

## Microbial Assessment Of Offices That Uses Fan And Air Conditioners In Rhema University, Aba.

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#### Abstract

The aim of this study was to determine the microbes present in the indoor air of offices that use fan and offices that use Air conditions (AC) in Rhema University, Aba. A total of 20 offices were used to carry out this study, Ten (10) offices with fan and ten (10) Air conditioners. Plates of nutrient agar, MacConkey agar and Czapex dox agar each, freshly prepared, where exposed in these offices at nose level for 20minutes. They were then aseptically wrapped and transported back to the laboratory immediately for incubation using standard microbiological procedures. Mean counts obtained from the AC offices showed a total heterotrophic count of 18-177cfu/m<sup>3</sup>, total coliform bacteria count of of 6-32 cfu/ml<sup>3</sup> while total fungi count had a range of 12-46 cfu/ml<sup>3</sup>. In the offices that uses fan, total heterotrophic bacteria count had a range of 26-144 cfu/m<sup>3</sup>, a total coliform bacteria count of 8-92cfu/m<sup>3</sup> while total fungi count was 2-46cfu/m<sup>2</sup>. The gram positive bacteria isolated were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* species, *Micrococcus Species and Bacillus species while the gram negative bacteria isolated were Acinetobacter, Klebsiella pneumoniae*, and *Escherichia coli*. The fungi isolated were *Aspergillus niger, Penicillium* species, *Rhizopus, Candida* species, *Trycophyton, and Cladosporium* species. *Staphylococcus aureus* (100%) was the bacteria isolates showed that out of 8 bacteria that was isolated, 7 (87.5%) were beta hemolytic. Antibiotic susceptibility showed that the bacteria were majorly sensitive to the drugs used in this study. 7 (87.5%) were beta hemolytic. Antibiotic susceptibility showed that the bacteria were majorly sensitive to the drugs used in this study. The presence of fungi and bacteria of public health importance in offices that uses fan and Air conditioners has been observed; therefore an intense cleaning plan should be put in place to reduce their proliferation.

Index Terms: Air Conditioners, Fans, Fungi, Bacteria, antibiotics susceptibility.

#### INTRODUCTION

Air Conditioners are widely used in tropical countries due to high temperature in these regions during the dry season. Air Conditioners are used in different places such as homes, offices, cars and industries (Gołofit. & Górny, 2010). Recently, there is an emergence of air conditioners built in cloths which could be worn around to help ease the heat. The quality of indoor air is one of the major factors affecting the health of people who inhale 10m<sup>3</sup> of air every day, and spend between 80-95% of their lives in indoor environment. The air inhaled by humans contains high quantity of microorganisms which form so-called bio aerosol. Bio- aerosol is a colloid like suspension whose constituent are liquid droplets and particles of solid matter in the air, whose components have viruses, fungal, spores, conidia, bacterial endospores, etc. attached to them. Major sources of bio aerosols in an enclosed area include human occupants, organic wastes, pets, house dust, as well as Air Conditioners (Gołofit. & Górny, 2010).

In situations where Air conditions are being used for a long time without cleaning, there could be accumulation of dusts and biofilms of microorganisms in the air filter of Air Conditioners. The microorganism present in the filters could be released to the environment when the Air Conditioner is working and when inhaled, some of them could be pathogenic (Gołofit. & Górny, 2010). A recent study has shown that there is increase in the number of allergic reactions, asthma, hay fever, pneumonia and many other health side-effects including infections arising from the inhalation of air contaminated by microorganisms (Bush,2016). A condition known as sick house

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syndrome has been noticed in people who stay in rooms where there is microbial proliferation of the air which may be as a result of Air Conditioners which have not been cleaned for a long time.

Variations in weather condition and climate change are increasingly issues of public health importance. The high level of heat exposure as a result of global climate change in cities and villages is a factor exposing millions of people to the risk of several health problems. People living in the urban areas are at higher risk due to higher population density. Heat is the top weather-related killer in the world. Air conditioners have however been used to reduce the prevalence of weather-related challenges in the world Many buildings in the city especially in public places have installed air conditioners to reduce heat exposure and improve indoor conditions. Air conditioners are indoor devices that provide an atmosphere with controlled temperature, humidity and purity at all times regardless of the weather conditions.

