**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**COLLEGE OF SCIENCE**

**DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY**



**IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF BACTERIA AND**

**FUNGI ON EARBUDS USED BY STUDENTS ON KNUST CAMPUS**

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**CHAPTER 3**

**3.0 MATERIALS AND METHODS**

**3.1 Study Area**

This study was conducted at the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi-Ghana, which has a total land area of 2512.96 acres and is around eight miles (13km) to the east of Kumasi, the Ashanti regional capital. It is comprised of six colleges and has 11 departments. With GPS coordinates of 6°40’23.4300N and 1°33’55.5228W, it is situated between latitude 6.673175 and longitude 1.565423 (Lartey et al., 2021).

**3.2 Study Design**

This cross-sectional study involved fifty (50) participants on KNUST campus. Students that wore earbuds gave their consents to partake in the study. Oral consent was sought from students whose earbuds were sampled. Additionally, participants were given semi-structured questionnaires to provide demographic information related to the research study.

**3.3 Sampling Approach**

In this study, fifty (50) samples were collected from five (5) different colleges using the snowballing technique on KNUST campus. Samples were taken from the earbuds owned by students using sterile moistened cotton swabs, which were then placed in airtight bottles containing 10ml of peptone water and sealed immediately to prevent contamination. Bottles containing the collected samples were labelled appropriately. These samples were labelled with the indication ‘ST’ that is for student, accompanied with a number. The collected samples were transported to the Microbiology laboratory at the Department of Theoretical and Applied Biology, KNUST for laboratory analyses.

**3.4 Sterilization of Laboratory Glassware**

Test tubes, petri dishes and other glassware used were washed and air dried. Petri dishes were arranged in canisters and sterilized in the hot air oven at 180°C for two (2) hours. Test tubes and glass bottles used for holding the peptone water were sterilized in an autoclave at 121°C for fifteen (15) minutes.

**3.5 Media Preparation**

Media used in all analyses were prepared according to instructions by the manufacturer.

**3.5.1 Preparation of Potato Dextrose Agar (PDA)**

A measuring cylinder with a volume of 1 liter of distilled water was used to dissolve 15g of powdered PDA which was weighed on an electric scale. The mixture was heated on an electric plate and swirled frequently to allow for even distribution. The mixture was later sterilized in the autoclave at 121°C for 15mins.

**3.5.2 Preparation of Plate Count Agar (PCA)**

A measuring cylinder with a volume of 1 liter of distilled water was used to dissolve 17.5g of powdered PCA which was weighed on an electric scale. The mixture was heated on an electric plate and swirled frequently to allow for even dissolution. The mixture was later sterilized in the autoclave at 121°C for 15mins.

**3.5.3 Preparation of peptone water**

An electric balance was used to weigh 1.5g of peptone powder which was transferred into a beaker. 100ml of distilled water was measured in a measuring cylinder and added to the beaker containing the peptone powder. The solution was stirred until evenly mixed, after which, 10ml each of the solution was pipetted and transferred into glass bottles with fitting lids and autoclaved at 121°C for 15mins.

### 3.5.4 Preparation of Nutrient Agar

A conical flask with 500ml of distilled water was used to dissolve 24g of the powdered agar which was weighed on an electric scale. The mixture was heated on an electric plate and swirled frequently to allow for even dissolution. The mixture was later sterilized in the autoclave at 121°C for 15mins.

### 3.5.5 Preparation of distilled water

Test tubes were filled with distilled water with a volume of 9ml and corked with cotton wool. The corked test tubes were then sterilized in the autoclave at 121°C for 15mins and was later used for serial dilution.

### 3.6. Serial Dilution

Samples taken with the cotton swabs were placed in glass bottles containing peptone water. The contents were then emptied into zip lock bags and pulsified for 15 (fifteen) seconds in the laboratory. Serial dilutions from 10-1 to 10-4 were prepared for each sample. The test tubes were labelled 10-1, 10-2, 10-3,10-4. The first dilution, 10-1 was prepared by adding 1ml of the sample stock to 9ml distilled water. The second dilution, 10-2 was prepared by adding 1ml of the 10-1 dilution to 9ml distilled water. This was done to the 10-4 dilution factor.

### 3.7 Sample Preparation

After the dilution, 1ml of each dilution was pipetted onto petri dishes and the various agars (with the exception of the nutrient agar) were added under the laminar flow hood. The prepared agars were left under the laminar flow hood to solidify and were covered afterwards. Each petri dish was labeled with the name of the agar and sample.

### 3.7.1 Potato Dextrose Agar

A capsule of chloramphenicol was added to the agar to suppress bacterial growth. The mixture was poured onto petri dishes containing 1ml of each dilution (10-1 to 10-4). This was swirled gently to enable even spread in the petri dishes. The mixtures were left to solidify after which the petri dishes were fastened together with masking tape and labeled. They were then inverted and incubated at 30°C for 48 hours.

