

The Effect of aerosols on the air microflora of the indoor air

ABSTRACT

This research work assessed the microflora of rooms sprayed with different insecticides and air fresheners 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: *Staphylococcus 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 *Staphylococcus 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

24 INTRODUCTION

25 **Background to the study**

26

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

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

48 from schools, hospitals, residences, and industrial buildings; However, in some instances
49 concentrations were as high as 10^4 CFU m³. Such information should be forthcoming as methods
50 for quantitative metagenomics analyses air samplers become more powerful (Shelton *et al.*,
51 2002; Frank *et al.*, 2011; Gilbert *et al.*, 2011; Duhaime *et al.*, 2012).

52 In confined environments geared for both industrial and non-industrial activities, the presence of
53 microbial pollutants may elicit the deterioration of indoor air quality (IAQ). Generally, in healthy
54 indoor occupational environments, microflora concentrations are lower than outdoor
55 concentrations (ACGIH 1989; Macheret *et al.*, 1995). In indoor environments, air from identifiable
56 sources may be responsible for exposure to microbial pollutants through phenomena like
57 diffusion, accumulation and concentration. As people spend 80–95% of their time indoors, air
58 pollution is frequently reported to cause health problems (WHO 1983, 1984). Diverse studies
59 have demonstrated that dust particles, macromolecular organic compounds, Gram-negative
60 bacteria and total volatile organic compounds may cause nasal, optical and physiological
61 changes and sensory symptoms exemplified by irritation, sluggishness, sleepiness, headache and
62 reduced ability to concentrate (Gyntelberg *et al.*, 1994; Pan *et al.*, 2000). The presence of any
63 type of micro-organism can be problematic to IAQ, particularly bacteria and fungi (Stetzenbach
64 *et al.*, 1998). In residential and public buildings like schools. Microbial growth is associated with
65 adverse health effects (Husman *et al.*, 1996; Haverinen *et al.*, 1999). Airborne concentrations of
66 *Cladosporium*, *Epicoccum* and *Coprinus* spores were associated with peak expiratory flow rates
67 (PEFRs) deficiency in children (Neaset *et al.*, 1996). The presence of moisture damage in school
68 buildings was a significant risk factor for respiratory symptoms in schoolchildren (Meklin *et al.*,
69 2002). Because of their lower water activity (*A_w*) requirements compared with bacteria, fungi
70 are the principal contaminant in various types of substrates. They tend to colonize a wide variety

71 of humid building materials wetted by floods, condensation or plumbing leaks. Consequently,
72 when fungal proliferation occurs, aerospores are abundantly distributed on and around the
73 surfaces, and the indoor environment becomes a source of exposure to occupants. Knowledge of
74 indoor environmental mycoflora is especially important from an allergologic view-point, which,
75 in many cases differs from that observed in outdoor environments. Although less frequent than
76 the possible dangers caused by exposure to pollen and acari, fungal exposure causes
77 hypersensitive reactions which characterize allergic respiratory pathologies like bronchial asthma
78 and rhinitis (Burge, 1989). Fungi may elicit allergic symptoms similar to those caused by pollen.
79
80 With an ever-increasing population utilizing different types of aerosols as insecticides and air
81 fresheners, in order to improve and sustain health and vitality; and consuming products in which
82 these supplements are used as room flavors, it is essential that these products are safe for human
83 use. A very critical indicator of safety is the microbiological quality of these products. To
84 improve the prediction of dispersion models and the environmental health assessment on the one
85 hand and to get an insight on the airborne micro-organisms in other relevant environments, e. g.
86 living spaces. However, these studies give insight in the internal structure of bio-aerosols and the
87 distribution of micro-organisms on airborne particles themselves for developing guidelines in
88 order to achieve and maintain safe microbial levels in these products.

89 Therefore, the aim of the study are to, isolate microorganism in air environment of rooms
90 sprayed with selected chemical aerosols and investigate the effect of the aerosols on the
91 load of microflora in the room environment

92

93

94 **MATERIALS AND METHODOLOGY**

95 **Study area**

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

98 **Collection of the samples**

99

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

104 **Experimental design**

105 The experimental design is 8x3; eight (8) rooms were sprayed with each of the eight selected
106 chemical aerosols, Petri-dishes were prepared aseptically in triplicates and exposed to each room
107 10 minutes after spraying with insecticides and air fresheners.

108

109 **Microbial isolation and determination of total viable counts**

110 The method used for isolation and identification of microorganisms was as described by Olutiola
111 *et al.* (1991). Twenty (20ml) of nutrient agar and acidified potato dextrose agar cooled to 45⁰C
112 was poured separately onto each of the plates in triplicate and the plates were gently swirled and
113 allowed to solidify. The plates were exposed to air in the room before and after spraying with
114 aerosols for 10 minutes. Thereafter, the nutrient agar plates were incubated in an inverted
115 position at 37⁰ ± 2⁰C for 24 hours for isolation of mesophilic bacteria while Potato Dextrose
116 Agar plates were incubated at 28⁰± 2⁰C for 72 hours. Anaerobic plates were inverted in the
117 anaerobic jar at 37⁰±2⁰C for 24 hours for isolation of anaerobic organisms present in the samples.