It cools air by reducing the humidity following condensation of the water vapours. The air conditioners have been discovered to cause some form of physiological discomforts and allergic symptoms which is also known as sick building syndrome that were noticed when occupants spend considerable time indoor in a room that is equipped with active air conditioners. The mucous membrane irritation, breathing difficulties, irritated skin, headache and light headedness, fatigue, feverish conditions, chest pain, persistent cold, sore throat, catarrh, cough, watery eye, prolonged muscle cramps and joint pain were common ailments that have been discovered to be suffered by individuals who have spent considerable time in air-conditioned rooms (Soto et al., 2009). Researchers have proven that air conditioners can be contaminated with organic pollutants, bacteria, fungi and particulate matter from mice, insects and the contaminating organisms can grow and survive in areas that meet their environmental requirements. Currently, most air conditioners use internal filters that extracts microorganisms and dust but sometimes they do not completely remove these microorganisms and they may remain viable and can be returned to the surrounding atmosphere during inefficient operation, period of maintenance and due to temporary malfunction. Particle pollutants are very dangerous to the human body; most of the particle pollutants in air are very small which enables them to easily enter into the respiratory canal deeply together with inhaled air. These pollutants are circulated in a close environment with the air from the air conditioning device. It is very possible for the surface of these particle pollutants to adsorb harmful or potential pathogens or microbes which increases their harmfulness to human body. Microbial contamination of air conditioners has been attributed to the operation of most air conditioners outside their designed parameters due to inappropriate filters, neglect or over use, poor maintenance and hygiene practices which results in dust accumulation (Soto et al., 2009). The dust accumulation coupled with humidity, especially at the downstream section of the cooling equipment leads to the proliferation of potential harmful microorganisms capable of surviving the prevailing conditions. Organisms like Legionella pneumophilia, Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae and Bacillus species have been isolated in air conditioner filters. Species of *Penicillium*, *Pneumocystis carinii* and *Aspergillus*, *Rhizopus*, *Fusarium and Alternaria* have been isolated too.

#### Need of the study

It is no news that indoor air are always compromised due to certain activities that take place within a building. Offices are places where individuals spend the majority of their day activities and hence it is important that the level of hygiene and quality of air they breathe in while in the office be monitored and evaluated. This has given rise to the study. Human activities such as sneezing cough, eating and moving from outdoor to indoor, tend to transport microbes and circulate them within the office. The use of AC in the offices has also limited the cross ventilation system which helps to purify the air naturally, hence making these microbes carve a habitat in these offices. The use of fan however allows the free flow of air, as the windows and doors are opened resulting in cross ventilation but at the same time allowing the influx of dust particles into these offices. It is therefore necessary to ascertain and determine the activity of these microbes in both systems.

#### Sample Area

Rhema University is located in the south eastern part of Nigeria, precisely in Abia State. The office building is the first 6 storey you will meet once you enter the school gate. Lecturer's offices are spread from the first floor to the last floor on both the left and right wings of the building.

A total of 20 offices randomly selected were used for the sampling, (10 offices with fan and 10 offices with AC's. This was randomly selected but focusing on offices with activities, these include, the lecturers offices (10), college secretary offices (3), HOD's offices (3), the bursary units (2), the Registrars offices and the SR offices (2). The table below describes the offices.

| Table 1: DESCRIPTION OF OFFICES USED FOR SAMPI | LE. |
|--|-----|
|--|-----|

| SN | OFFICES           | FAN | AC | NO OF WINDOWS | FREQUENCY OF |
|----|-------------------|-----|----|---------------|--------------|
|    |                   |     |    |               | CLEANING     |
| 1  | Lecturers office  | +   |    | 2             | DAILY        |
| 2  | Lecturer's office | +   |    | 2             | DAILY        |
| 3  | Lecturer's office | -   | -  | 2             | DAILY        |
| 4  | Lecturer's office | -   | -  | 1             | DAILY        |
| 5  | Lecturer's office | -   | -  | 1             | DAILY        |
| 6  | Lecturer's office | +   |    | 1             | DAILY        |
| 7  | Lecturer's office | +   |    | 1             | DAILY        |
| 8  | Lecturer's office | -   | -  | 2             | DAILY        |
| 9  | Lecturer's office | -   | -  | 2             | DAILY        |
| 10 | Lecturer's office | +   |    | 1             | DAILY        |
| 11 | HOD's office      | +   |    | 1             | DAILY        |

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| 12 | HOD's office               | + |   | 1 | DAILY |
|----|----------------------------|---|---|---|-------|
| 13 | HOD's office               | + |   | 1 | DAILY |
| 14 | Busary unit 1              | - | - | 1 | DAILY |
| 15 | Bursary unit 1             | - | - | 1 | DAILY |
| 16 | SR 's Office 1             | - | - | 1 | DAILY |
| 17 | Registrar's office 1       | + |   | 1 | DAILY |
| 18 | Registrar's office 2       | - | - | 1 | DAILY |
| 19 | College secretary's office | + |   | 1 | DAILY |
| 20 | College secretary's office | - | - | 1 | DAILY |
| -  |                            |   |   |   |       |

Key: + = present; - = Absent

#### Sample collection

Samples were collected using swab sticks moistened in peptone water and the dust particles were rubbed off using the moistened swab sticks from the AC filters and from the fan blades. Using the passive air sampling method, the settle plate technique was used to expose freshly prepared sterile Nutrient agar, MacConkey agar and Blood agar at three different positions (edge, centre and end) of the rooms for 20 minutes during working hours (9am - 4pm).

#### Work bench preparation

To avoid contamination the work bench was cleaned with a cotton wool soaked in 70% alcohol. The clean, sterile containers were labelled appropriately while they were still closed.

#### Media preparation

The culture media used include Nutrient agar (NA), MacConkey agar(MA), Czapex Dox agar(CDA), Blood Agar(BA) and Muller hinton agar(MHA), they were prepared according to the manufacturer's instructions. Culture media were all prepared by weighing out required grams of the agar powder and dissolving in specific volume of distilled water according to the respective Manufacturers' specification and sterilized in an autoclave at 121°C at 15 psi for 15 minutes. The autoclaved media were carefully poured into different new sterile disposable petri dishes that were 60.123ml in size. For Blood agar, the blood agar base which was sterilized was allowed to cool to 45°C, then blood was added to the base and rocked till it was evenly distributed. This was then dispensed into new sterile petri dishes. Sterilized CDA was also allowed to cool to 45°C then it was seeded with chloramphenicol before it was dispensed, to avoid bacteria growth.

