Agar** | **Eosin****Methylene Blue Agar** | **Hektoen Enteric Agar** | **Salmonella Shigella Agar** | **MacConkey Agar** |
| Arts and Science ground floor (female) | ― | ― | *Escherichia coli* | ― | *―* | ― |
| Arts and Science ground floor (male) | ― | ― | ― | *―* | *―* | ― |
| Arts and Science First floor (female) | *Enterobacter aerogenes* | ― | *Escherichia coli* | *Escherichia coli* | *―* | *Enterobacter aerogenes* |
| Arts and Science First floor (male) | *Escherichia coli* | ― | ― | ― | *―* | ― |
| Arts and Science second floor(female) | *Enterobacter aerogenes* | ― | *Enterobacter aerogenes* | ― | *―* | ― |
| Arts and Science Second floor (male) | *Enterobacter aerogenes* | ― | *Enterobacter aerogenes* | ― | *―* | *Enterobacter aerogenes* |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Library ground floor | *Escherichia coli, Enterobacter**aerogenes* | ― | *Escherichia coli, Enterococcus**faecalis* | ― | *―* | *Enterobacter aerogenes, Escherichia coli* |
| Library first floor | *Escherichia coli, Enterobacter aerogenes* | ― | *Enterococcus faecalis* | *Escherichia coli* | *―* | ― |
| Library second floor | *Escherichia coli* | ― | *Escherichia coli, Enterobacter aerogenes, Enterococcus**faecalis* | *Escherichia coli* | *―* | ― |
| Cafeteria | *Enterobacter aerogenes* | ― | ― | ― | *―* | *Enterobacter aerogenes* |

*Antimicrobial susceptibility analysis*

All the samples obtained were subjected to three antibiotics; chloramphenicol, ampicillin, and tetracycline. These antibiotics were used because they are frequently used by people as well as students in AUN to treat bacterial infections. The procedure was done by replicate plating the samples on LB agar with antibiotics. The table below shows the results obtained from the antimicrobial susceptibility test (Table 4).

Table 4. Susceptibility test of bacterial colonies against antibiotics

|  |  |  |  |
| --- | --- | --- | --- |
| **Source of Bacteria** | **Chloramphenicol** | **Ampicillin** | **Tetracycline** |
| Arts and Science ground floor | Susceptible | Resistant | Susceptible |
| Arts and Science first floor | Susceptible | Susceptible | Susceptible |
| Arts and Science second floor | Susceptible | Resistant | Susceptible |
| Library ground floor | Susceptible | Resistant | Susceptible |
| Library first floor | Susceptible | Susceptible | Susceptible |
| Library second floor | Susceptible | Resistant | Susceptible |
| Cafeteria doors | Susceptible | Resistant | Susceptible |
| Cafeteria restrooms | Susceptible | Resistant | Susceptible |

All the bacteria were susceptible to chloramphenicol and tetracycline. For the samples that had resistant strains of bacteria, they were further subjected to biochemical tests so as to identify them. From the table above (Table 3), it can be seen the bacteria were only resistant to ampicillin while chloramphenicol and tetracycline were very effective in killing them. Surfaces that harbored the ampicillin resistant strains were study tables in the library, faucets in the restrooms, and door handles in the cafeteria. Endo agar and Eosin Methylene Blue agar were used to confirm that the bacteria were coliform. Out of the 8 locations sampled in school, 6 were found to have resistant strains of

bacteria. The petri-dishes from these 6 locations were tested using replica plating and were incubated for 24 hours. Seven of the petri-dishes had resistant strains with 6 of them being coliform bacteria. The coliform bacteria were identified to be *E. coli* and

*E. aerogenes*.

There are different mechanisms for antibiotic resistance. The common mechanism for resistance to ampicillin is by the production of beta lactamase. This enzyme hydrolyzes the beta lactam ring of ampicillin. The gene that produces beta lactamase may be located on the bacterial chromosome. Alternatively, it may be on a plasmid instead of the chromosomal DNA. That was why a plasmid extraction was done to find out if the bacteria were resistant to ampicillin because of the presence of a plasmid. After the plasmid extraction, a nano spectrophotometer machine was used to confirm if indeed there was DNA in the samples before running them on gel. The nanodrop indicated that there was DNA in the samples and it was further confirmed by gel electrophoresis. The readings can be seen in the table below (Table 5). The readings being 0 means there is no plasmid, and if it happens to be more than 0, then there is plasmid present in the bacteria. The presence of the plasmid could be the reason for the resistance. The samples tested for the presence of plasmid are from the Arts and Science building, cafeteria, and the library.