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

123

124 **Determination of microbiology of the air samples**

125

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

133

134 **Identification of fungal Isolates**

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

140

141

142 Calculation of Percentage frequency of the isolates

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

146

$$147 \text{ Frequency of occurrence (\%)} = \frac{\text{Number of isolates of a genus} \times 100}{\text{Total number of samples collected}}$$

149 Data Analysis

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

152

153 RESULTS AND DISCUSSION

154 This present study was conducted to isolate and identify airborne microbes in some rooms
155 sprayed with insecticides and air fresheners with a view to identify the microflora of the rooms
156 and determine their sensitivity to the aerosols . A total of ten organisms were isolated from eight
157 rooms during the course of this study. Seven bacterial genera were identified from the sampling
158 sites as shown in table 2 comprising *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus*
159 *coagulans*, *micrococcus spp.*, *Aerococcus viridans*, *Pediococcus cerevisiae* and *Streptococcus*
160 *spp* while *Aspergillus* was the only mould generally identified *Aspergillus niger*, *Aspergillus*
161 *flavus*, *Aspergillus fumigatus* are the specific species of *Aspergillus* reported. The result of eight
162 different rooms sprayed with different aerosol as Insecticide and air fresheners are as follows:

163 Table 1 revealed the bacteria Isolated before and after spraying all the rooms with different
164 aerosols are: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Micrococcus*
165 *spp.*, *Aerococcus viridans*, *Pediococcus cerevisiae*, *Streptococcus spp.* Table 2 shows the fungi

166 isolated before and after spraying; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*
167 and *Aspergillus niger*. Before spraying the room with Mobil Insecticides, the microorganisms
168 isolated were: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus*
169 *flavus* and *Aspergillus niger*, after spaying the room with Mobil, the Insecticide was able to
170 inhibit the growth of *Lactobacillus jensenii*, *Bacillus coagulans*, However, there was an
171 introduction of a new organisms (*Micrococcus spp*) which was not present initially. The
172 microorganisms isolated were able to inhibit the growth of *Lactobacillus jensenii*, *Bacillus*
173 *coagulans* and *Aspergillus flavus* that were present in the room after spraying. However, there
174 was an introduction of new organisms (*Micrococcus spp.*) which was not present initially.
175 Before spraying the room with Raid microbes reported were: *Staphylococcus aureus*, *Aerococcus*
176 *viridans*, and *Pediococcus cerevisiae*. *Streptococcus spp*, *Aspergillus fumigatus*, *Aspergillus*
177 *flavus*, after spraying there was inhibition of *Streptococcus spp* only by Morten (year).
178 Insecticide thereafter before spraying Rambo into the rooms, microorganism isolated
179 were: *Staphylococcus aureus*, *Aerococcus viridans*, *Pediococcus cerevisiae*. *Streptococcus spp*,
180 *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* after spraying it was discovered that
181 Rambo Insecticide was able to inhibit all the organisms present initially except *Staphylococcus*
182 *aureus* and *Aspergillus flavus*.
183 Similarly, before spraying Febreze air fresheners, the microorganisms reported were:
184 *Staphylococcus aureus*, *Streptococcus spp*, *Aspergillus fumigatus* and *Aspergillus niger*. Then
185 after spraying it was discovered that Febreze air freshener was not able to inhibit all the initial
186 organisms present. There was an introduction of three new organisms which are: *Lactobacillus*
187 *jensenii*, *Bacillus coagulans*, *Aspergillus flavus*, likewise before spraying with Air wick,
188 microorganism present were: *Staphylococcus aureus*, *Streptococcus spp*, *Aspergillus flavus* and

189 *Aspergillus niger*, and after spraying; it was discovered that There was no difference between the
190 type of organism present before and after spraying the room with Air wick. Similarly, before
191 spraying both glade and top breeze into the rooms this are the microorganism are:
192 *Staphylococcus aureus*, *Streptococcus spp*, *Pediococcus cerevisiae*, *Aspergillus flavus* and
193 *Aspergillus niger* and for Top breeze we have *Staphylococcus aureus*, *Pediococcus cerevisiae*.
194 *Aspergillus fumigatus*, and *Aspergillus niger* However, after spraying the room, it was
195 discovered that there was no difference between the type of organism present before and after
196 spraying the room with Glade. Similarly, there was no difference between the type of organism
197 present before and after spraying the room with Top breeze. However, there was an introduction
198 of *A. flavus*. The occurrence of *Staphylococcus aureus* (100%) was highest in all the rooms
199 followed by *Aspergillus niger* (87.5) and *A. flavus* (75%). *Lactobacillus jensenii*, *Bacillus*
200 *coagulans* and *Micrococcus spp* had the lowest frequency of occurrence (12.5%) as shown on
201 table 3 and figures:1-8. The result of the morphological, microscopic and biochemical
202 characterization of all the organisms isolated before and after spraying are shown in table 4-6

The highest percentage occurrence (100%) is *Staphylococcus aureus* 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 organisms 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 correlate with several and similar findings of the studies conducted by 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. This study also supported the finding of Sheik *et al.* (2015), in which the occurrence was reported to be 38% in a research conducted to detect the airborne microorganism from a college in Saudi Arabia. In a review of indoor bioaerosols, Nazaroff *et al.* (2014) 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 spraying 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 *Aspergillus niger*. However, after spraying the room with Mobile, the Insecticide was able to inhibit the growth of *Lactobacillus jensenii*, *Bacillus coagulans*, from the report, there was an introduction of a new organisms (*Micrococcus spp*) which was not present initially. Further more the microorganisms isolated were 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: *Staphylococcus aureus*, *Aerococcus viridans*, *Pediococcus cerevisiae*, *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: *Staphylococcus aureus*, *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 *Staphylococcus aureus* and *Aspergillus flavus*.

Similarly, before spraying febreze air fresheners microorganisms identified were: *Staphylococcus aureus*, *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: *Staphylococcus aureus*, *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 microorganism that were isolated were: *Staphylococcus aureus*, *Streptococcus spp.*, *Pediococcuscerevisiae*. *Aspergillus flavus* and *Aspergillus niger* and for top breeze, the isolates are; *Staphylococcus aureus*, *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 *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Micrococcus spp.*, *Aerococcus viridans*, *Pediococcus cerevisiae* 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.

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.*, *Epicoccum spp.*, 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 in conformity 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 *Staphylococcus aureus* 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). 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,

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 (Cyrus *et. al.*, 2004)

CONCLUSION

Conclusively, it was important to determine the type of microflora present in the built environment. The outcome of this research revealed that some aerosols were able to inhibit some organisms that were initially present in experimental rooms while there were

introduction of another organisms from some aerosols into some rooms. This shows that, airborne microbiome can be emitted into any environment through the use of aerosols.

