Original Research Article

A study of the microflora of air environment of rooms sprayed with different aerosols

ABSTRACT

This research work assessed the microflora of rooms sprayed with different insecticides and air freshners with the aim of investigating the effect of the aerosols on the types of microflora in the room environment. Eight (8) different samples of chemical aerosols were used they are: Mobile insecticide, Raid multipurpose insect killer, Morten Insecticide, Rambo Insecticide. as categorized as Insecticides, while Febreze, Air wick, Glade and Top breeze were purchased as air fresheners/fragrance and eight (8) different rooms were used. Microorganisms isolated from the rooms before and after spraying with aerosols were: *Staphylococus aureus, Lactobacillus jensenii, Bacillus coagulans, Aspergillus flavus, Aspergillus niger, micrococcus spp., Aerococcus viridans, Pediococcus cerevisiae, Streptococcus spp, Aspergillus fumigatus and Aspergillus niger*. The result of eight different rooms sprayed with different aerosol as Insecticide and air fresheners showed that, some aerosols were able to inhibit some organisms that were initially present in some rooms while there were introduction of another organisms from some aerosols into some rooms. The occurrence of *Staphylococus aureus* (100%) was the highest in all the rooms followed by *Aspergillus niger* (87.5) and *A. flavus* (75%). *Lactobacillus jensenii*, *Bacillus coagulans* and *micrococcus spp* had the lowest frequency of occurrence (12.5%).

Keywords: Air environment; aerosols; microflora; Indoor; microbial load

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INTRODUCTION

Background to the study

Each day people are exposed to millions of bio aerosols, including whole microorganisms, which can have both beneficial and detrimental effects. Assessment of the indoor of the built environment, the aerobiomes is important and they are bacteria, viruses, fungi and their spores are examples of bio aerosols present in the air, inhaled by human beings. According to Smith*et al.* (2013) major sources of these bioaerosols are: humans; pets; plants; plumbing systems; heating, ventilation, and air-conditioning systems, dust, suspension; aesthetic pollutant and the outdoor environment. Recent advances in molecular sequencing have generated a rush to characterize the microbiome of various environments including indoor and outdoor air (Smith*et al.,* 2012; Kelley *et al*.,2013;Smith*et al.,* 2013; DeLeon-Rodriguez*et al.,* 2013) This is because humans spend over 90 % of their time indoors (Klepeis*et al.,* 2001) Researchers have observed that there are diverse microbial communities in indoor environments such as schools, houses, and hospital (Tringe *et al.,* 2008;Rintala*et al.,* 2008; Kembel*et al.,* 2012*)* rooms within the same building. For instance, Dunn*et al.* (2013) and Adams*et al.* (2014) revealed that microbial isolates in the bedroom differs from that of the bathroom within the same building.

Despite rapid advances in the characterization of airborne microbial communities through rRNA surveys, metagenomics, proteomics, and metabolomics, limited information is available about actual concentrations of airborne microorganisms in built environments. In one of the few studies of concentrations of total bacteria and viruses in indoor air, Prussin*et al*. (2015) found virus-like and bacteria-like particle concentrations of approximately 10^5 and 10^6 particles m^3 in various indoor and outdoor air environment, respectively (Shelton *et al.,* 2002). Moreover an average viable airborne fungi concentration of 80 CFU/m^3 were reported in samples collected from

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schools, hospitals, residences, and industrial buildings; However, in some instances concentrations were as high as 10^4 CFU m^3. Such information should be forthcoming as methods for quantitative metagenomics analyses become more powerful (Shelton*et al.,* 2002;Frank *et al*., 2011*; Gilbert* et *al*., 2011;Duhaime*et al.*, 2012).

In confined environments geared for both industrial and non-industrial activities, the presence of microbial pollutants may elicit the deterioration of indoor air quality (IAQ). Generally, in healthy indoor occupational environments, microflora concentrations are lower than outdoor concentrations (ACGIH 1989; Macher*et al*., 1995). In indoor environments, air from identifiable sources may be responsible for exposure to microbial pollutants through phenomena like diffusion, accumulation and concentration. As people spend 80–95% of their time indoors, air pollution is frequently reported to cause health problems (WHO 1983; WHO 1984). Diverse studies have demonstrated that dust particles, macromolecular organic compounds, Gram-negative bacteria and total volatile organic compounds may cause nasal, optical and physiological changes and sensory symptoms exemplified by irritation, slugginess, sleepiness, headache and reduced ability to concentrate (Gyntelberg*et al*., 1994; Pan *et al*.,2000). The presence of any type of micro-organism can be problematic to IAQ, particularly bacteria and fungi (Stetzenbach*etal*., 1998). In residential and public buildings like schools. Microbial growth is associated with adverse health effects (Husman*al*.,1996; Haverinen*etal*., 1999). Airborne concentrations of *Cladosporium, Epicoccum and Coprinus* spores were associated with peak expiratory flow rates (PEFRs) deficiency in children (Nease*t al*., 1996). The presence of moisture damage in school buildings was a significant risk factor for respiratory symptoms in schoolchildren (Mekline*t al*., 2002). Because of their lower water activity (Aw) requirements compared with bacteria, fungi are the principal contaminant in various types of substrates. They

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tend to colonize a wide variety of humid building materials wetted by floods, condensation or plumbing leaks. Consequently, when fungal proliferation occurs, aerospores are abundantly distributed on and around the surfaces, and the indoor environment becomes a source of exposure to occupants. Knowledge of indoor environmental mycoflora is especially important from an allergologic view-point, which, in many cases differs from that observed in outdoor environments. Although less frequent than the possible dangers caused by exposure to pollen and acari, fungal exposure causes hypersensitive reactions which characterize allergic respiratory pathologies like bronchial asthma and rhinitis (Burge 1989). Fungi may elicit allergic symptoms similar to those caused by pollen.

With an ever-increasing population utilizing different types of aerosols as insecticides and air fresheners, in order to improve and sustain health and vitality; and consuming products in which these supplements are used as room flavors, it is essential that these products are safe for human use. A very critical indicator of safety is the microbiological quality of these products. To improve the prediction of dispersion models and the environmental health assessment on the one hand and to get an insight on the airborne micro-organisms in other relevant environments, e. g. living spaces. However these studies give insight in the internal structure of bio-aerosols and the distribution of micro-organisms on airborne particles themselves for developing guidelines in order to achieve and maintain safe microbial levels in these products.

Therefore, the objectives of the study are to;

(i) Isolate microorganism in air environment of rooms sprayed with selected chemical aerosols

(ii) Determine the microbial load of air environment of rooms sprayed with selected chemical aerosols

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(iii) Evaluate the microbial load of microorganisms present in living room environment

(iv) Investigate the effect of the aerosols on the load of microflora in the room environment

MATERIALS AND METHODS

Study area

The sampling area was an inbuilt living rooms in a house at Akure and the aerosols were purchased from Shoprite shopping mall located at alagbaka, Akure, Ondo State, Nigeria.

Collection of the samples

Eight (8) different samples of chemical aerosols were purchased from shoprite shopping mall, alagbaka, Akure, Ondo State, Nigeria. The selected aerosols were; Mobil insecticide, Raid multipurpose insect killer, Morten Insecticide, Rambo Insecticide. as categorized as Insecticides, while Febreze, Air wick, Glade and Top breeze were purchase as air fresheners/fragrance,

Experimental design

The experimental design is 8x3; sixteen (8) rooms were sprayed with each of the eight selected chemical aerosols, Petri-dishes were prepared aseptically in triplicates and exposed to each room 10 minutes after spraying with insecticides and air fresheners.