#### **Sterility test:**

A sterility check was carried out by incubating the freshly prepared plates for 24 hours inside the incubator. This is to ensure that the plates were sterile. The plate without growth in it was termed sterile and used to for the culturing.

#### Inoculation

The samples exposed where then closed and sent in for incubation, while the swabs taken where further diluted using the 10 fold serial dilution method, then 0.1ml from the 6<sup>th</sup> diluent were then inoculated onto the petri dishes using the spread plate method. This was achieved using an L-shaped glass rod flamed after being soaked in ethanol, it was then used to evenly distribute the 0.1ml onto the surface of the agar. This was allowed to stand for 20minutes on a flat surface.

#### Incubation

The Nutirent, MacConkey and Blood Agar plates were incubated at 37<sup>o</sup>C in an incubator for 24hours while the Czapex Dox Agar plates were incubated at 28<sup>o</sup>c for 3days.

#### **Determination of microbial density**

Following incubation of culture plates, bacterial and fungal colony forming units (CFU) were enumerated. Afterwards, the mean colony forming units per cubic meter (CFU/m<sup>3</sup>) of air of the plates collected in triplicates were determined using the following equation as described by (Borrego et al.,2010; Gutarowska, 2010).

#### $N = 5a \times 10^4 (bt)^{-1}$ ,

Where N: microbial CFU/m3 of indoor air; a = number of colonies per Petri dish; B = dish surface area, cm2; t= exposure time of the petri dish, minutes.

#### **Isolation of pure cultures**

Bacteria:

After 24hours, plates were inspected for visible growth, then distinct colonies were observed and picked for sub-culturing. They were then inoculated onto freshly prepared nutrient agar plates using a sterile wire loop, and then incubated again at 37<sup>o</sup>C for 24hours. This was repeated until pure cultures were obtained (Cheesbrough,2010).

#### Fungi:

Sub-culture was done to isolate individual fungal in their pure form on freshly prepared SDA. These were incubated for 3-5 days (Larone *et al.*, 2016).

#### Storage of pure isolates:

Agar slants were prepared by making a preparation of Nutrient Agar according to manufacturer's instruction. Homogenized agar were dispensed into McCartney bottles and sterilized and afterwards slanted and allowed to set. Pure cultures of bacteria were streaked on agar slants prepared. The slants were incubated for 24 hours and after growth was observed, the McCartney bottles were then stored in the refrigerator at  $4^{\circ}$ C for further studies.

#### **Characterization and Identification of isolates**

#### **Bacteria:**

Bacteria were identified using gram's stains and biochemical tests as described by Cheesbrough (2004) while characterization was done by observing their morphology on agar plates which included their shape, color, size, texture, and elevation.

#### Gram staining:

This was done to identify the bacteria isolates as either gram positive or gram negative and the method described by Cheesbrough (2004) was used. A thin smear of the 24hour culture was made on a clean grease free slide, allowed to air dry and heat fixed. It was flooded with crystal violet and allowed to stand for 1minute. It was rinsed with slow running tap water then flooded again with grams iodine for 30seconds. It was rinsed with water again then decolorized with Acetone for 5 seconds and again rinsed under slow running tap water till no color came off. Then safranin which is the secondary dye was flooded on the slide for 30seconds and washed off under slow running water. It was air dried and immersion oil was dropped, this was viewed under the X100 objective lens of the microscope. Bacteria that retained the pink/red color were recorded as the gram negative while the ones that retained the purple color were recorded as the gram positive bacteria.

#### **BIOCHEMICAL TESTS**

#### A. Catalase test:

This was done according to the methods described by Cheesbrough (2004). 2-3 drops of 3% hydrogen peroxide was placed on a clean grease free slide. Using a glass rod, colonies were taken from a 24hour old culture and dropped on the slide, Bubbling within 5 seconds were recorded as positive.

#### B. Coagulase test:

The methods described by Cheesbough (2004) were also adopted. 2-3 drops of serum was dropped onto a clean grease free slide and then a loopful of the organism was added, visible clumping within 5-10 seconds was recorded as positive.

#### C. Oxidase test:

This was done according to the method described by Cheesbough (2004). A few drops of 1% aqueous solution of tetramethyl-pphenylene-diamine hydrochloride reagent was added to a piece of filter paper in a petri dish smear of the bacteria was made onto the impregnated filter paper in a petri dish using a glass rod. A purple coloration appearing within 5-10 seconds is a positive result.

#### D. Citrate test:

This was done as described Cheesbrough (2004). Simmons citrate agar was prepared according to the manufacturer's instruction in test tubes and allowed to gel. The tubes were inoculated by streaking the isolates using a sterile wire loop. It was then incubated at  $37^{\circ}$ C for 24-48 hours. A colour change from green to blue indicated a positive test.