UNDER PEER REVIEW

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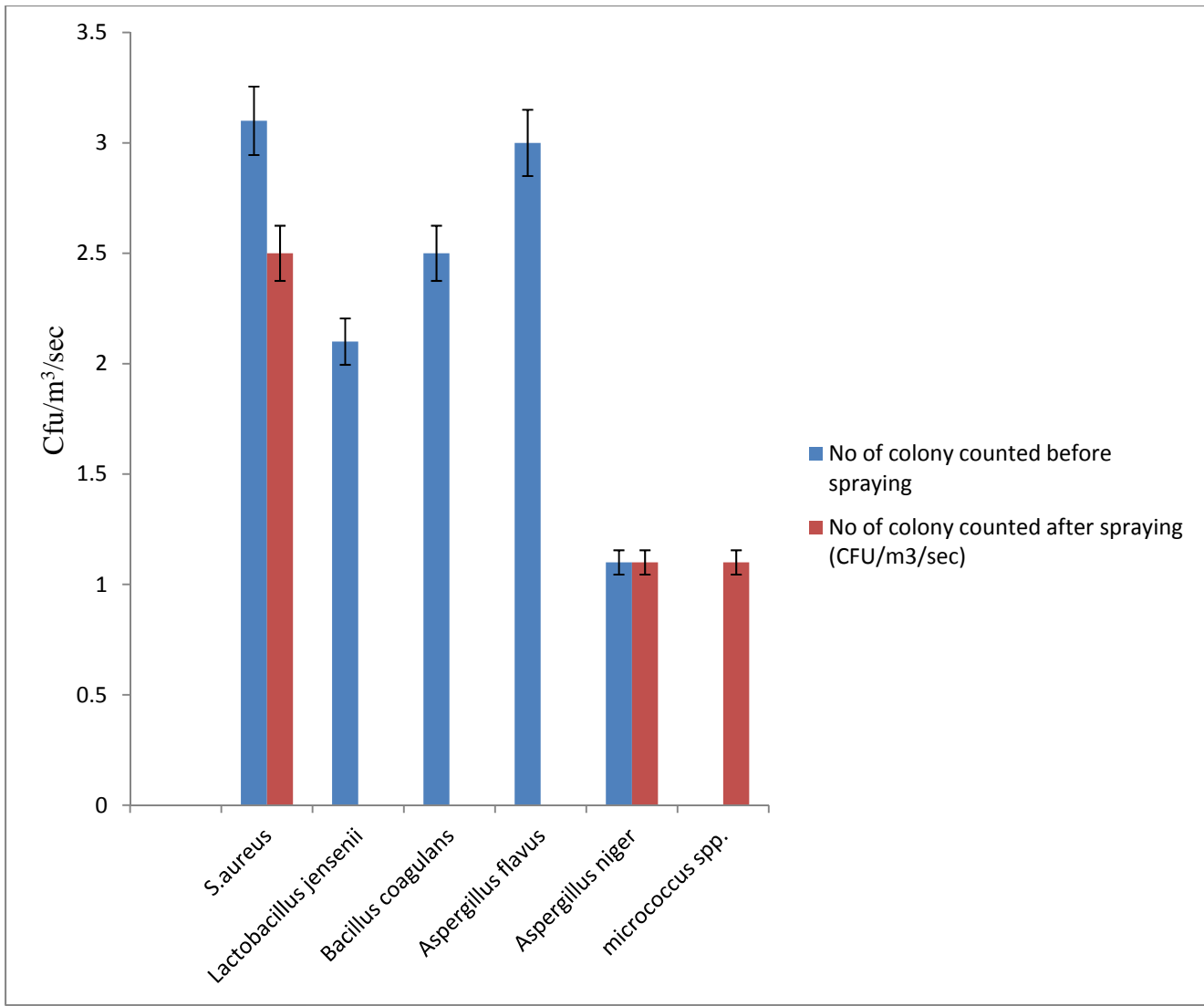


Figure 1: The mean values of colony counted from each room before and after spraying with mobile aerosol