Sterilization of material used

All glass wares were washed with detergent, rinsed with clean tap water, air-dried and then oven sterilized at 160^0C for 2 hours. Inoculating loop, used were usually flamed to red hot, dipped into 70 % ethanol, reflamed and allowed to cool before used. Laboratory benches were also swabbed with cotton wool moisten with 95 % ethanol before and after investigation Laboratory coat was

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washed with detergent, rinsed with clean tap water. The hands were also washed with detergent, rinsed dried and cleaned with 70 % ethanol before and after every inoculation.

Inoculating chamber was swarbed with 95% ethanol, thereafter the UV light was on for 2 hours before and after inoculation. The can of the aerosols were cleaned with ethanol. Thereafter, the cans were handled aseptically and the aerosols were released into the air environment of each rooms for10 minutes.

Preparation and sterilization of culture media

Nutrient Agar (NA),Manitol salt agar for isolation of *Staphylococcus aureus* and Potato Dextrose Agar (PDA) were the culture medium used for the investigation. Nutrient agar was prepared by dissolving 28g of the dehydrated powder (Oxoid) into 1 Litre of distilled water in a conical flask. Potato dextrose agar was prepared by dissolving 39g in 1000ml of distilled water in a conical flask. Thereafter, each of the mixtures was placed on hot plates for 20 minutes to ensure proper dissolution of the agar. These were autoclaved at 121^0C for 15 minutes.

Microbial isolation and determination of total viable counts

The method used for isolation and identification of microorganisms was as described by Olutiola*et al.* (1991). Twenty (20ml) of nutrient agar and acidified potato dextrose agar cooled to 45^0C was poured separately onto each of the plates in triplicate and the plates were gently swriled and allowed to solidify. The plates were exposed to air in the room before and after spraying with aerosols for 10 minutes. Thereafter, the nutrient agar plates were incubated in an inverted position at $37^0 \pm 2^0$C for 24 hours for isolation of mesophilic bacteria while Potato Dextrose Agar plates were incubated at $28^0 \pm 2^0$C for 72 hours. Anaerobic plates were inverted in the anaerobic jar at $37^0 \pm 2^0$C for 24 hours for isolation of anaerobic organisms present in the

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samples. After incubation, colonies on the plates were counted using colony counter and the number of viable cells obtained to be the total viable counts of the isolates. The viable colonies were sub cultured from mixed culture plate to obtain a pure culture. The colonies were then identified directly by their size, shape, colour of the pigment (chromogenesis), opacity, elevation, surface, edge and consistency and stored on agar slants for further biochemical tests.

Determination of microbiology of the air samples

Microbiological analysis were determined according to the procedure of (Buchaman, and Gibbons, 1975,Gerhardt, (1981).The microbiological analysis includes isolation of microorganisms from the air samples, direct and microscopic observation of the isolates, biochemical identification of the isolates (*Olutiola et al* 1991*)*. (which include gelatin hydrolysis, a starch hydrolysis, casein hydrolysis, catalase test, coagulase test, indole test, urease test, nitrate reduction test, sugar fermentation test, oxidative fermentation (O/F) test, methyl red voges-proskaur test, citrate test and oxidase test and motility test.

Identification of fungal Isolates

Moulds were identified based on cultural and morphological features using light microscope also number of colony isolated was recorded (Barnett and Hunter, 1998; Labbe and Garcia, 2001). Cultural characterization was based on the rate of growth, presence of aerial mycelium, colour of aerial mycelium as well as colour on the obverse and reverse of the plates. Microscopic identification was based on spore and conidiophore morphology.

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Calculation of Percentage frequency of the isolates

The isolation frequency (Fq) of each isolate from the eight rooms was calculated according to the formula by Gonzalez *et al*. (1999). This was used to determine the distribution of the isolates in the eight sample rooms.

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of isolates of a genus} \times 100}{\text{Total number of samples collected}}$$

Data Analysis

The experiment was conducted using a completely randomized design. Means of three replicates were computed using computer software Microsoft Excel.

RESULT

The result of eight different rooms sprayed with different aerosol as Insecticide and air fresheners are as follows:

Table 1 revealed the bacteria Isolated before and after spraying all the rooms with different aerosols are: *Staphylococusaureus, Lactobacillus jensenii, Bacillus coagulans,Micrococcus spp., Aerococcus viridans, Pediococcus cerevisiae, Streptococcus spp.* Table 2 shows the fungi isolated before and after spraying; *Aspergillus flavus, Aspergillus niger, Aspergillus fumigates* and *Aspergillus niger*. Before spraying the room with Mobil Insecticides, the microorganisms isolated were: *Staphylococcus aureus, Lactobacillus jensenii, Bacillus coagulans, Aspergillus flavus* and *Aspergillus niger*, after spaying the room with Mobil, the Insecticide was able to inhibit the growth of *Lactobacillus jensenii, Bacillus coagulans,* However, there was an introduction of a new organisms (*Micrococcus spp)* which was not present initially. The microorganisms isolated were able to inhibit the growth of *Lactobacillus jensenii, Bacillus*

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coagulans and *Aspergillus flavus* that were present in the room after spraying. However, *there* was an introduction of new organisms (*Micrococcus spp)* which was not present initially.

Before spraying the room with Raid microbes reported were: *Staphylococcus aureus, ,Aerococcus viridans, and Pediococcus cerevisiae. Streptococcus spp, Aspergillus fumigatus, Aspergillus flavus,* after spraying there was inhibition of *Streptococcus spp* only by Morten Insecticide thereafter before spraying Rambo into the rooms, microorganism isolated were: *Staphylococcus aureus, Aerococcus viridans, Pediococcus cerevisiae. Streptococcus spp, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* after spraying it was discovered that Rambo Insecticide was able to inhibit all the organisms present initially except *Staphylococusaureus and Aspergillus flavus.*

Similarly, before spraying Febreze air fresheners microorganisms reported were: *Staphylococcus aureus, Streptococcus spp, Aspergillus fumigatus and Aspergillus niger*. Then after spraying it was discovered that Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: *Lactobacillus jensenii, Bacillus coagulans, Aspergillus flavus,* likewise before spraying with Air wick, microorganism present were: *Staphylococcus aureus, Streptococcus spp, Aspergillus flavus* and *Aspergillus niger,* and after spraying; it was discovered that There was no difference between the type of organism present before and after spraying the room with Air wick. Similarly, before spraying both glade and top breeze into the rooms this are the microorganism are: *Staphylococcus aureus, Streptococcus spp, Pediococcus cerevisiae. Aspergillus flavus* and *Aspergillus niger* and for Top breeze we have *Staphylococcus aureus, Pediococcus cerevisiae. Aspergillus fumigatus,* and *Aspergillus niger* However, after spraying the room, it was discovered that there was no difference between the type of organism present before and after spraying the room with Glade.

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Similarly, there was no difference between the type of organism present before and after spraying the room with Top breeze. However, there was an introduction of *A. flavus.* The occurrence of *Staphylococcus aureus* (100%) was highest in all the rooms followed by *Aspergillus niger* (87.5) and A. flavus (75%). *Lactobacillus jensenii, Bacillus coagulans* and *micrococcus spp* had the lowest frequency of occurrence (12.5%) as shown on table 3 and Fig:1-8. The result of the morphological, microscopic and biochemical characterization of all the organisms isolated before and after spraying are shown in table 4-6

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DISCUSION

This present study was conducted to isolate and identify airborne microbes in some rooms sprayed with insecticides and air fresheners with a view to identify the microflora of the rooms and determine their sensitivity to the aerosols . A total of ten organisms were isolated from eight rooms during the course of this study. Seven bacterial genera were identified from the sampling sites as shown in Table 2 comprising *Staphylococcus aureus*, *Lactobacillus jensenii, Bacillus coagulans, micrococcus spp., Aerococcusviridans, Pediococcuscerevisiae*and *Streptococcus spp* while *Aspergillus* was the only mould generally identified *Aspergillusniger, Aspergillus flavus, Aspergillus fumigates* are the specific species of*Aspergillus* reported.The highest percentage occurrence (100%) is Staphylococusaureus followed by *Aspergillus niger* (87.5) and *A. flavus* (75%). while *Lactobacillus jensenii, Bacillus coagulans and micrococcus spp* had the lowest frequency of occurrence (12.5%). These pathogens could be linked with several infectious organisms responsible for gastroenteritis, respiratory tract infections, urinary tract infections and skin disorders. As *Staphylococcus aureus* belong to the normal flora of the human skin and nose, revealed that these organism may be originated from the nose and skin flora of the occupant of the rooms.