### 3.7.2 Plate Count Agar

Plate count agar was poured onto its labeled petri dishes containing 1ml of each dilution (10-1 to 10-4). These mixtures were swirled gently to enable even spread in the petri dishes. The mixtures were left to solidify after which the petri dishes were fastened together with masking tape and labeled. They were then inverted and incubated at 37°C for 24 hours.

### 3.8 Plate Count

### 3.8.1 Enumeration of total bacteria count

The colonies formed after the incubation periods were counted manually on the petri dishes illuminated by transmitted light from a counting chamber. Each plate was placed under the magnifying lens of the colony counter and the colonies formed on the plate were counted. The morphological features of the colonies were also described and recorded (mainly the bacterial colonies). The number of colony-forming units in each original sample was obtained by multiplying the number of colonies found on the plate by the reciprocal of the dilution factor of the sample. The final result was recorded in colony-forming units per milliliters (CFU/ml).

**3.8.2 Enumeration of Fungal Colonies**

After the incubation period, the petri dishes were sent to the laboratory at the Agric Department for morphological identification. The colonies formed on the plate were counted identified manually using their morphology and color. The number of colony-forming units in each original sample was obtained by multiplying the number of colonies found on the plate by the reciprocal of the dilution factor of the sample. The final result was recorded in colony-forming units per milliliters (CFU/ml). The fungal species identified included yeast, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus terreus*.

### 3.10 Biochemical Tests

### 3.10.1 Catalase test

This test is used to differentiate bacteria that produce catalase from bacteria that does not produce catalase. A small isolate was picked up from a day-old culture using a sterilized inoculating loop. This isolate was placed on a slide and a drop of hydrogen peroxide was added to it. A catalase positive test showed a bubble formation while a catalase negative test showed no change.

### 3.10.2 Indole test

This is to test for bacteria that can degrade tryptophan and produce indole. Test tubes were filled with 5ml of tryptophan broth and were sterilized in the autoclave at 121°C for 15 minutes. They were then inoculated with isolates from bacterial colonies and incubated at 37°C for 24 hours. After the incubation period, few drops of Kovac’s reagent were added to the test tubes. Indole positive tests showed a pink or red ring at the top of the broth while indole negative tests showed no change.

### 3.10.3 Citrate test

This test is used to identify bacteria that use citrate as an energy source. Test tubes were filled with 5ml of citrate broth and were sterilized in the autoclave at 121°C for 15 minutes. They were then inoculated with isolates from bacterial colonies and incubated at 37°C for 24 hours. A control test was set aside with no bacterial isolate. After the incubation period, test tubes are shaken slightly and compared to the control test. A citrate positive test gave a cloudy appearance and a citrate negative test gave no change.

### 3.10.4 Methyl red test

This test is to identify bacteria that ferment glucose. Test tubes were filled with 5ml of peptone water and were sterilized in the autoclave at 121°C for 15 minutes. They were then inoculated with isolates from bacterial colonies and incubated at 37°C for 24 hours. After the incubation period, about 5 drops of methyl red indicator solution was added to the test tubes. A positive reaction showed a color change to red after a few minutes and a negative reaction showed a pale-yellow color.

### 3.11 Gram Staining

Gram staining is a staining technique that can be used to identify microorganisms such as bacteria. In the test, a drop of saline solution was placed on a clean, sterile glass slide, after which a small portion of the isolates were picked from a day-old culture using a sterilized inoculation loop. The picked isolates were then emulsified on the drop of saline solution. The prepared smear was allowed to air dry and then sterilized and fixed to the glass slide by heat flaming with a Bunsen burner.

To begin the staining process, the heat fixed smear was covered with crystal violet stain for two minutes and then washed off with clean water. This was followed by Lugol’s iodine solution for another two minutes, rinsed with ethanol and subsequently washed off under a slowly running tap. Lastly, the smear was covered in a counterstain (safranin) for one minute and then rinsed off with clean running water. The smear was then air dried and viewed under a light microscope using the oil immersion lens.

### 3.12 Microscopy

After the Gram staining, the prepared slide was first placed under the low power objective lens (×10) for viewing. Afterwards, a drop of immersion oil was placed directly on the smear and examined under the oil immersion lens (×100) of the light microscope. Purple cells observed indicated the presence of Gram-positive bacteria while Gram-negative bacteria were seen as pink or red.

### 3.13 Data Management

Data were entered into Microsoft Excel 2010 and analyzed using Statistical Package for Social Sciences version 25 (SPSS 25.0). An alpha analysis (p-value) less than or equal to 0.05 was deemed statistically significant. Basic descriptive analyses such as frequencies and percentages were used to describe and determine the knowledge of students on the maintenance and diseases associated with the use of their earbuds.

**REFERENCES**

Lartey, O., & Marful, A. B. (2021). Campus Planning and Architecture : A comparative Study of Kwame Nkrumah University of Science and Technology ( KNUST ) and University of Ghana ( LEGON ). Preprint.Org, 1(June), 29. https://doi.org/10.20944/preprints202106.0712.v1.