Table 5. Amount of DNA in each sample

|  |  |
| --- | --- |
| **Samples** | **Readings (ng/µl)** |
| Sample 1 | 74.1 ng/µl |
| Sample 2 | 70.2 ng/µl |
| Sample 3 | 73.8 ng/µl |
| Sample 4 | 119.7 ng/µl |
| Sample 5 | 54.9 ng/µl |

The presence of DNA was checked using gel electrophoresis. The result showed DNA in all 5 samples (Fig. 3). This indicates that there is plasmid DNA in all samples.



Figure 3. Gel electrophoresis under UV light

To further confirm if the presence of plasmid in the bacteria is the reason for the resistance, the plasmid DNA were used for the transformation of competent bacterial cells. If bacteria grow on the LB agar after putting ampicillin, then the presence of

plasmid is the reason for the resistance. However, after transformation, no growth was seen on the LB/ampicillin plates, as well as on the negative control plates. There was only bacterial growth on the positive control plates which confirms that the presence of plasmid DNA is not the reason for the resistance to ampicillin. A positive control was used so as to know if the transformation procedure worked.

# CHAPTER 4 DISCUSSION

This study showed that the certain surfaces in three American University of Nigeria facilities are contaminated with microbes that can potentially harm students and staff. The results showed that more bacteria were present in the female restrooms than in the male restrooms. This could be due to women practicing a poorer personal hygiene than men or less effective cleaning in the female restrooms.

Researches on microbial contamination in universities appear sparse and have generally focused on public areas such as public restrooms and supermarkets. However, few researches conducted in universities in the United States showed the presence of pathogens such as the ones found in AUN on surfaces. A research conducted in a university in the United States confirmed the presence of pathogenic microorganisms on surfaces such as keyboards, faucets, and telephone mouthpieces. The surfaces sampled were frequently used surfaces and the results showed the probability of students getting infected (Brooke, Annand, Hammer, Dembkowski, & Shulman, 2009). In another study conducted in a Canadian university, the results showed that fomites were indeed contaminated, especially in the co-ed dormitories. The study also showed that students were concerned about the presence of pathogens such as H1N1 virus and were considering a change in personal hygiene (Decker & Slawson, 2012).

Some of these microbes were found to be resistant to ampicillin, posing a potential threat to the health of staff and students. The type of bacteria found on the surfaces are bacteria that are commonly found in congregated places as well as restrooms. All the

bacteria identified were found to be coliform bacteria. From the study conducted, *Escherichia coli*, *Enterococcus faecalis*, and *Enterobacter aerogenes* were found in all the buildings sampled. However, there were other microbes that could not be identified due to lack of resources.

Microbes are ubiquitous and therefore expected to be found on most surfaces, however, not all microbes are expected to be harmful. In the case of American University of Nigeria, these microbes could be potentially harm students and staff. This becomes a public health concern especially if the bacteria found have developed a resistance.

Most of the surfaces sampled in AUN harbored *E. coli*, *E. faecalis*, and *E. aerogenes*. Even though not all strains of *E. coli* are harmful, some strains (such as O157:H7) cause problems such as kidney failure, anemia, bloody diarrhea, and ultimately death (CDC, 2016). Also, other strains of *E. coli* could cause urinary tract infections (CDC, 2016). *E. coli* is a coliform bacterium which means it is gotten from feces of humans or animal origin. Contamination can occur if good personal hygiene practices such as washing of hands after a visit to the toilet are not practiced. Some strains of *E. coli* found on the AUN campus surfaces are resistant to ampicillin, which is most likely due to the production of beta-lactamase.

*Enterococcus faecalis* could also be lethal if not treated properly. This bacterium is part of the normal gut flora of animals. It has been recognized to be important to the human body but could still be harmful depending on the strain. Infections caused by

*E. faecalis* include UTIs, bacteremia, and soft tissue infections. These infections

caused by *E. faecalis* can be treated with antibiotics but some strains have developed a resistance to beta-lactam antibiotics. However, the strain found on campus was susceptible to all the three antibiotics tested.