However, this higher incidence of *Staphylococcus aureus* obtained from this study correlatewith several and similar findings of the studies conductedby several researchers. A study conducted by Yaghoub and Elagbash (2010) at Omdurman and El-Rhibat hospital Sudan found that *Staphylococcus aureus* was the predominant bacteria isolated from these hospitals. Thisstudy also supported the finding of Sheik *et al.* (2015), inwhich the occurrence was reported to be 38% in a researchconducted to detect the airborne

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microorganism from a college in Saudi Arabia. In a review of indoor bioaerosols, Nazaroff*et al.* (2014s) suggested that the penetration efficiency of bioaerosols is close to 100 % in a naturally ventilated building, meaning that all bioaerosols flowing through leaks and openings in the building environment arrive indoors. In fact, Prussin*et al.* (2015) showed that concentrations of bacteria-like and virus-like particles were approximately two times higher in outdoor air than in indoor air, suggesting that human occupant might not be the only component shaping the microbial structure of indoor air environment.

The microbial community structure of indoor air varies geographically, depending on the external factors such as temperature, humidity, oxygen etc.However, some specific chemical air pollutants insecticides and fresheners like Mobil, Raid multipurpose insecticides, Morten insecticide, Rambo insecticide, Febreze air freshener, Air wick, Glade, Top breeze used in the experiment, affected the distribution of some microorganisms because microorganisms were discovered before spaying and some of the microbes found before spraying might not be seen after spraying due to the fact that the chemical aerosols inhibited the growth of some of these microbes, this shows that these microbes are very sensitive to the aerosols. For those microbes that were seen after spraying, they were not inhibited by the chemical aerosols, this means they adapt or tolerate the condition, so the spray do not have effect on the microbes.

From Mobile Insecticides the microorganisms reported were: *Staphylococcus aureus,Lactobacillus jensenii,Bacillus coagulans, Aspergillus flavus*and *Aspergillusniger.* However, after spaying the room with Mobile,the Insecticide was able to inhibit the growth of

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Lactobacillus jensenii, Bacillus coagulans, from the report, there was an introduction of a new organisms (*micrococcus spp)* which was not present initially. Furthermorethe microorganisms isolatedwere able to inhibit the growth of *Lactobacillus jensenii, Bacillus coagulans*and *Aspergillus flavus* that were present in the room after spraying. However, *there* was an introduction of a new organisms (*Micrococcus spp)* which was not present initially.

Before spraying the room with Raid, the microbes isolated were:*Staphylococusaureus, Aerococcusviridans, Pediococcuscerevisiae. Streptococcus spp, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and after spraying there was inhibition of *Streptococcus spp* only by Morten Insecticide. Before spraying Rambo into the rooms, microorganism identified were:*Staphylococusaureus, Aerococcusviridans, Pediococcuscerevisiae. Streptococcus spp, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* after spraying it was discovered that Rambo Insecticide was able to inhibit all the organisms present initially except *Staphylococusaureus and Aspergillus flavus.*

Similarly, before spraying febreze air fresheners microorganisms identified were:*Staphylococusaureus, Streptococcus spp, Aspergillus fumigatus and Aspergillus niger* and after spraying it was discovered that Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: *Lactobacillus jensenii, Bacillus coagulans,Aspergillus flavus,* And also before spraying with Air wick microorganism present are: *Staphylococusaureus, Streptococcus spp, Aspergillus flavus* and *Aspergillus niger,* and after spraying the it was discovered that There was no difference between the type of organism present before and after spraying the room with Air wick. Similarly before spraying both glade and top breeze into the rooms the microorganismthat were isolated were:*Staphylococusaureus, Streptococcus spp, Pediococcuscerevisiae. Aspergillus flavus*

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and *Aspergillus niger* and for top breeze, the isolates are; *Staphylococusaureus, Pediococcuscerevisiae. Aspergillus fumigatus,* and*Aspergillus niger* after spraying it was discovered that There was no difference between the type of organism present before and after spraying the room with Glade and There was no difference between the type of organism present before and after spraying the room with Top breeze. However, there was an introduction of *A. flavus,* so a single community profile cannot be applied to all indoor settings to account for the influence of outdoor air.

Adams *et al.,* (2015) sought to determine how outdoor air and human occupancy affected bacterial microbial communities in a mechanically ventilated, office-like building. Although the authors found that human occupancy was associated with increased levels of bioaerosols associated with the human body, occupancy did not have the most profound effect on the microbiome. Rather, microbial communities observed in indoor air were closely related with those in outdoor air, and changes in microbial communities in outdoor air were mirrored by changes in indoor air. The observation recorded in this study showed an overlap in the microbial taxa in aerosol samples collected in indoor air. The observation indicated high abundances of *Staphylococusaureus, Lactobacillus jensenii, Bacillus coagulans, Micrococcus spp., Aerococcusviridans, Pediococcuscerevisiae* and *Streptococcus spp*., which are typically classified as outdoor-associated microorganism. This study led to the conclusion that outdoor air might exert a stronger influence on microbial communities than does human occupancy in the built environment that is well ventilated and has moderate occupancy.

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Compared to airborne bacteria, fungi are even more strongly correlated between indoor and outdoor air Adams *et al.*, (2013). Typically most airborne fungi found indoors are presumed to originate from outdoors, except in water-damaged buildings. In residential homes, Adams *et al*., (2013) showed that indoor and outdoor air were dominated by *Cryptococcus victoriae*, *Cladosporium spp.*, *Epicoccumspp.,and Penicillium spp.* and that the fungal community structure varied seasonally contrary to this finding. Lee *et al*., (2005) found an indoor/outdoor (I/O) ratio of 0.345 for total fungal spores and 0.025 for pollen grains. Additionally, indoor fungal and pollen concentrations followed trends in outdoor air concentrations. The low I/O ratio for pollen grains reflected the low penetration efficiency of large particles into the built environment compared to smaller spores.

This result is also inconformity with the result obtained by Badri*et al.* (2016), who reported *Staphylococcus aureus* as the highest bacteria isolated from their study.

In the present study *Staphylococcusaureus* was the dominant isolated organism and this bacterium is a common causative agent of various human diseases, it is responsible for many gastrointestinal tract infections, respiratory tract infections and skin disorders (Yaghoub and Elagbash, 2010). Besides the isolation of *Streptococcus species* is of great concern due to the fact that these bacteria are responsible for many cases of meningitis, endocarditis, bacterial pneumonia and necrotizing fasciitis. The reasons for high percentage frequency of occurrence of bacteria in this study could be due to low minimal usage of disinfection procedures against airborne pathogens,

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It is well known that microorganisms is able to penetrate effectively from outdoor air into the built environment (Chen and Zhao 2011) In fact, in some cases variation in outdoor microorganisms explains the majority of variation in microorganism in the built environment (Cyrys*et. al*., 2004)

CONCLUSION

Conclusively, it was important to determine the type of microflora present in the built environment. Ten different types of microorganisms had been identified in the room environment exposed to chemical aerosols. While some qualitative and quantitative information is presently available about humans as a source, much less is known about other source probable. A more complete understanding of the airborne microbiome will require knowledge about the emission rates from these sources. Since airborne particles are a major cause of respiratory ailments of humans, causing allergies, asthma, and pathogenic infections of the respiratory tract. Airborne fungal spores are also important agents of plant disease, and the means for dissemination of many common saprotrophic (saprophytic) fungi. Although a lot has been done in the arena of household air pollution, there is still room for further understanding the newer sources of indoor air pollution. Given the knowledge we have regarding in built microbiology air pollution, long-term measures to curb its health effects have remained grossly insufficient. Stringent implementation of WHO guidelines on indoor air quality and a combined effort from the healthcare profession, industry, and healthcare policy makers can reinforce ways to curb household air pollution and, to an extent, limit its effects on health.The development of a more quantitative approach in characterizing the airborne microbiome in the built environment will open new opportunities for probing fundamental relationships between specific

sources and human health, designing interventions to improve building health and human health, or even for providing evidence for forensic investigations.

