

Bacteriological Assessment of Indoor Air-Conditioner and Grills in Academic Institutions in South Western Nigeria

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Abstract: Air Conditioners are widely used devices that are used in enclosed areas to remove heat and lower the temperature of the air in such enclosed areas. Air conditioners are widely used in homes, offices, laboratories, cars and even in recent times there is an emergence of Air-Conditioners built in cloths. Samples were taken from 500 Air Conditioners from selected offices in primary, secondary and tertiary institutions in the southwestern region of Nigeria. Swab sticks were rubbed on the grill surfaces of the Air Conditioners used in this research, after the Air Conditioner has been switched on for 15 minutes Nutrient Agar plates were also exposed for two seconds to the ventilation coming from the Air Conditioners. This study has revealed that Air conditioners often harbor microorganisms which could be pathogenic. *Bacillus sp.*, *Streptococcus sp.*, *Pseudomonas sp.* and *Staph sp.* were present in the samples taken from the Air Conditioners. The population of *Bacillus species* were observed to be higher in dry season (particularly between November 2015- March 2016), while in the other organisms, no seasonal variation was observed

Keywords: Air Conditioner, Bioaerosols, Biofilms, Grill, Institutions, Pathogenic, Temperature, Ventilations.

1. INTRODUCTION

Air Conditioners are widely used in tropical countries due to high temperature in these regions during the harmatan season. Air Conditioners are used in different places such as homes, offices, cars and industries [1]. 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 10m³ of air every day, and spend between 80-95% of their lives in indoor environment. The air inhaled by humans contain high quantity of microorganisms which form so-called bioaerosol. Bioaerosol 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, e.t.c attached to them. Major sources of bioaerosols in an enclosed area include human occupants, organic wastes, pets, house dust, as well as Air Conditioners [6].

In situations where AC are being used for a long time without cleaning, there could be accumulation of dusts and biofilms of microorganism 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 [1]. 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 [4]. A condition known as "sick house 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 [1].

Allergic symptoms that accompany the “sick house syndrome” are: headache, watery eyes, skin disorders and weakness [1, 14].

Some strains of the organisms isolated from air conditioners has been reported to be pathogenic. Strains of *Bacillus* has been implicated in pulmonary infections [2] [12] [13] and nosocomial infections and these species has also been reported to be virulent. Some nosocomial infections have been traced to the Air Conditioners in the hospitals which has not been cleaned for a long time [2]. *Streptococcus species* has been reported to be the causative agent of pneumonia [3] [10]. *Staphylococcus species* has also been implicated in respiratory infections when inhaled [5] [11]. *Pseudomonas species* has been observed to cause chronic pulmonary infections [8] [9]. The aim of this research is to ascertain if there are potential pathogenic microorganisms in the Air Conditioners sampled in the South western region of Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection:

The research was carried out between January 2014 - May 2016. Samples were collected four times weekly and a total of 500 samples were collected. The grill surfaces of the Air Conditioners used were swabbed and the organisms on the swab sticks were transferred into sterilized maximum recovery diluent and incubated for six hours. Pour plate method was used in enumerating the heterotrophic bacteria present after serial dilutions were carried out on the incubated samples. Swab sticks were also used to make primary smear on sterilized Nutrient Agar plates. Prepared petri dishes containing sterilized Nutrient Agar were as well exposed to the Air Conditioner ventilation for two seconds. The petri dishes were incubated for 24 hours at 37°C.

2.2 Morphological identification of isolates:

Isolates were grown on fresh Nutrient Agar plates and the colonial characteristics which include shape, size, colour, elevation, surface, opacity and edge of the colonies were observed and recorded.

2.3 Isolation of pure colonies:

After 24 hours of incubation, the plates were checked for growth and colonies were counted and carefully picked from the mixed colonies on the plates using sterilized inoculating loop and plated out on freshly sterilized Nutrient Agar plates. This was repeated until pure colonies were gotten.

2.4 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°C for further studies.

2.5 Microscopic observation of isolates:

Characterization of isolate began with staining; a procedure that dictates subsequent biochemical tests that is needed to be done for traditional identification of bacteria.

2.5.1 Gram Staining:

Clean, grease free microscope slides were labelled appropriately. A drop of distilled water was placed on the slide and with the aid of a sterile wire loop, organisms were picked from a colony of a 24 hours old pure culture and transferred to the slide. A thin film smear of bacteria isolate was then made on the grease free microscope slide. The smear was heat fixed by passing through a Bunsen burner flame. The heat fixed smear was then stained with crystal violet solution for 1 minutes after which it was rinsed with water. The slide was then flooded with Gram's iodine solution and also left for 1 minute. This served as a mordant; the smear was then washed with 95 % alcohol for 5 seconds and rinsed with running tap water. The smear was counter stained with safranin for 1 minute and rinsed under running tap water. The stained smear was blotted dry and examined under oil-immersion objective. Gram-positive cells appeared violet while Gram-negative cells appeared pink. Cell shapes were also observed.