#### E. Indole test:

The method described by Cheesbrough (2000). A loopful of the bacteria isolates was inoculated into sterile peptone water and incubated for 24 hours at 37°C. Then to this culture, 0.5ml of kovacs reagent (p-dimethyl amino benzaldehyde) was added and thoroughly mixed, and allowed to stand. Presence of a deep colour indicated a positive result.

#### F. Urease test

This was prepared according to the methods described by Cheesbrough (2004). Christensen's urea agar was prepared according to the manufacturer's instructions. It was dispensed in tubes, slanted and allowed to gel. The slope was then inoculated with the bacteria isolates using the streaking method; this was incubated at 37°C for 24 hours. A positive test was indicated by a colour change from yellow to pink.

#### G. Methyl red test

This was done according to the method described by Cheesbrough (2000). 5mls of peptone water was dispensed into tube and a loop full of the bacteria isolate as inoculated into the medium. It was incubate at 37°C for 3-5 days. 5 drops of the indicator, methyl red, was then added. A colour change indicative of a positive test, from yellow to red.

#### H. Sugar fermentation test:

Triple Sugar Iron agar (TSI) containing lactose, glucose and sucrose was used to carry out this test. The required quantity of the agar was weighed, homogenized and dispensed into test tubes and then sterilized at 121° C for 15 min. After sterilization, the tubes were arranged sideways and were left for some time to allow the agar to form slants. Using a sterile inoculating wire, the slants were stabbed by inserting the inoculating wire into 24 hour old broth culture and incubated at optimum temperature. The agar was checked for color change and gas production. The tubes where the organism produced hydrogen sulphide showed a black coloration i. Fungi:

The fungi were identified based on their microscopic and macroscopic characteristics as described by Larone *et al.*, (2016). Macroscopic assessment was done based on their morphological features on the SDA plates while Microscopy was done based on their direct preparations stained with lactophenol cotton blue (de Hoog *et al.*, 2000).

#### Analysis

Results were analysed using Ms-Excel and presented in tables using Percentage (%) for better clarification.

#### **RESULT AND DISCUSSION**

| AC OFFICES | THBC | TCC | TFC |
|------------|------|-----|-----|
| 1          | 80   |     | 12  |
| 2          | 63   | 16  | -   |
| 3          | 116  | 15  | 14  |
| 4          | 162  | 27  |     |
| 5          | 80   | 6   | 46  |
| 6          | 177  | 32  | 24  |
| 7          | 36   | 12  | 14  |
| 8          | 18   | -   | 15  |
| 9          | 31   | -   | 22  |
| 10         | 86   | -   | 12  |

Table 2: MICROBIAL DENSITY IN OFFICES THAT USE AIR CONDITIONS

Key: THBC- Total heterotrophic bacterial count; TCC- Total coliform count; TFC- Total fungi count

Table 2 shows the microbial densities observed in the offices that uses AC. It was observed that heterotrophic bacteria was present in the 10 offices used for the study, having a range of 18-177cfu/m3 and a total coliform bacteria occurred in 6 offices with a range of 6-32cfu/m3 while fungi was recorded in 8 offices having a range of 12-46cfu/m3. From this study, heterotrophic bacteria were observed to have the highest microbial density.

Table 3 show the microbial densities observed in the offices that uses fan. It was observed that heterotrophic bacteria was present in the 10 offices used for the study, having a range of 26-144 (cfu/m<sup>3</sup>) and coliform bacteria occurred in 5 offices with a range of 8-92 (cfu/m<sup>3</sup>) while fungi was recorded in 8 offices having a range of 2-46 (cfu/m<sup>2</sup>). From these study heterotrophic bacteria was observed to have the highest microbial density just like the offices with AC.

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| FAN OFFICES | THBC | TCC | TFC |
|-------------|------|-----|-----|
| 1           | 110  | -   | 12  |
| 2           | 44   | 8   | -   |
| 3           | 44   | 32  | 4   |
| 4           | 52   | -   | -   |
| 5           | 119  | 80  | 46  |
| 6           | 108  | 92  | 24  |
| 7           | 144  | 62  | 14  |
| 8           | 26   | -   | 5   |
| 9           | 37   | -   | 22  |
| 10          | 46   | -   | 2   |

#### TABLE 3: MICROBIAL DENSITY OF OFFICES THAT USES FAN

Key: THBC- Total heterotrophic bacterial count; TCC- Total coliform count; TFC- Total fungi count

Characterization of the bacteria isolates (table 4) was done using gram staining and biochemical test which includes, catalase test, coagulase test, citrate test, urease test, sugar fermentation test, Oxidase, methyl red and Indole test. It was observed that most of these isolates were gram positive and they were mainly in cocci form. They were all catalase positive, and negative to oxidase test.

*E. coli* was the only isolate positives for Indole test while the rest were negative. The gram positive isolates were, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus*, *Micrococcus* species and *Bacillus* species while the gram negative bacteria isolated were *Acinetobacter*, *Klebsiella pneumonoiae*, and *Escherichia coli*.