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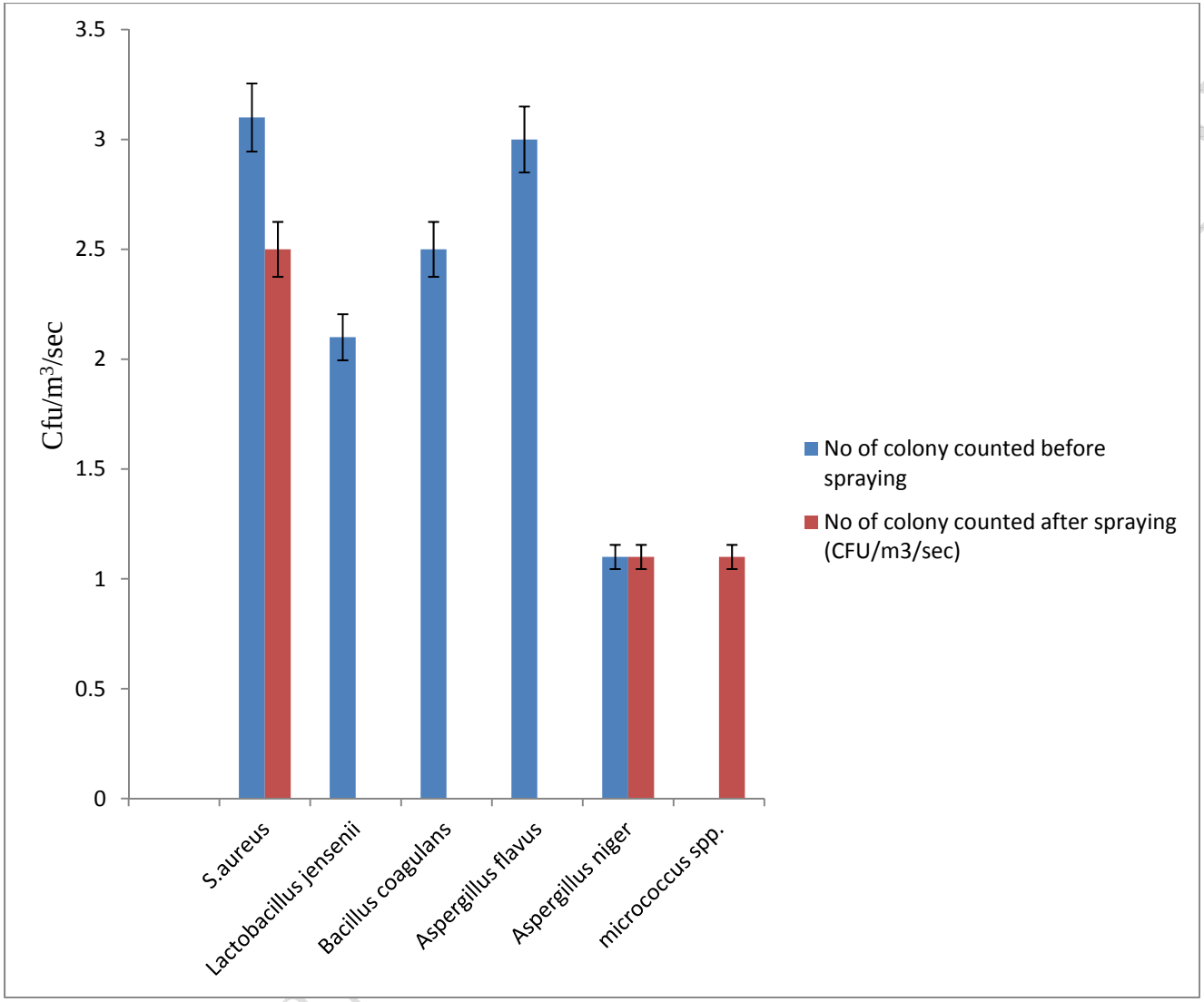

Figure 1: The mean values of colony counted from each room before and after spraying with mobile aerosol

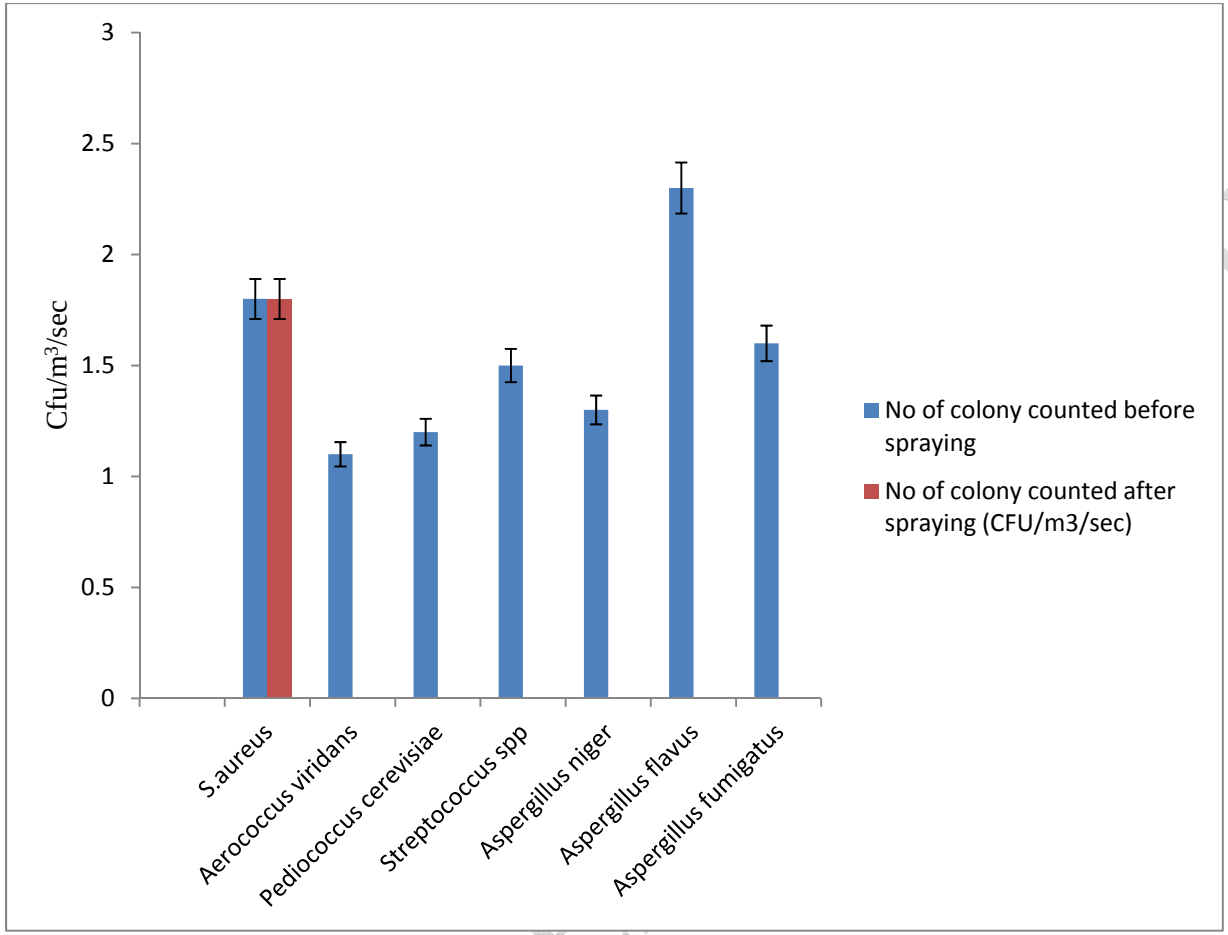

Figure 2: The mean valuesof colony counted from each room before and after spraying with raid aerosol