2.5.2 Spore Staining

Heat fixed smear of the 5 days old culture of the test organism on a grease free slide was stained with malachite green solution and steamed for 5-10 minutes ensuring that the stain did not dry out. The malachite green smear was carefully rinsed under tap water. The smear counter stained with Safranin solution for 15 seconds and washed with water. The slides was blotted dry and examined under oil-immersion objective lens. Bacterial endospores stained green while the vegetative cells stained red.

2.6 Biochemical characterization of bacterial isolates:

The following biochemical tests were performed for the identification of the isolates;

2.6.1 Catalase test:

A loopful of 24 hours old culture was emulsified with a drop of 3.0 % hydrogen peroxide (H₂O₂) on a clean microscope slide. The formation of gas bubbles indicated the presence of enzyme catalase which constituted a catalase positive reaction and the absence of gas bubbles indicated a catalase negative reaction. A drop of hydrogen peroxide with no isolate added represent a negative control.

2.6.2 Citrate utilization test:

Koser's citrate medium was prepared according to the manufacturer's instruction and dispensed into clean bijou bottles. The medium was sterilized under standard conditions. Sterile medium was inoculated using a straight inoculating wire with 24h old broth culture of the test organism. The inoculated medium was incubated at 35⁰C for 24 hours against an uninoculated medium which served as control. A change in colour of the medium from green to blue indicated positive reaction showing that the organism was able to utilize citrate as a sole carbon source while no colour change indicated negative reaction and this means the organism was unable to utilize citrate as a sole carbon source.

2.6.3 Starch hydrolysis:

The test organisms were streaked once across the center of the surface of sterile starch agar plate and incubated at 35⁰C for 24hours. The culture plates were then flooded with 5.0ml of Lugol's iodine solution. Area where hydrolysis of starch occurs appeared as a clear zone around the line of streak and unhydrolysed starch formed a blue-black colouration with the iodine.

2.6.4 Oxidase test:

Filter paper was moistened with a few drop of 1 % tetramethyl-p-phenylenediaminedihydrochloride. With the aid of a sterile wire loop, each test organism was picked and smeared on the filter paper. Oxidase production is indicated by the appearance of a purple colour within 5 to 10 seconds. Absence of purple colour indicates a negative result.

2.6.5 Sulphide Indole and Motility test:

Sulphide Indole Motility medium was prepared according to manufacturer's specifications and dispensed into labeled test tubes. The tubes were corked and sterilized at 121^oc for 15 minutes and allowed to set. Using a sterile inoculating wire, sterile media were carefully stab-inoculated with a 24 hours old broth culture of test organism and incubated at 37⁰C for 24 hours. Motile organisms spread out from the line of stab while non-motile organisms grew along the line of stab. A stabbed but un-inoculated medium served as control. Observation of black colouration in the test tubes indicated a sulphide positive reaction while the absence of black colouration indicated a negative reaction.

About 0.5ml of Kovac's indole reagent was added to each test tube of Sulphide Indole Motility agar and the test tubes were shaken gently and allowed to stand for two minutes. A pink colouration which separates out in alcohol layer indicated indole production i.e. positive result, while no colour change indicated negative reaction.

2.6.6 Sugar Fermentation and Hydrogen Sulphide production 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 121⁰C 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 colouration.

2.6.7 Mannitol Fermentation:

Phenol red mannitol broth, was prepared and dispensed into test tubes. The tubes were sterilized at 121⁰C for 15 minutes. Each tube was inoculated with the test organism and was incubated at 37⁰C for 24 hours. A positive test shows a colour change from red to yellow, indicating a pH change to acidic, while a negative test shows no colour change.

2.6.8 Methyl Red and Voges Proskauer test (MRVP):

Sterile MRVP medium (glucose phosphate broth) was prepared and dispensed into test tubes which were inoculated with 0.1ml of 24 hours old nutrient broth culture of the test organism and incubated for 5 days at 35⁰C. The content of each test tube was divided into two portions and labeled MR and VP respectively. About 5 drops of methyl red solution was added to the test tubes labeled MR and examined for a colour change. A red colour indicated a positive reaction while yellow coloration indicated a negative reaction. To the VP tubes, 0.5ml of 6% naphthol solution was added and 0.5ml of 16% potassium hydroxide (KOH) solution was also added. The tubes were shaken and left for 5minutes. Development of red coloration indicated a positive reaction (i.e. acetoin production), no coloration indicated negative reaction.