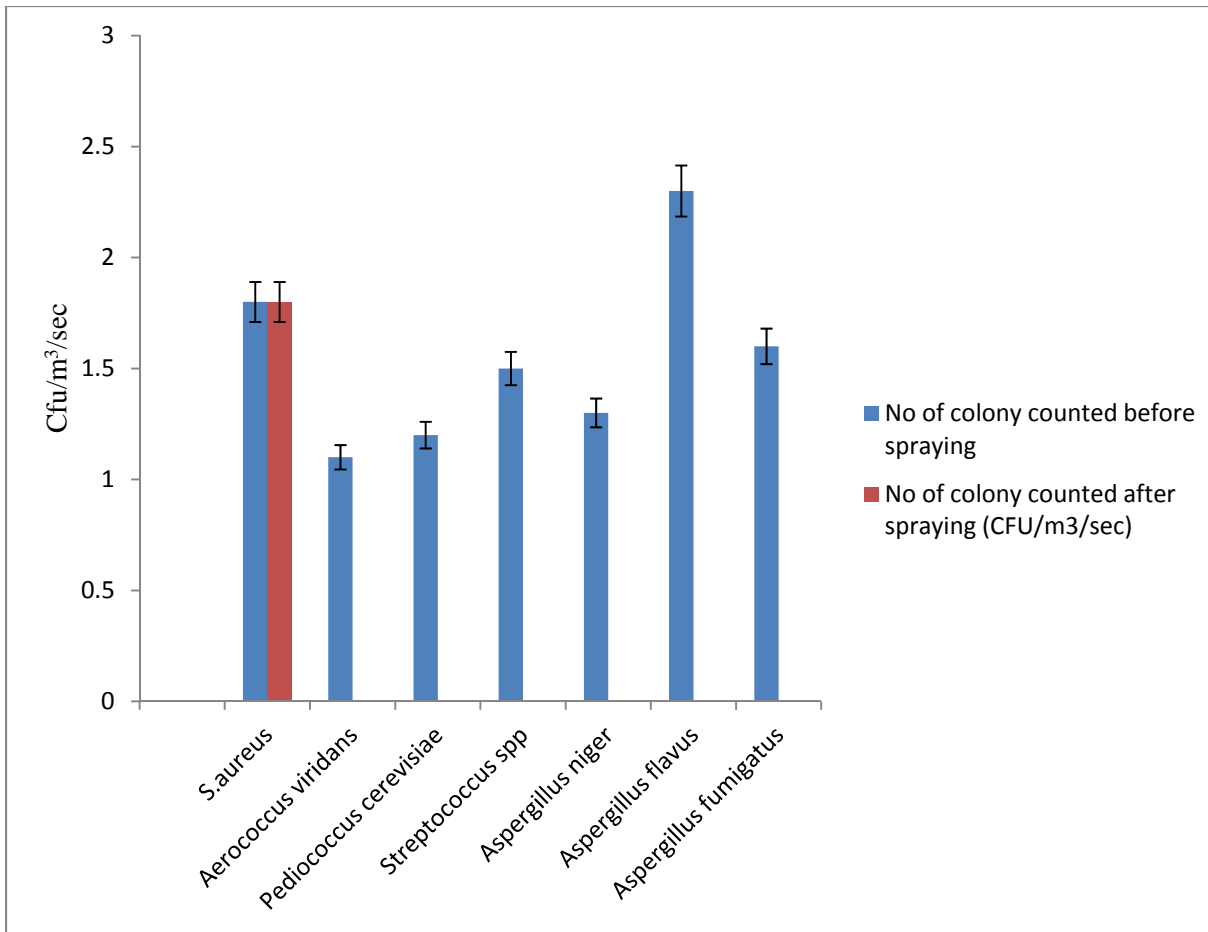


Figure 2: The mean values of colony counted from each room before and after spraying with raid aerosol

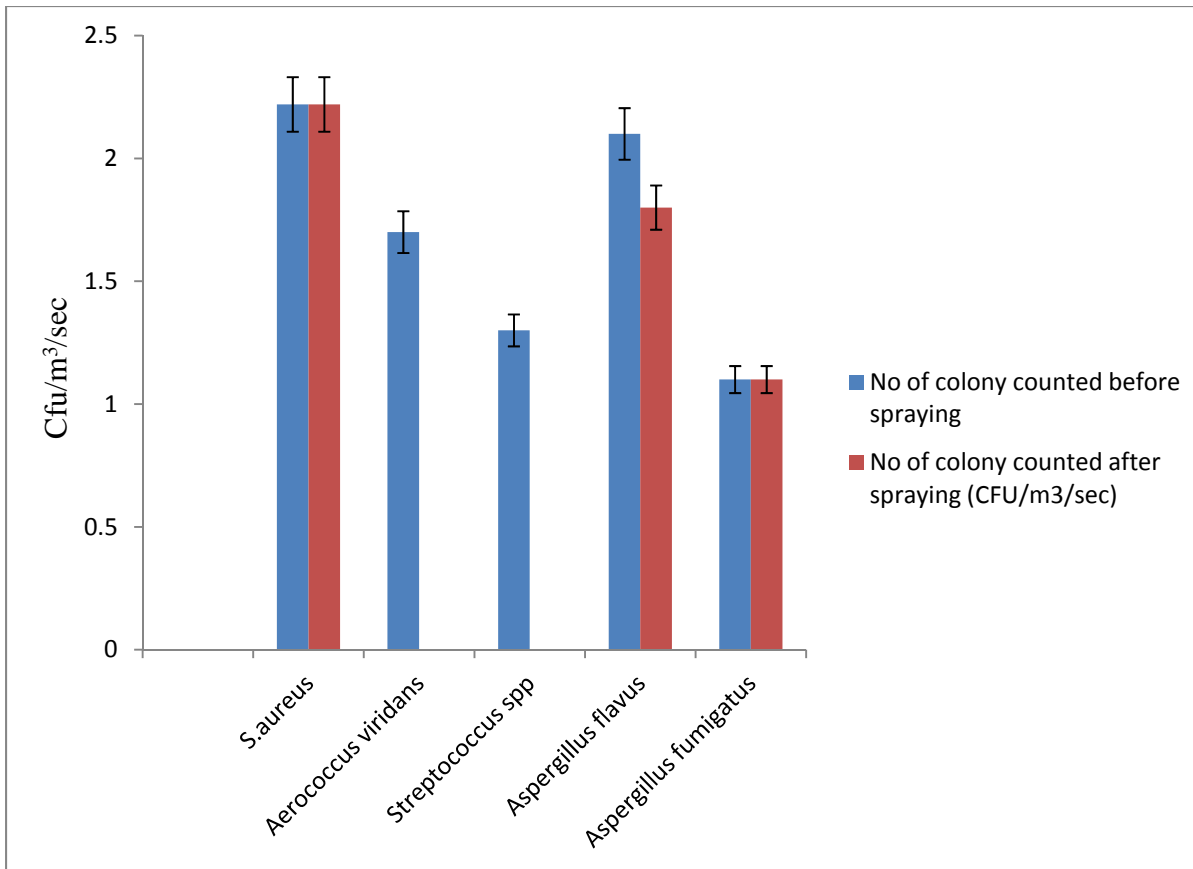


Figure 3: The mean values of colony counted from each room before and after spraying with Morten aerosol