UNDER REVIEW

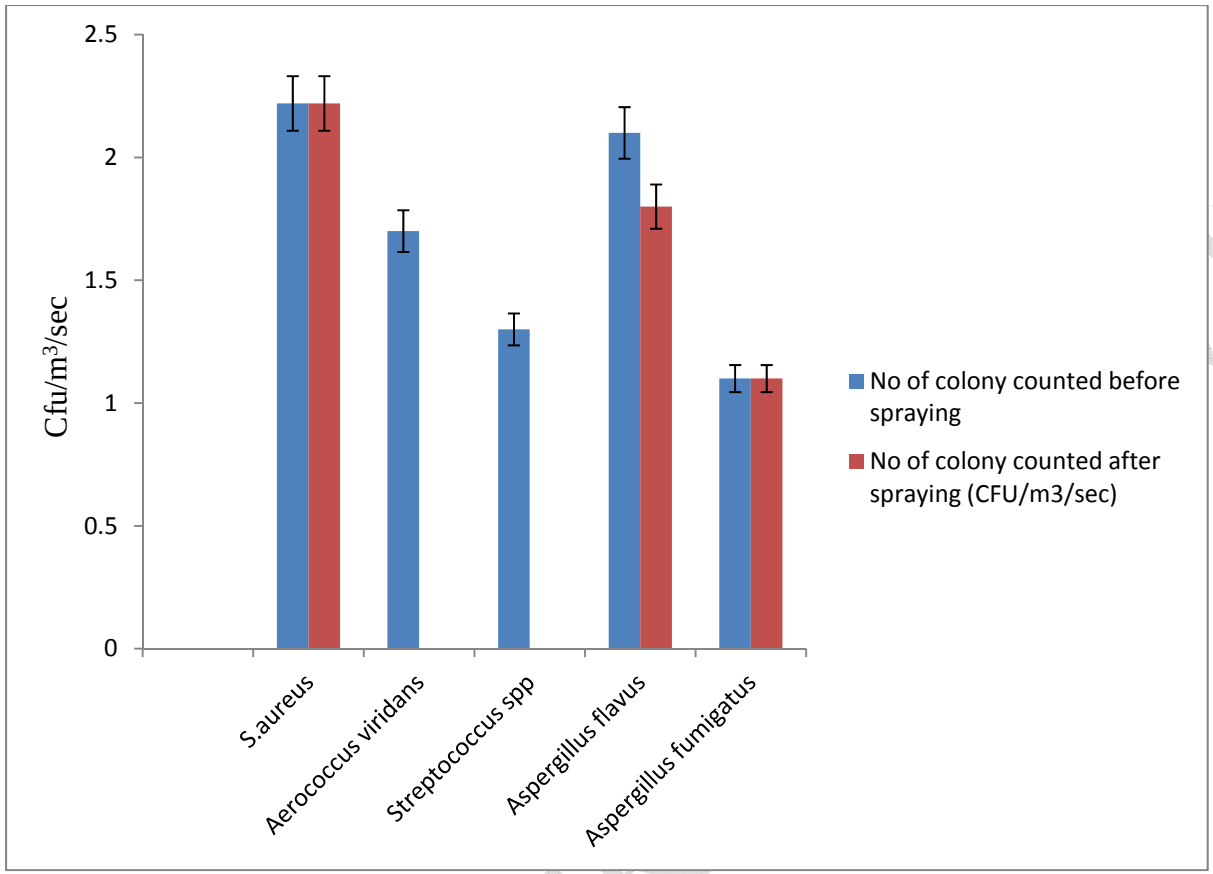

Figure 3: The mean values of colony counted from each room before and after spraying with Morten aerosol

UNDER REVIEW

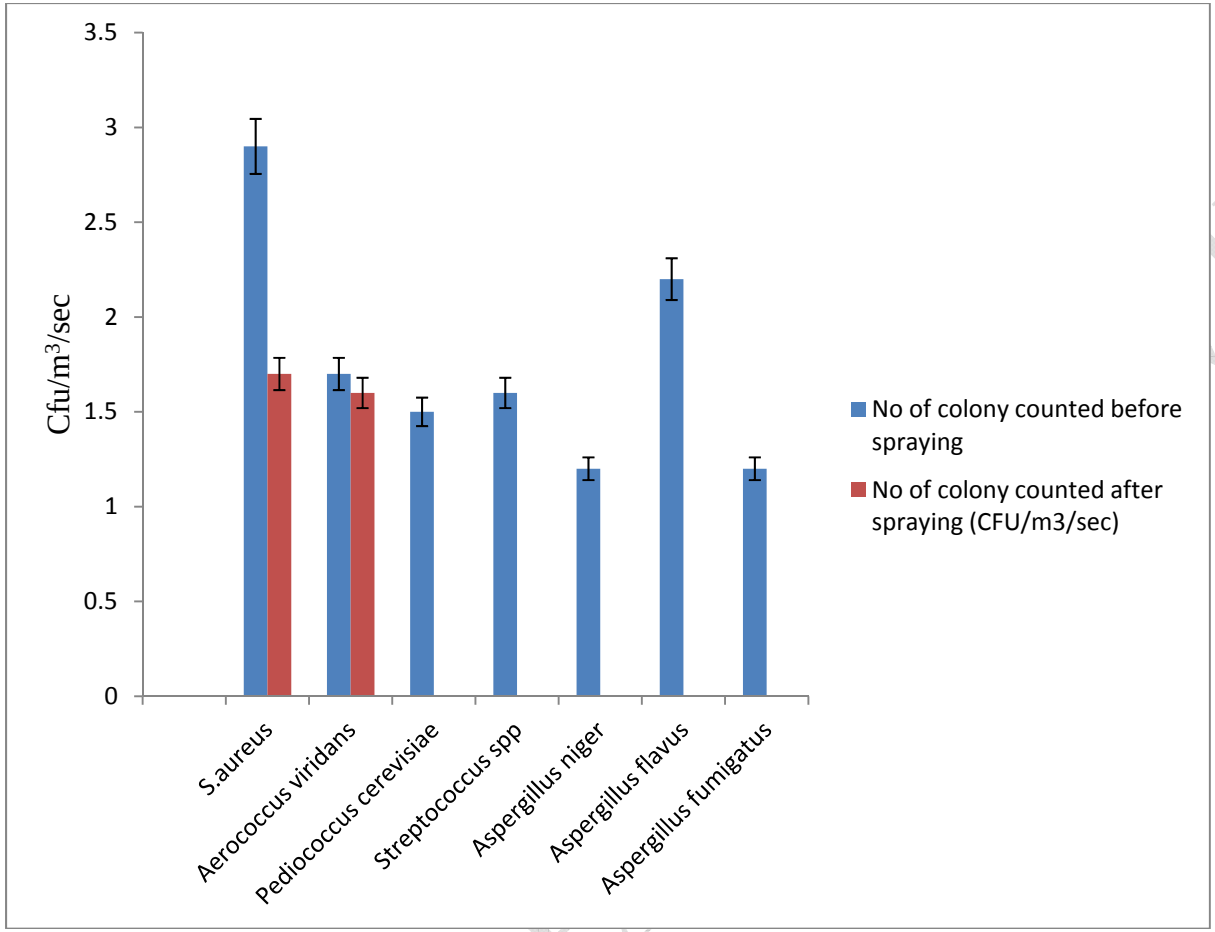

Figure 4: The mean valuesof colony counted from each room before and after spraying with Rambo aerosol