2.6.9 Urease test:

Urease basal medium was prepared and sterilized at 121⁰C for 15 minutes and cooled to 45⁰C. Urea solution was sterilized by membrane filtration and then added to the basal medium to give a final concentration of 2 % urea. The solution was dispensed into test tubes. The tubes, while the contents were still warm were slanted and the content was allowed to cool and set in a slanting position. The test organisms were streaked on the slant and also stabbed into the agar using an inoculating loop and wire loop respectively. The slants were incubated at 37⁰C for 24 hours and observed after 24 hours. The production of urease indicated by the change in colour from yellow to pink and indicated a positive reaction while no colour change indicated that the tests were negative.

3. OBSERVATIONS AND RESULTS

The population of the organism observed in the Air Conditioner ventilation were more than those that were observed on the Grills of the Air Conditioners. From the gram staining and biochemical tests carried out on the organisms recovered from the Air Conditioner grills and ventilations, the bacteria observed are: *Bacillus sp.*, *Pseudomonas sp.*, *streptococcus sp.* and *Staph sp.* which has the least population. *Bacillus species* was also observed to have a higher population during the dry season that is between November 2015- March 2016 compared to the rainy season. The highest population of organism found in the Air Conditioner ventilation during the rainy season was found in *Bacillus sp.* (45%), followed by *Pseudomonas sp.* (23%), *Streptococcus sp.* (18%) and *Staphylococcus sp.* (14%). On the grill of the Air Conditioner during the rainy season, *Bacillus sp.* (38%) was the most occurring organism followed by *Pseudomonas sp.* (23%), *Streptococcus sp.* (21%) and *Staphylococcus sp.* (18%). The highest population of organism recorded in the Air Conditioner during the dry season was *Bacillus sp.* (53%) which was the highest population found throughout the study period and this population was followed by the population of *Pseudomonas sp.* (20%), *Streptococcus sp.* (15%) and *Staphylococcus sp.* with a population of 12%. On the grill of the Air Conditioner during the dry season, the percentage of *Bacillus sp.* was 45% which was the highest population recorded on the grill during this sampling period. The next population recorded was in *Pseudomonas sp.* with a population of (23%), *Streptococcus sp.* with a population of 18% and *Staphylococcus sp.* with a population of 14%.

TABLE.I: Showing the population (%) of organisms observed in the air conditioner ventilation in rainy and dry season.

Organism	% found in AC ventilation in the rainy season	% found on the AC grill in the rainy season	% found in the AC ventilation in the dry season	% found in the AC grill in the dry season
<i>Bacillus sp.</i> (%)	45%	38%	53%	45%
<i>Pseudomonas sp.</i> (%)	23%	23%	20%	23%
<i>Streptococcus sp.</i> (%)	18%	21%	15%	18%
<i>Staphylococcus sp.</i> (%)	14%	18%	12%	14%

4. DISCUSSION

The population of the organisms observed from the Air Conditioner ventilation were more than the those observed on the grill and this indicates that the organisms can be traced to the interior part of the Air Conditioners and only a portion of them settle on the grill 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[6]; Gołofit-Szymczak and Górny, 2010 [7] and Nwaugo *et al.*, (2006) [1] reported that *Bacillus 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.

Bacillus sp. was observed to be higher in the dry season than in the rainy season while other organisms shows no seasonal variation, Nwaugo *et al.*, (2006) [1] also reported the increase in the population of *Bacillus sp.* in the dry season. The increase in population of *Bacillus sp.* during the dry season is due to the ability of the species to survive adverse conditions by producing spore, might be due to the ability of the organism to produce enzymes that could lyze other organisms or might be due to the fact that the temperature during the dry season supports the growth of *Bacillus sp.* better than the growth of other organisms.

Legionellae sp. was absence from the organisms isolated and this finding agrees with the results observed by Modebolu and Modebolu, (2013) [6], who reported the absence of *Legionellae sp.* in Air Conditioners and further explained that the absence of this species may be because the environmental conditions in southwestern Nigeria does not support the growth of this species in the Air Conditioners.

5. CONCLUSION

Air Conditioners has been very useful in cooling the temperature especially in tropical countries and this study has isolated some potential pathogenic organisms in the Air Conditioner ventilations and grills and this implies that air conditioners could serve as a reservoir of harmful pathogenic organisms. Hence, Air Conditioners should be cleaned often to avoid it being a source of infection to humans.

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