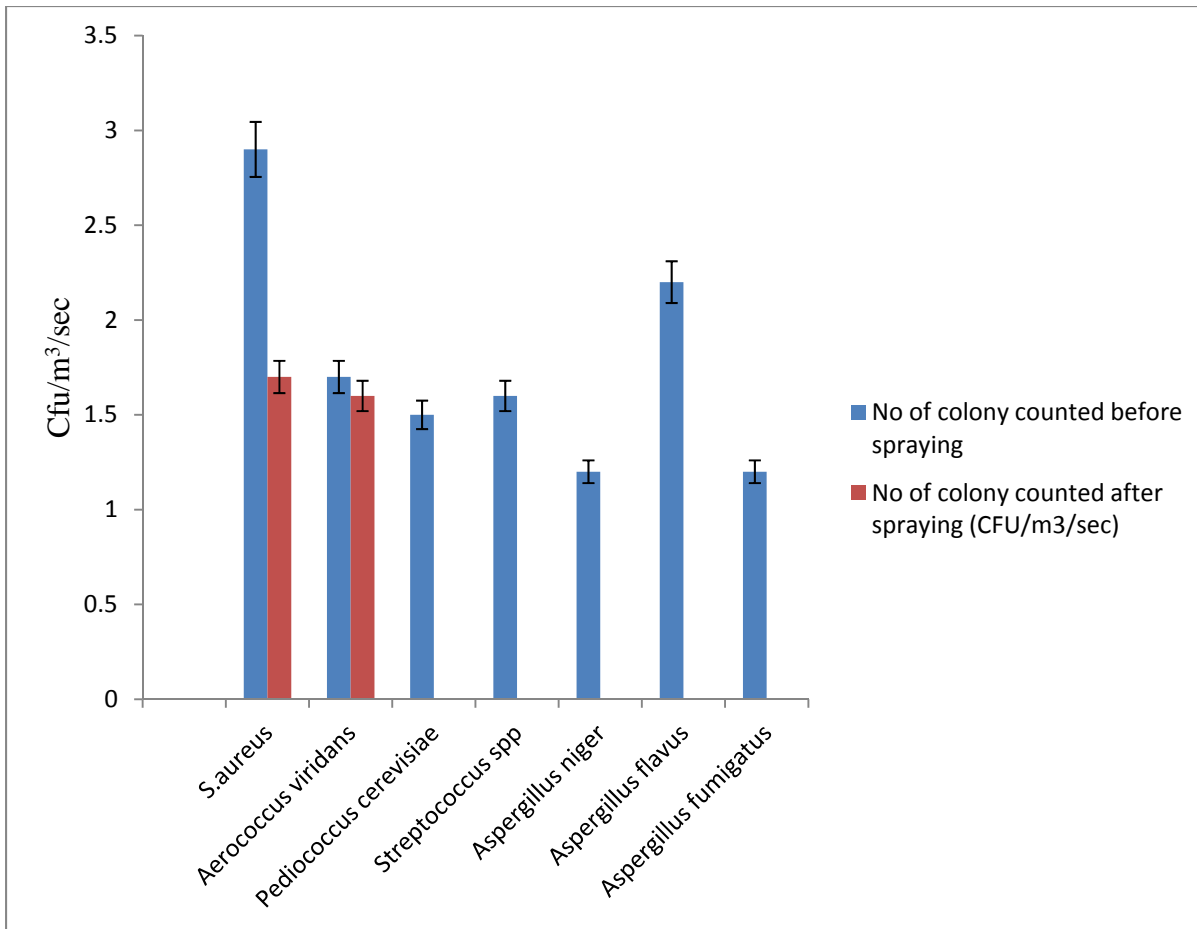


Figure 4: The mean values of colony counted from each room before and after spraying with Rambo aerosol