UNDER PEER REVIEW

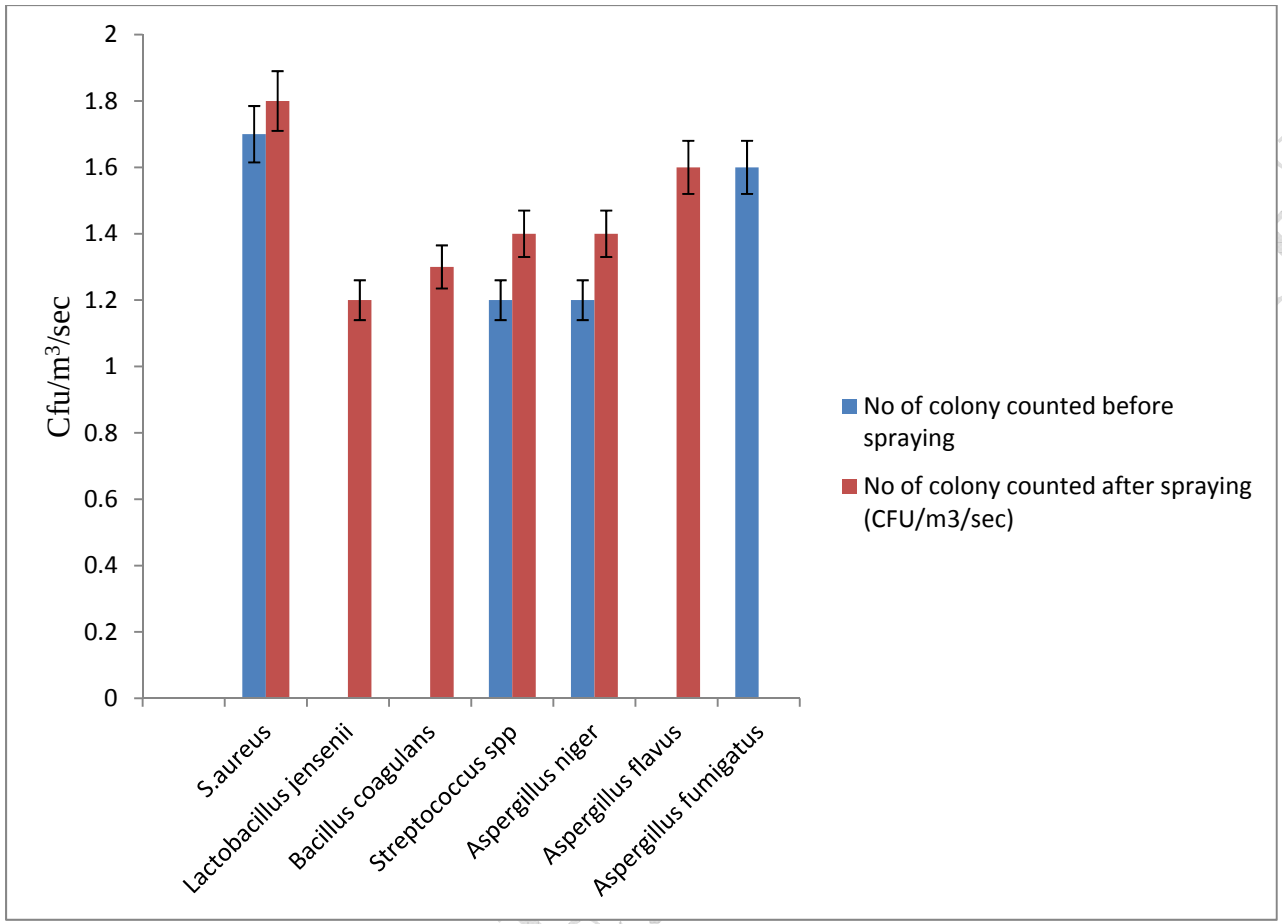

Figure 5: The mean values of colony counted from each room before and after spraying with Febreze aerosol

UNDER REVIEW

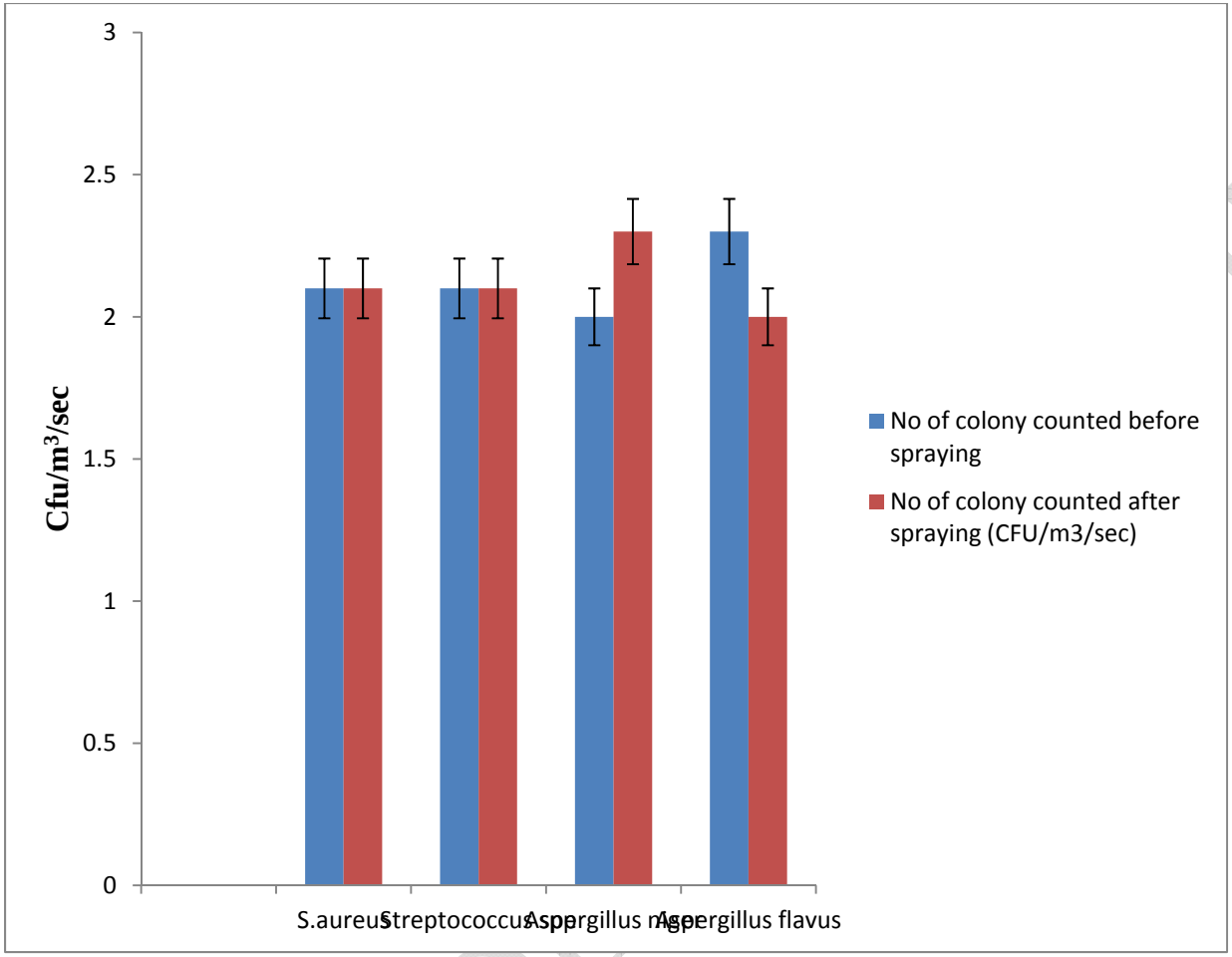

Figure 6: The mean values of colony counted from each room before and after spraying with Air wick aerosol