*Enterobacter aerogenes* was the most prevalent bacterium found on the AUN campus. It was mostly found in the restrooms which was no surprise as it is a coliform bacteria. This bacteria could be pathogenic depending on the strain. Infections such as UTIs, bacteremia, endocarditis, septic arthritis, etc., are caused by *E. aerogenes* (Thiolas, Bollet, La Scola, Raoult, & Pagès, 2005). Getting infected with this bacteria could be a cause for prolonged hospitalization and could also lead to death if not treated properly (Thiolas et al., 2005). The strain found on campus was confirmed to be resistant to ampicillin. This is an issue because infections can be persistent if the right antibiotic is not used.

From this study, *E. coli* and *E. aerogenes* were found to be resistant to ampicillin; this was an expected finding. There were other bacteria that were also resistant, however, they could not be identified due to lack of resources. The presence of a plasmid DNA in a bacteria could be the reason for a resistance to antibiotics. The plasmids of the resistant strains were extracted and were further subjected to transformation. It was confirmed that the presence of the plasmid was not the reason for the resistance. The reason for the resistance to ampicillin could be due to a mutation on the bacterial chromosome.

Ampicillin is a beta-lactam antibiotic which contains a beta-lactam ring in its structure. Ampicillin has a broad-spectrum of bacteria it can kill. Although it is theoretically

expected to kill *E. coli* and *E. aerogenes,* ampicillin did not kill the strains found in AUN. These antibiotics target the penicillin-binding proteins which are found in the bacterial cell membrane. The beta-lactam ring prohibits cell wall formation of the bacteria and eventually leads to the death of the bacterial cell. Many bacteria have developed a mutation that enables them to neutralize the effect of beta-lactam antibiotics. They produce an enzyme called beta-lactamase which inactivates the antibiotic. The enzyme breaks the beta-lactam ring in antibiotics which deactivates its antibacterial properties. If such antibiotics are coupled with beta-lactamase inhibitors, the efficiency of the drug will increase as these inhibitors block the activity of the enzyme.

A research that was conducted in a New York University has shown that the resistant bacteria such as the ones found in AUN developed a resistance to beta-lactam antibiotics many years ago (Shanks & Peteroy-Kelly, 2009). Also, the strains resistant to antibiotics, especially beta-lactam antibiotics are indeed harmful to health. To effectively treat the antibiotic resistant bacteria, other antibiotics such as triclosan, chloramphenicol, can be used in place of ampicillin. The issue of antimicrobial resistance should be a major concern to the institution because staff and students could potentially get harmed. The hygiene practices of the people in the university if poor, can increase the probability of getting infected and could facilitate transmission of these pathogens. It can be inferred that microorganism transmission occurs throughout the university as the bacteria were found in places frequently visited by people. However, transmission of these pathogens can be reduced by encouraging frequent hand washing and making available hand sanitizers in public areas.

*Recommendations*

Evidently, microbes are present in all surfaces but could be reduced by effective cleaning. Surface disinfection is beneficial to human health as it eliminates the probability of getting infected with pathogens. The institution could implore the use of stronger cleaning agents, and clean surfaces more frequently, especially surfaces touched frequently by people. Also, the importance of hand washing should be emphasized as this could be effective in stopping the spread of microbes. Using soaps free of antimicrobial agents will be the best option as the frequent use of antimicrobial agents promotes resistance to antibiotics. Researches have shown the connection between the frequent use of antimicrobial agents and the development of resistance to those agents (Courvalin, 1994).

Even though no link between the microorganisms on campus and the health cases in the AUN clinic have been made, it is very possible some of these cases are caused by the microbes found. Nevertheless, the AUN management needs to be aware of the health risks associated with the microbes found in the AUN campus. Also, the presence of resistant strains of bacteria in the AUN campus is a major concern and should be taken seriously. The clinic should be aware so as to enable them make provisions for stronger antibiotics. Generally, there should be awareness on campus about the importance of hand washing as well as the use of hand sanitizers.

# CHAPTER 5 CONCLUSION

The American University of Nigeria (AUN) is indeed contaminated with microbes that could potentially harm students and staff. The microorganisms found could be detrimental to health and if not properly treated could be lethal. The results show that there is need for concern because some of the bacteria have developed a resistance. Although the effectiveness of cleaning was not determined, the results show that a need for a more effective way of cleaning is needed. AUN definitely has the interests of its students and staff at heart and should do everything possible to reduce the amount of bacteria found on surfaces. This might be hard to maintain but with the right cleaning agents and awareness programs on campus, this can be achieved. The results of this research will be shared with the institution so as to create awareness for the potential health risks on campus.

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