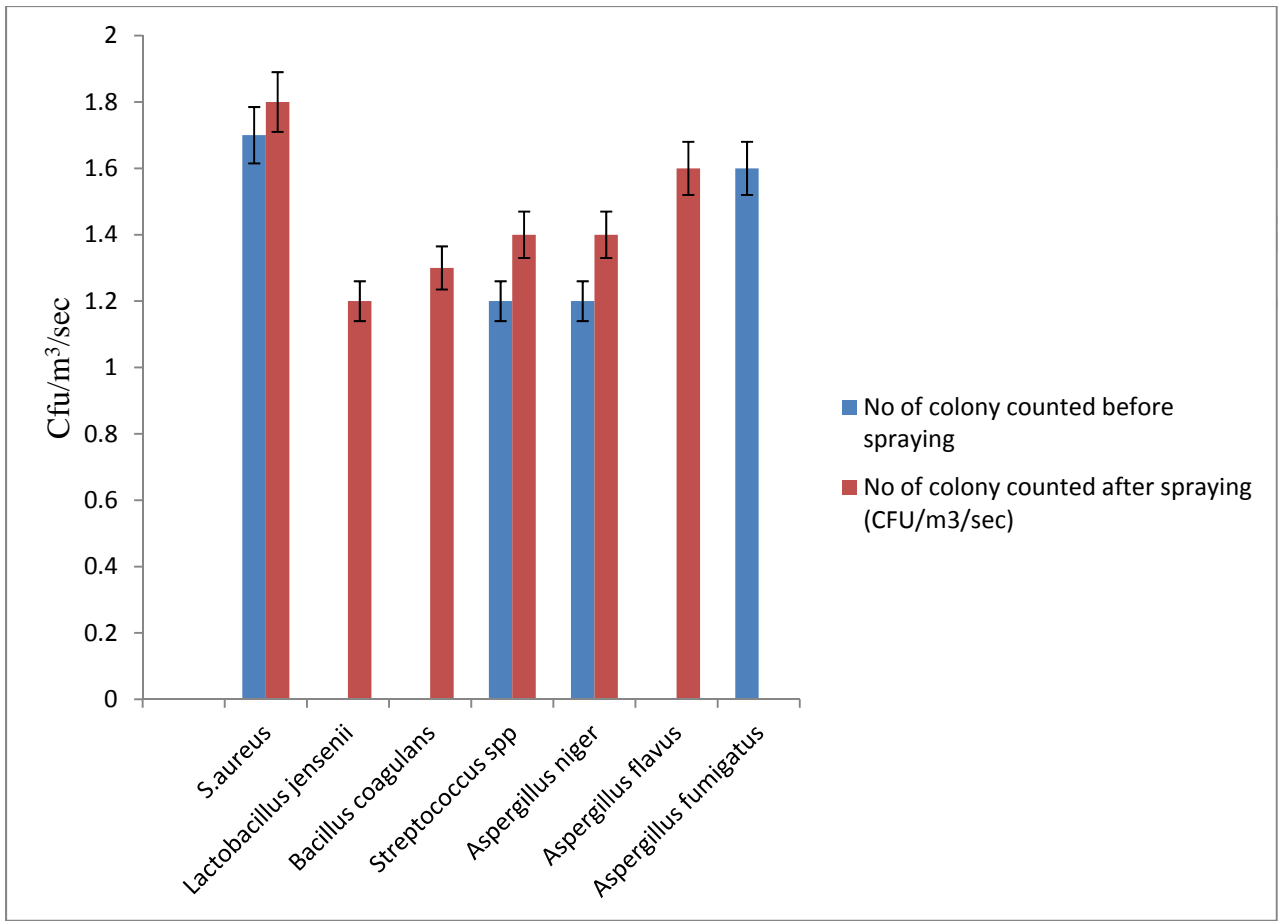


Figure 5: The mean values of colony counted from each room before and after spraying with Febreze aerosol

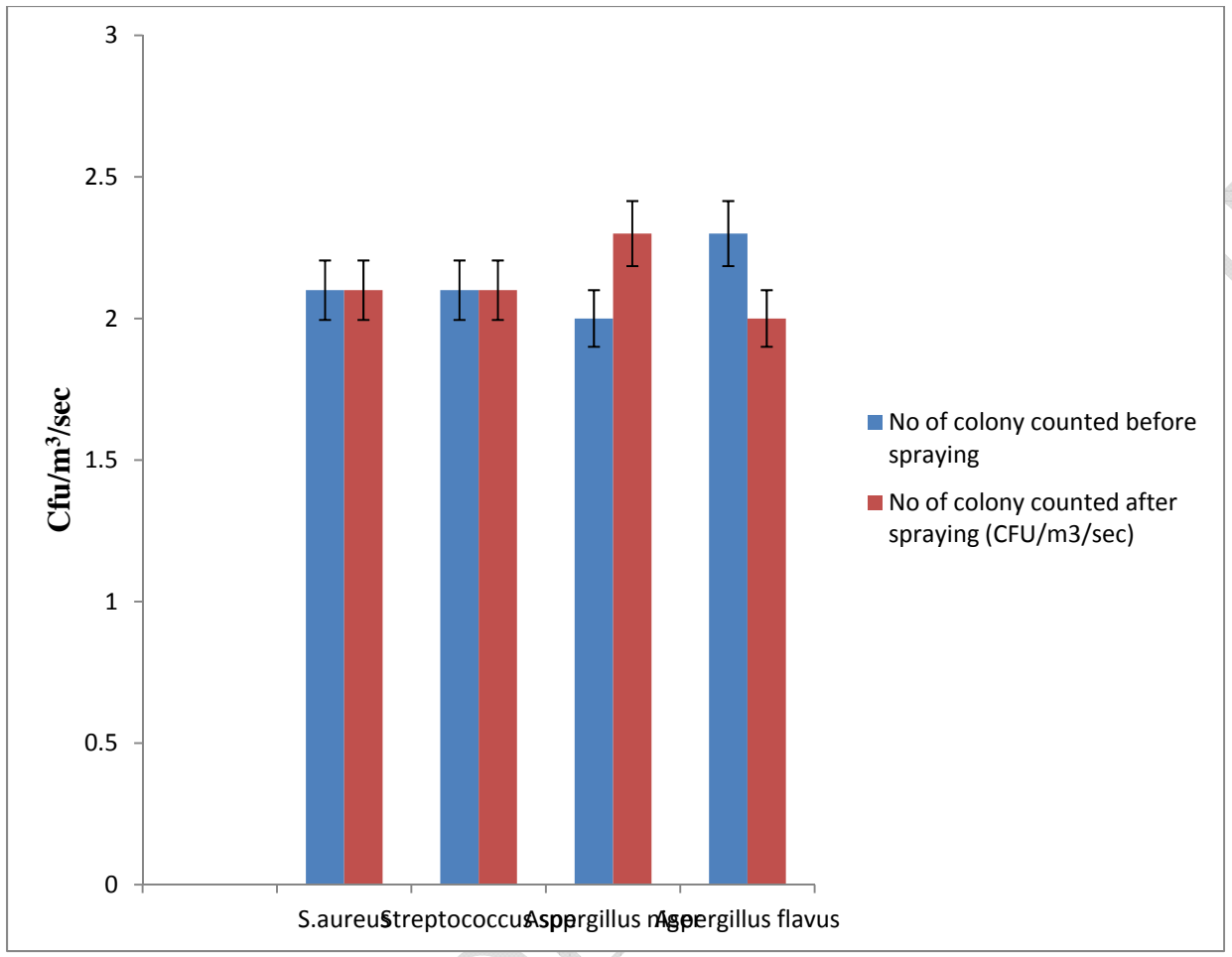


Figure 6: The mean values of colony counted from each room before and after spraying with Air wickaerosol