UNDER REVIEW

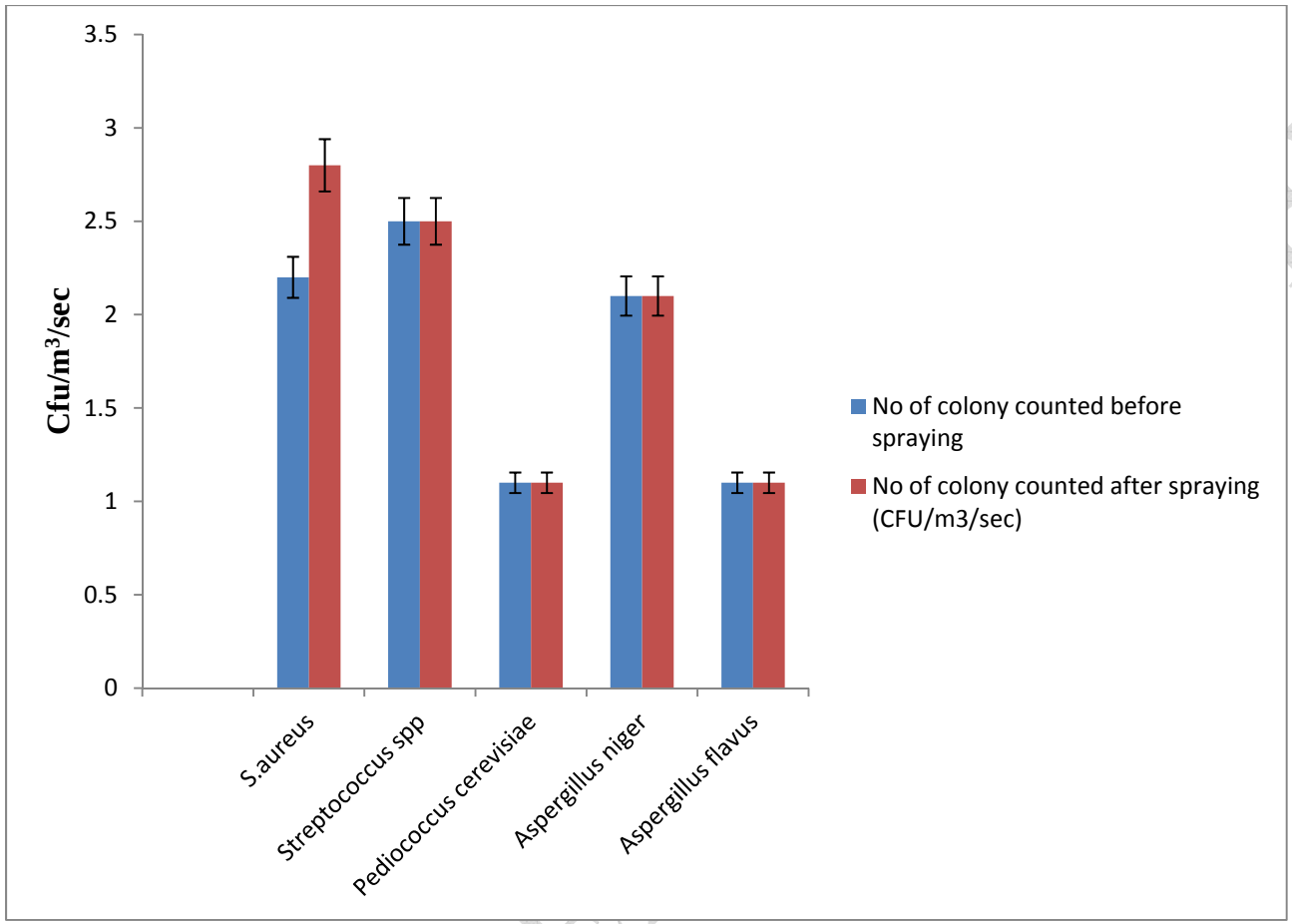

Figure 7: The mean values of colony counted from each room before and after spraying with Glade aerosol

UNDER REVIEW

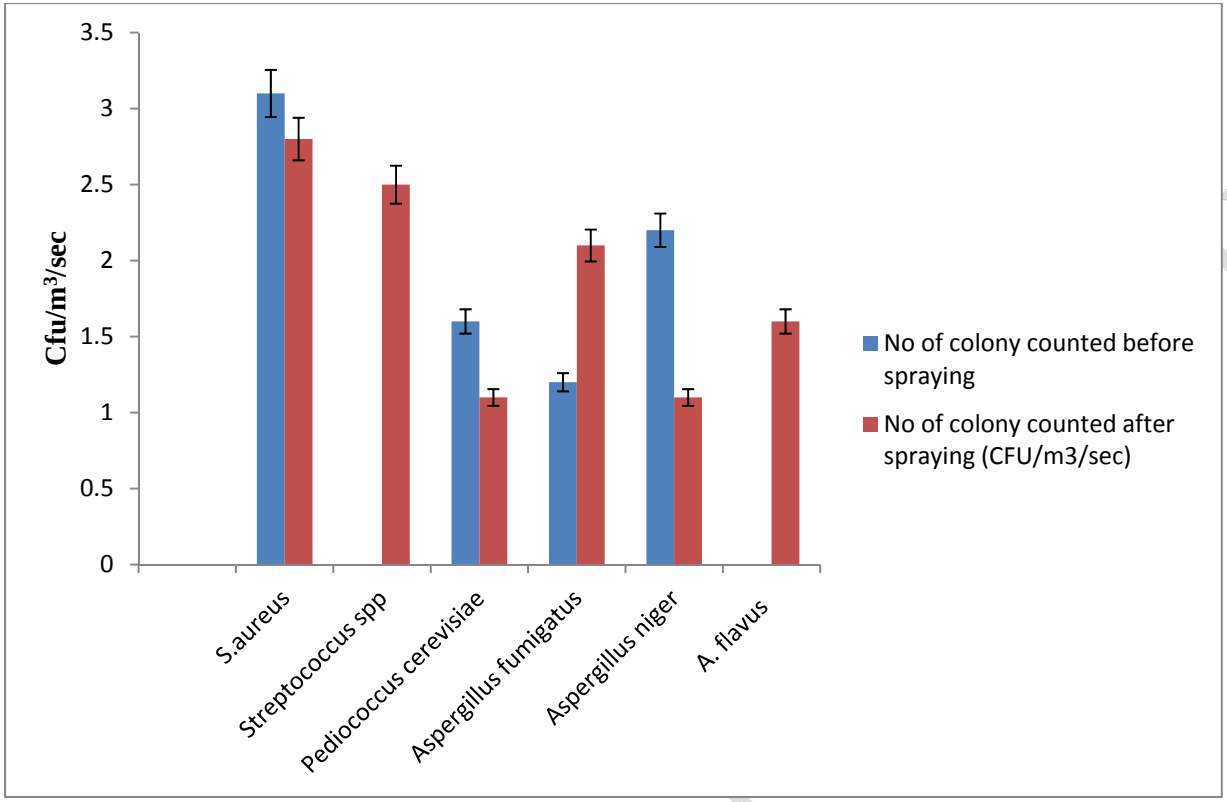

Figure 8: The mean values of colony counted from each room before and after spraying with Top breeze aerosol