The identification of bacteria from the colonies showed that out of 8 bacteria isolated, 5 of the isolates from the sampling points were gram positive and only 3 gram negative cells. On the other hand, morphological studies showed that cocci were also more predominant over the bacillary shape bacteria even though *Bacilli* can be found in almost every environment. They contain spores that enable them to survive for a long period of time in the environment. The bacterial species isolated in this study are similar to those isolated by Stryjakowska-Sekulsa et al. (2007) and Vlad et al. (2013).

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#### TABLE 4 : CHARACTERIZATION OF BACTERIA ISOLATED

| ISOLATE | MACROSCOPIC CHARACTERISTICS                |     |     |       |     |       |                     |      | Sugar        | uspected organism      |
|---------|--|-----|-----|-------|-----|-------|---------------------|------|--------------|------------------------|
| CODE    |  | CAT | COA | MR    | DXI | ND    | JRE                 | CIT  | fermentation |                        |
|         |  |     |     |       |     |       | $( \cdot , \cdot )$ |      | G S M        |                        |
| Α       | Bright golden yellow colored small         | +   | +   | - ) ( | -   | - 🔍 . | +                   | +    | +            | taphylococcus aureus   |
|         | round colonies, 2mm, shiny and elevated.   |     |     |       |     |       |                     | · A  |              |                        |
|         | Gram positive cocci in clusters            |     |     |       |     |       |                     |      |              |                        |
| В       | Whitish colored small round colonies,      | +   | +   | -     | -   | - ) / | +                   | +    | +            | taphylococcus          |
|         | 2mm, shiny and elevated. Gram +ve          |     |     |       |     |       |                     |      |              | epidermidis            |
|         | cocci in cluster                           |     |     |       |     |       |                     |      |              |                        |
| C       | Whitish, shiny, small round raised         | +   | -   | +     | -   | - 7   | +                   | +    | + - +        | <i>Aicrococcus</i> sp  |
|         | colonies, abut 2mm, elevated, gram +ve     |     |     |       |     |       |                     |      |              |                        |
| -       | cocci in tetrads                           |     | / . |       |     |       |                     |      |              |                        |
| D       | Small, dome, mucoid colonies, about 1-     | +   | -   |       | -   | -     | -                   | +    | + + +        | <i>cinetobacter</i> sp |
|         | 2mm. gram negative rods in pairs           |     |     |       |     |       |                     |      |              |                        |
| E       | Shiny bright whitish colonies, that are    | -   | +   | +     | -   | -     | -                   | -    | +            | <i>treptococcus</i> sp |
|         | smooth, elevated, and about 2mm. gram      |     |     |       |     |       | 1000                |      |              |                        |
| -       | positive cocci in chains                   |     | -   |       |     |       |                     | 1    |              |                        |
| F       | mucoid large colonies that are creamy in   | +   | +   | 1     | -   | -     | +                   |      | + - +        | lebsiella pneumoniae   |
|         | color about 2mm. lactose fermenters,       |     |     |       |     |       |                     |      |              |                        |
| 0       | gram positive rods.                        |     |     |       |     |       |                     |      |              | 7 1 • 1 • 1 •          |
| G       | Pale colored, sniny colonies that are      | +   | -   | +     | -   | +     | -                   | -    | +            | scherichia coli        |
|         | shooti, elevated and about 211111, having  | _   |     |       |     |       |                     |      | _            |                        |
|         | a characteristic shieli, ferments factose, | te  | 00  | PIO(  |     | Re    | 100                 | 100h | OUC          |                        |
| н       | Large slightly round dry flat colonies     | _   |     |       |     |       |                     |      | + +          | Racillus caraus        |
| 11      | pale in color with a characteristic small  |     |     |       | -   |       |                     | т    | тт <b>-</b>  | ucilius cereus         |
|         | Gram positive long rods with central       |     |     |       |     |       |                     |      |              |                        |
|         | spores rods                                |     |     |       |     |       |                     |      |              |                        |
|         | pp1051046.                                 |     |     |       |     |       |                     |      |              |                        |

Key: +-Present, -+ Absent, sp- species, CAT-Catalase, COA-Coagulase, MR-Mehtyl red, OXI-oxidase, IND URE-Urease, CIT-Citrate test, G-Glucose, M-Maltose, S-Sucrose

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Table 5 shows the characterization of the fungi isolated. From this study it was observed that the most predominant organisms were Aspergillus niger, Penicillium species, Rhizopus species Candida species, Trycophyton species, and Cladosporium species.

#### TABLE 5: CHARACTERIZATION OF FUNGI ISOLATES AND THEIR PROBABLE IDENTITY

| ISOLATE | MACROSCOPY  | MICROSCOPY   | SUSPECTED<br>ORGANISM |
|---------|---|--|-----------------------|
| A       | Powdery radial dark brown<br>with white edges growth.<br>Reverse is colourless.   | Non septate hyphae, condiospores<br>scattered round, spores colour is<br>brown   | Aspergillus niger     |
| В       | Powdery Concentric to radial<br>blush with white edges.<br>Reverse is white to cream.                                     | Septate hyphae with condiospores<br>arranged in rows of 8 on phalides.<br>Spore colour is light green<br>to colourless | Penicillium sp        |
| С       | Woolly, fluffy white then grey later growth. Reverse is pale brown.   | Non septate hyphae, unbranched sporangiospores, stolons. Spore colour is blue.   | Rhizopus sp           |
| D       | Pasty smooth bulging cream coloured growth. Reverse is white.   | Septate hyphae with spherical<br>short pseudophyphae not<br>conscripted.   | Candida sp            |
| Ε       | White colonies, waxy or<br>slightly downy; heaped or<br>folded. Reverse is colourless or<br>pale yellowish orange to tan. | Septate hyphae. Chlamydoconidia<br>are numerous. Microconidia and<br>macroconidia are present.                         | Trichophyton sp       |
| F       | Greenish brown velvety ,<br>heaped and folded, reverse side<br>is black   | Dark Septate hyphae with oval conidia present  | Cladosporium sp       |