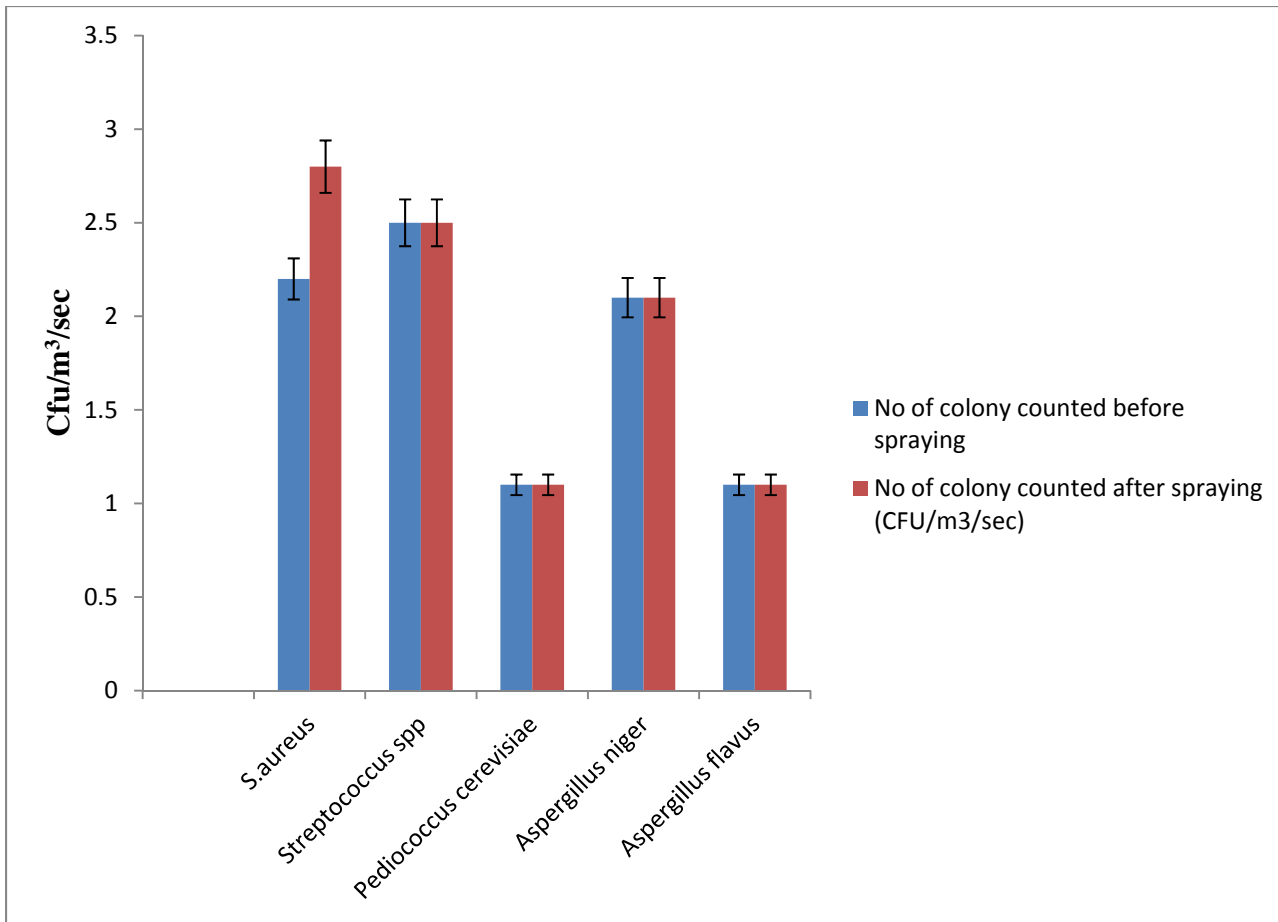


Figure 7: The mean values of colony counted from each room before and after spraying with Glade aerosol