UNDER PEER REVIEW

Table 1: Morphology and microscopic characteristic of the bacterial isolates

Code	Shape on Plates	Chromogenesis	Opacity	Elevation	Surface	Edge	Consistency	Gram reaction	shapes	Arrangement of cells	Spore	Spore position	Motility
1	Circular	Insoluble	Opaque	Low Convex	Smooth/ glistering	Entire	Smooth	tve	rod	Chains	-ve	-ve	-ve
2	Circular	Insoluble	Opaque	Raised	Dull	Tentate	friamble	tve	rod	singly	Oval Spore	Central	tve
3	filamentous	Insoluble	Opaque	Effuse	Smooth	Rhizoid	Friamble	tve	cocci	Pairs/ cluster	-ve	-ve	-ve
4	filamentous	Slightly soluble	translucent	raised	Dull	Rhizoid	friamble	tve	cocci	Pair/tetrad	-ve	-ve	-ve
5	Circular	Slightly soluble	Opaque	Raised	Smooth/ glistering	Entire	Smooth	tve	cocci	cluster	-ve	-ve	-ve
6	Circular	Slightly soluble	Opaque	Raised	Smooth/ glistering	Entire	smooth	tve	cocci	tetrad	-ve	-ve	-ve
7	Circular	Insoluble	Opaque	Raised	Smooth	Entire	smooth	tve	cocci	chains	-ve	-ve	-ve

Key:
1= Lactobacillus jensenii, 2= *Bacillus coagulans, 3= Aerococcus Viridans, 4= Pediococcus cerevisiae, 5=Staphylococus aureus, 6= micrococcus spp,* 7=*Streptococcus spp*

+ve positive
-ve negative

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Table 2: Morphological identification of the fungi isolates

Isolate	Morphological Characteristics	Microscopic Identification
Aspergillus flavus	Obverse: yellow-green becoming green with age. Reverse: creamish-yellow	Conidial head showing verrucose stipe, domed-shaped vesicle and philades borne directly on vesicle
Aspergillus fumigatus	Obverse: bluish-green Reverse: creamish-green.	Conidia head with philiades, metulae is absent.
Aspergillus niger	Obverse: blackish-brown often with yellow mycelium Reverse: creamish-yellow to yellow.	conidial head with metulae and philades, brownish colour of stipe.

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Table 3: Biochemical characteristic of the bacterial isolates.

ASP	GA	GL	MN	SC	LA	MA	AR	XY	RA	SO	LM	GH	SH	CA	CO	UR	IN	CI	PROBABLE ORG
-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-Ve	-Ve	Lactobacillus *Jensen*
-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	*Bacillus coagulans*
-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	*Aerococcus Viridans*
-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	*Pediococcus cerevisiae*
-ve	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	*Staphylococcus aureus*
Tve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	ND	ND	ND	ND	ND	ND	ND	ND	*Streptococcus spp*
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	*micrococcus spp*

Keys:

ND- not determined, +ve - positive, -ve -negative, ASP- ascospore, GA-galactose

GL- Glucose, MN-manitol, SC-Sucrose, LA- Lactose, MA -Maltose , AR- Arabinose , XY- Xylose ,

RA- Raffinose, SO- Sorbitol , LM- Litmus Milk, GH-Gelatin, SH -Starch Hydrolysis, CA- Catalase, CO-Coagulase, UR -Urease, IN -Indole, CI- Citrate.

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Table 4: List of bacteria isolates from rooms before and after spraying with aerosol

Room code	Type of aerosol used	Type of microorganisms isolated from the room before spraying with aerosol (control rooms)	Type of microorganisms isolated from the room after spraying with aerosol for 10 minutes	Remarks
A	Mobil	*Staphylococus aureus, Lactobacillus jensenii, Bacillus coagulans*	*Staphylococus aureus, and Micrococcus* spp.	The Insecticide was able to inhibit the growth of *Lactobacillus jensenii, Bacillus coagulans,* However, there was an introduction of a new organisms (*Micrococcus spp)* which was not present initially
B	Raid multipurpose insect killer	*Staphylococus aureus, Aerococcus viridans, Pediococcus cerevisiae. Streptococcus* spp	*Staphylococus aureus*	Raid was able to inhibit all organisms presents initially except *Staphylococus aureus*
C	Morten Insecticide	*Staphylococus aureus, , Aerococcus viridans, Streptococcus spp*	*Staphylococus aureus, Aerococcus viridans*	There was inhibition of *Streptococcus spp* only by Morten Insecticide
D	Rambo Insecticide	*Staphylococus aureus, Aerococcus viridans, Pediococcus cerevisiae. Streptococcus* spp	*Staphylococus aureus*	Rambo Insecticide was able to inhibit all the organisms present initially except *Staphylococus aureus*
E	Febreze air freshener	*Staphylococus aureus, Streptococcus* spp	*Staphylococus aureus, Streptococcus* spp, *Lactobacillus jensenii, Bacillus coagulans,*	Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: *Lactobacillus jensenii, Bacillus coagulans*
F	Air wick	*Staphylococus aureus, Streptococcus* spp	*Staphylococus aureus, Streptococcus* spp	There was no difference between the type of organism present before and after spraying the room with Air wick.

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G	Glade	*Staphylococus aureus, Streptococcus spp, Pediococcus cerevisiae.*	*Staphylococus aureus, Streptococcus spp, Pediococcus cerevisiae.*	There was no difference between the type of organism present before and after spraying the room with Glade
H	Top breeze	*Staphylococus aureus, Pediococcus cerevisiae.*	*Staphylococus aureus, Pediococcus cerevisiae.*	There was no difference between the type of organism present before and after spraying the room with Top breeze.

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Table 5: fungi isolates from rooms before and after spraying with aerosol

Room code	Type of aerosol used	Type of microorganisms isolated from the room before spraying with aerosol	Type of microorganisms isolated from the room after spraying with aerosol for 10 minutes	Remarks
A	Mobil	*Aspergillus flavus*, *Aspergillus niger*	*Aspergillus niger*	The Insecticide was able to inhibit the growth of *Aspergillus flavus*.
B	Raid multipurpose insect killer	*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*		Raid was able to inhibit all organisms presents
C	Morten Insecticide	*Aerococcus viridan*, *Aspergillus fumigatus*, *Aspergillus flavus*	*Aerococcus viridans* *Aspergillus fumigatus* and *Aspergillus flavus*	There was no inhibition of any microorganism
D	Rambo Insecticide	*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*	*Aspergillus flavus*	Rambo Insecticide was able to inhibit all the organisms present initially except *Aspergillus flavus*
E	Febreze air freshener	*Aspergillus fumigatus and Aspergillus niger*	*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*	Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of a new organisms which is*Aspergillus flavus*,
F	Air wick	*Aspergillus flavus* and *Aspergillus niger*	*Aspergillus flavus* and *Aspergillus niger*	There was no difference between the type of organism present before and after spraying the room with Air wick.
G	Glade	*Aspergillus flavus* and *Aspergillus niger*	*Aspergillus flavus* and *Aspergillus niger*	There was no difference between the type of organism present before and after spraying the room with Glade
H	Top breeze	*Aspergillus fumigatus*, and *Aspergillus niger*	*Aspergillus fumigatus*, *A. flavus* and *Aspergillus niger*	There was no difference between the type of organism present before and after spraying the room with Top breeze. However, there was an introduction of *A. flavus* after spraying

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Table 6: percentage (%) occurrence of bacteria isolates

Isolates	No of rooms	No of occurrence	% Occurrence
Staphylococcus aureus	8	8	100
Lactobacillus jensenii	8	1	12.5
Bacillus coagulans	8	1	12.5
Micrococcus spp.	8	1	12.5
Aerococcus viridans	8	3	37.5
Pediococcus cerevisiae	8	5	62.5
Streptococcus spp	8	6	75

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Table 7: Percentage occurrence (%) of fungi isolates

Isolates	No of rooms	No of occurrence	% Occurrence
Aspergillus flavus	8	6	75
Aspergillus niger	8	7	87.5
Aspergillus fumigatus	8	5	62.5

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