Key: sp- species

Table 6 shows the frequency of the potential pathogenicity testing of the bacteria isolated. It was observed that 7 of the isolate were 87.5% beta haemolytic in reaction while one (I) was about 12.5% with an alpha hemolytic reaction. None of the bacteria isolates was gamma haemolytic. This implies that these isolates found in these rooms have the potential to cause illness when they get access into the body system.

#### TABLE 6: FREQUENCY OF THE POTENTIAL PATHOGENICITY TESTING

| ISOLATE                             | A <mark>LPHA</mark> | BETA    | GAMMA  |
|-------------------------------------|---------------------|---------|--------|
| Staphylococcus a <mark>ureus</mark> | -                   | +       | -      |
| s.epidermidis                       | +                   | -       |        |
| Micrococcus sp                      | -                   | +       | -      |
| Acinetobacter sp                    | reere               | +       | 'oug n |
| Streptococcus sp                    | -                   | +       | _      |
| Klebsiella pneumoniae               | -                   | +       | -      |
| Escherichia coli                    | -                   | +       | -      |
| Bacillus cereus                     | -                   | +       | -      |
| Frequency (%)                       | 1(12.5)             | 7(87.5) | 0(0,0) |

Key: numbers in parenthesis () are the percentages of the numbers

Table 7 shows the frequency of occurrence of bacteria and fungi isolated from the offices in Rhema University, Aba. It was observed that *S. aureus* had the highest occurrence (100%) while *E.coli* had the least occurrence with 55%. While for the fungi, it was observed that *Aspergillus* was the highest occurrence of 100% and the least occurrence of 15% was observed with *Trichophyton* species.

|                      |     |      | В  | ACTI | ERIA |     |     |    | OTAL |     |    | FUN | GI  |    |    | ГОТА<br>L |
|----------------------|-----|------|----|------|------|-----|-----|----|------|-----|----|-----|-----|----|----|-----------|
| Isolates<br>/offices | А   | В    | C  | D    | E    | F   | G   | Н  |      | A1  | B1 | C1  | D1  | E1 | F1 |           |
| 1                    | +   | +    | +  | +    | -    | -   | +   | +  | 75   | +   | +  | -   | -   | -  | +  | 50        |
| 2                    | +   | +    | -  | +    | +    | +   | -   | -  | 62.5 | +   | -  | +   | +   | -  | +  | 50        |
| 3                    | +   | -    | +  | +    | +    | -   | +   | +  | 75   | +   | +  | +   | -   | -  | +  | 50        |
| 4                    | +   | +    | +  | +    | +    | +   | -   | +  | 87.5 | +   | +  | +   | -   | -  | +  | 66.6      |
| 5                    | +   | -    | +  | -    | +    | +   | +   | +  | 75   | +   | -  | +   | -   | -  | +  | 50        |
| 6                    | +   | +    | +  | +    | +    | +   | +   | -  | 87.5 | +   | -  | +   | +   | +  | +  | 83.3      |
| 7                    | +   | +    | +  | +    | +    | +   | +   | -  | 87.5 | +   | +  | +   | +   | +  | +  | 100       |
| 8                    | +   | -    | +  | +    | -    | -   | +   | +  | 62.5 | +   | -  | +   | -   | -  | -  | 33.3      |
| 9                    | +   | +    | +  | -    | +    | -   | -   | +  | 62.5 | +   | +  | +   | -   | -  | +  | 66.6      |
| 10                   | +   | -    | -  | +    | +    | +   | -   | -  | 50   | +   | -  | +   | +   | -  | +  | 66.6      |
| 11                   | +   | +    | +  | +    | +    | +   | +   | -  | 87.5 | +   | -  | +   | -   | -  | +  | 50        |
| 12                   | +   | +    | +  | +    | +    | +   | -   | +  | 87.5 | +   | +  | +   | +   | -  | +  | 83.3      |
| 13                   | +   | +    | +  | +    | +    | +   | - ( | +  | 87.5 | +   | +  | +   | -   | -  | +  | 66.6      |
| 14                   | +   | -    | +  | +    | +    | +   | +   | +  | 87.5 | +   | 6  | +   | - ( | -  | +  | 50        |
| 15                   | +   | -    | +  | -    | +    | +   | +   | +  | 75   | +   | -  | +   | -   | 1  | +  | 50        |
| 16                   | +   | -    | +  | +    | +    | +   | +   | +  | 87.5 | +   | +  | +   | -   | -  | +  | 66.6      |
| 17                   | +   | +    | +  | + <  | +    | -   | -   | -  | 62.5 | +   | +  | +   | +   | -  | +  | 83.3      |
| 18                   | +   | +    | +  | +    | +    | +   | - \ |    | 75   | +   | +  | +   | +   | -  | +  | 83.3      |
| 19                   | +   | +    | +  | +    | +    | +   | -   | +  | 87.5 | +   | +  | +   | +   | -  | +  | 83.3      |
| 20                   | +   | - (  | +  | +    | +    | -11 | +   | +  | 75   | +   | -  | +   | -   | +  | +  | 66.6      |
| DCCURRENC            | 100 | 60   | 80 | 75   | 80   | 70  | 55  | 65 |      | 100 | 55 | 95  | 40  | 15 | 95 |           |
| E(%)                 |     | 1000 |    |      |      |     |     |    |      |     |    | _   |     |    |    |           |