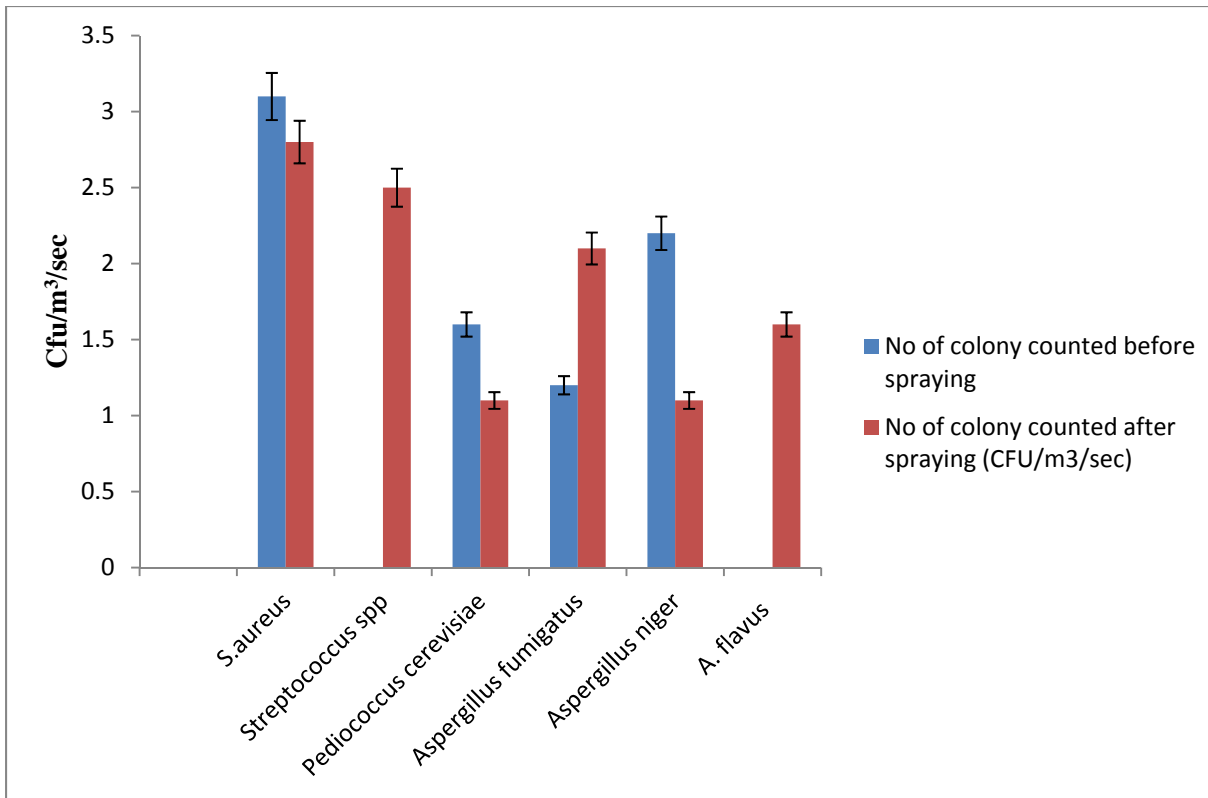


Figure 8: The mean values of colony counted from each room before and after spraying with Top breeze aerosol

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/glistening	Entire	Smooth	+ve	rod	Chains	-ve	-ve	-ve
2	Circular	Insoluble	Opaque	Raised	Dull	Tentate	friamble	+ve	rod	singly	Oval Spore	Central	+ve
3	filamentous	Insoluble	Opaque	Effuse	Smooth	Rhizoid	Friamble	+ve	cocci	Pairs/cluster	-ve	-ve	-ve
4	filamentous	Slightly soluble	translucent	raised	Dull	Rhizoid	friamble	+ve	cocci	Pair/tetrad	-ve	-ve	-ve
5	Circular	Slightly soluble	Opaque	Raised	Smooth/glistening	Entire	Smooth	+ve	cocci	cluster	-ve	-ve	-ve
6	Circular	Slightly soluble	Opaque	Raised	Smooth/glistening	Entire	smooth	+ve	cocci	tetrad	-ve	-ve	-ve
7	Circular	Insoluble	Opaque	Raised	Smooth	Entire	smooth	+ve	cocci	chains	-ve	-ve	-ve

Key:
1=

1= *Lactobacillus jensenii*, 2= *Bacillus coagulans*, 3= *Aerococcus Viridans*, 4= *Pediococcus cerevisiae*, 5= *Staphylococcus aureus*, 6= *Micrococcus spp*, 7= *Streptococcus spp*

+ve positive
-ve negative

Table 2: Morphological identification of the fungi isolates

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

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 <i>Jensen</i>
-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Bacillus coagulans</i>
-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Aerococcus viridans</i>
-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Pediococcus revisiae</i>
-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	<i>Staphylococcus aureus</i>
Tve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	ND	ND	ND	ND	ND	ND	ND	ND	<i>Streptococcus spp</i>
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	<i>Micrococcus spp</i>

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.

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	<i>Staphylococcus aureus</i> , <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i>	<i>Staphylococcus aureus</i> , and <i>Micrococcus</i> spp.	The Insecticide was able to inhibit the growth of <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i> , However, there was an introduction of a new organisms (<i>Micrococcus spp</i>) which was not present initially
B	Raid multipurpose insect killer	<i>Staphylococcus aureus</i> , <i>Aerococcusviridans</i> , <i>Pediococcuscerevisiae</i> . <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i>	Raid was able to inhibit all organisms presents initially except <i>Staphylococcus aureus</i>
C	Morten Insecticide	<i>Staphylococcus aureus</i> , , <i>Aerococcusviridans</i> , <i>Streptococcus spp</i>	<i>Staphylococcus aureus</i> , <i>Aerococcus viridans</i>	There was inhibition of <i>Streptococcus spp</i> only by Morten Insecticide
D	Rambo Insecticide	<i>Staphylococcus aureus</i> , <i>Aerococcusviridans</i> , <i>Pediococcuscerevisiae</i> . <i>Streptococcus spp</i>	<i>Staphylococcus aureus</i>	Rambo Insecticide was able to inhibit all the organisms present initially except <i>Staphylococcus aureus</i>
E	Febreze air freshener	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i>	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i> , <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i> ,	Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i>
F	Air wick	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i>	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i>	There was no difference between the type of organism present before and after spraying the room with Air wick.

G	Glade	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i> , <i>Pediococcus cerevisiae</i> .	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i> , <i>Pediococcus cerevisiae</i> .	There was no difference between the type of organism present before and after spraying the room with Glade
H	Top breeze	<i>Staphylococcus aureus</i> , <i>Pediococcus cerevisiae</i> .	<i>Staphylococcus aureus</i> , <i>Pediococcus cerevisiae</i> .	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	Mobile	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	<i>Aspergillus niger</i>	The Insecticide was able to inhibit the growth of <i>Aspergillus flavus</i> .
B	Raid multipurpose insect killer	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>		Raid was able to inhibit all organisms presents
C	Morten Insecticide	<i>Aerococcus viridan</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	<i>Aerococcus viridans</i> <i>Aspergillus fumigatus</i> and <i>Aspergillus flavus</i>	There was no inhibition of any microorganism
D	Rambo Insecticide	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	Rambo Insecticide was able to inhibit all the organisms present initially except <i>Aspergillus flavus</i>
E	Febreze air freshener	<i>Aspergillus fumigatus</i> and <i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	Febreze air freshener was not able to inhibit all the initial organisms present. There was an introduction of a new organisms which is <i>Aspergillus flavus</i> ,
F	Air wick	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	There was no difference between the type of organism present before and after spraying the room with Air wick.
G	Glade	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	There was no difference between the type of organism present before and after spraying the room with Glade
H	Top breeze	<i>Aspergillus fumigatus</i> , and <i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i> , <i>A. flavus</i> and <i>Aspergillus niger</i>	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 <i>A. flavus</i> after spraying

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Table 6: percentage (%) occurrence of bacteria isolates

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

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Table 7: Percentage occurrence (%) of fungi isolates

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

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