#### Table 7: OCCURRENECE OF THE ISOLATES FROM THE DIFFERENT OFFICES

#### Occurrence = <u>number of times the isolate was present</u> X <u>100</u> Total number of offices sampled 1

The bacteria isolates were all subjected to antibiotic susceptibility testing test, the drugs of choice was the popular commercial antibiotic disc purchased from the market. Table 8 and 9 showed that these drugs were active against the gram positive bacteria and gram negative isolates from the air in these offices. However a moderate level of resistance was observed with majority of the bacteria isolated to common drugs such as gentamycin (CN), Erythromycin (E), Ampiclox (APX) and so on.

Low level of resistance was observed with *Bacillus* species and *S.epidermidis* for the gram positive bacteria, then for the gram negative bacteria, low resistance was observed with *Acinetobacter* species

#### Table 8: ANTIMICROBIAL SENSITIVITY OF THE GRAM POSITIVE BACTERIA ISOLATED

| isolate       | Susceptibility | CPX | NB | CN | AMC | S  | RD | Е  | СН | APX | LEV |
|---------------|----------------|-----|----|----|-----|----|----|----|----|-----|-----|
| Micrococcus   | R              | 9   | 3  | 4  | 8   | 4  | 2  | 5  | 8  | 7   | 6   |
| Sp            | Ι              | 3   | 10 | 2  | 4   | 2  | 2  | 1  | 3  | 5   | 5   |
| N=18          | S              | 6   | 5  | 16 | 6   | 10 | 14 | 12 | 7  | 6   | 7   |
|               |                | 0   |    |    |     | :  |    |    |    |     |     |
| S.aureus      | R              | 3   | 7  | 8  | 11  | 5  | 2  | 1  | 8  | 4   | 8   |
| N=20          | Ι              | 5   | 3  | 2  | 4   | 5  | 5  | 4  | 5  | 2   | 2   |
|               | S              | 12  | 10 | 10 | 5   | 10 | 13 | 15 | 7  | 12  | 10  |
|               |                |     |    |    |     |    |    |    |    |     |     |
| S.epidermidis | R              | 5   | 4  | 5  | 4   | 1  | 0  | 0  | 0  | 0   | 0   |
| N=12          | Ι              | 2   | 4  | 1  | 4   | 2  | 4  | 2  | 1  | 6   | 2   |
|               | S              | 5   | 4  | 6  | 4   | 10 | 8  | 10 | 11 | 6   | 10  |
|               |                |     |    |    |     |    |    |    |    |     |     |
| Streptococcus | R              | 10  | 4  | 10 | 6   | 5  | 4  | 2  | 4  | 3   | 4   |
| N=18          | Ι              | 4   | 2  | 2  | 3   | 2  | 2  | 2  | 2  | 2   | 2   |
|               | S              | 4   | 12 | 6  | 9   | 11 | 12 | 14 | 12 | 13  | 12  |

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| Bacillus sp | R | 8 | 0  | 2 | 0 | 0  | 0  | 0  | 0  | 0  | 0  |
|-------------|---|---|----|---|---|----|----|----|----|----|----|
| N=13        | Ι | 3 | 2  | 5 | 4 | 0  | 2  | 0  | 1  | 3  | 0  |
|             | S | 2 | 11 | 6 | 9 | 13 | 11 | 13 | 12 | 11 | 13 |
|             |   |   |    |   |   |    |    |    |    |    |    |

Key: CPX-Ciprofloxacin; NB- Norfloxacin, CN-Gentamycin, AML- Amoxil, S-streptomycin, RD- Rifampicin, E-Erythromycin, CH-Chloramphenicol, APX-Ampiclox, LEV-levofloxacin.

#### Table 9: ANTIMICROBIAL SENSITIVITY OF THE GRAM NEGATIVE BACTERIA ISOLATED

| ISOLATES             | SUSCEPTIBILITY |     |     |     | DRI | UGS U | JSED | 1   |    |     |    |
|----------------------|----------------|-----|-----|-----|-----|-------|------|-----|----|-----|----|
|                      |                | OFX | PEF | CPX | AU  | CN    | S    | CEP | NA | SEP | PN |
| <i>Klebsiella</i> sp | Resistant      | 6   | 0   | 4   | 4   | 2     | 0    | 2   | 4  | 3   | 2  |
| N=14                 | Intermediate   | 4   | 4   | 3   | 6   | 2     | 2    | 4   | 4  | 5   | 2  |
|                      | Sensitive      | 4   | 10  | 7   | 4   | 10    | 12   | 12  | 6  | 6   | 10 |
| Escherichia coli     | Resistant      | 3   | 2   | 5   | 2   | 1     | 9    | 4   | 2  | 0   | 2  |
| N=12                 | Intermediate   | 1   | 5   | 4   | 4   | 2     | 1    | 0   | 2  | 1   | 1  |
|                      | Sensitivity    | 8   | 5   | 3   | 6   | 9     | 2    | 8   | 8  | 11  | 10 |
| Acinetobacter        | Resistant      | 0   | 0   | 0   | 0   | 0     | 0    | 0   | 0  | 0   | 0  |
| sp                   | Intermediate   | 5   | 4   | 2   | 1   | 2     | 0    | 0   | 0  | 0   | 0  |
| N=17                 | Sensitive      | 12  | 11  | 15  | 16  | 15    | 17   | 17  | 17 | 17  | 17 |

| Key: OFX-Tarvid  | , PEF-Reflacine, | CPX-Ciprofloxacin, | AU, | Augum <mark>ent</mark> in, | CN-Gentamycin, | <mark>S-Stre</mark> ptomycin, | CEP- | Ceporex, | NA- |
|--|------------------|--------------------|-----|----------------------------|----------------|-------------------------------|------|----------|-----|
| Nalidixix Acid. SXT-Sept <mark>rin, PN –Am</mark> picilin. N=number of times pres <mark>ent</mark> . |                  |                    |     |                            |                |                               |      |          |     |

- The population of the organisms observed from the offices with Air Conditioner ventilation were more than the those observed on the fan and this indicates that the organisms can be traced to the interior part of the Air Conditioners and only a portion of them while the other higher population are dispersed into the environment due to force that drive the air from within the Air Conditioner. Modebolu and Modebolu, 2013, Gołofit-Szymczak and Górny, 2010 and Nwaugo et al., (2006) reported that *Baciilus* sp., *Pseudomonas species, Streptococcus* sp. and Staphylococcus sp. which were the organisms isolated during research were also observed to be associated with Air Conditioners in their findings and this was in agreement with our findings.
- *Bacillus* sp. was observed to be higher in their research conducted by Nwaugo et al., (2006). Microorganisms are well adapted to aerial transmission through nasopharyngeal secretions and saliva drops and can easily survive dehydration; therefore, they can be easily transmitted from one host to another (Brooks et al., 1998). Although bacteria are part of normal skin and nasal passages flora, some species can cause a large range of illnesses from minor skin infections (furuncles, pimples, impedigo, abscesses) to life-threatening diseases (pneumonia, meningitis, sepsis) (Kluytmans et al., 1997). Airborne microorganisms affect human health, especially generating respiratory allergies, and infectious lung diseases (Fracchia et al., 2006). The species of *Aspergillus* and *Penicillium* are often associated with allergic symptoms in the respiratory system. In recent years, the role of indoor *Penicillium* as a cause of allergies in some people has been proven, Higher concentrations of *Cladosporium* and *Penicillium* indoor could cause allergic disease. Fungal genera that most commonly cause allergies are *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium* species (Enitan et al., 2017).
- Penicillium chrysogenum may cause central nervous system infection, otomycoses, endophthalmitis, keratitis and endocarditis (Twaroch et al.,2015). Mycotoxin producers common in indoor fungi are species of Aspergillus, Penicillium and Fusarium. In addition, Alternaria and Aspergillus flavus isolated in the present study are potential mycotoxin producers. In dust extracts there are few reports on the airborne fungi and fungal aeroallergens of organic dusts including airborne fungi (Oliviera et al., 2005). Detection of allergenic and potentially pathogenic fungi in the air does not necessarily indicate that all may cause problems, it addresses the potential risk of diseases and sensitivity in individuals. Staphylococcus aureus has been associated with different clinical conditions. For example, it is one of the most frequently encountered single bacteria species in hospitals and continues to be the frequent cause of infections in burns and sepsis (Obiazi, et al., 2007). It produces pustules, carbuncles, boils and impetigo. It frequently causes septicaemia, osteomyelitis, bacteraemia and otitis (Udeze et al., 2012).
- Susceptibility patterns of the isolates were determined by the disk agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Humphries, et al., 2018) Adopting the Kirby Baeur method of recording results, antimicrobial sensitivity carried out showed that majority of the organisms were sensitive mostly to Gentamycin, Tarvid, erythromycin, Streptomycin. Least level of resistance was observed with *Bacillus* species and S.*epidermidis*. S. *epidermidis*. showed highest sensitivity to Erythromycin, Streptomycin, levofloxacin.

#### 4.2 Conclusion

Indoor air pollution assessment is very important as it has been linked to public health problems. This study has shown the presence of fungi and bacteria of public health importance in offices that uses fan and the ones that uses AC. Bacteria and fungi were higher in

offices that uses AC than offices that uses fan. The isolates were mainly potentially pathogenic, therefore, Indoor Air Quality Plan and Procedures should be outlined and awareness created so that the office users will ensure that the offices are effectively cleaned.

#### Conflict of interest

The researchers declare that there is no conflict of interest.

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