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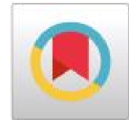
CORRELATION ANALYSIS BETWEEN INDOOR AIRBORNE BACTERIAL LOAD AND OCCUPANT DENSITY IN HOSPITAL INDOOR AIR



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Abstract:
A study of the quantity and types of airborne bacteria and its correlation with human presence was conducted. Air samples were collected from different units for three days and three sessions (morning, afternoon and evening) for the enumeration and identification of bacterial isolates. Walk-through exercise was also conducted prior to every sampling to gather information on the number of occupants present, activities going on, and room characteristics. Isolation study revealed higher bacterial load in the afternoon and evening sessions; with Male Ward and Operating Theatre recording the highest and lowest bacterial loads respectively, as compared to the morning session that was done immediately after cleaning and before influx of people. The Spearman's Correlation Coefficient showed a positively direct linear correlation between the bacterial load and occupant population irrespective of the three sessions (r = 0.84, 0.88 and 0.93). Identification study showed that the isolates are representatives of normal microflora of the skin, respiratory and gastro-intestinal tracts which includes the following; Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus roseus, Klebsiella pneumoniae, Proteus mirabilis, Bacillus subtilis, Aspergillus, Penicillium, Mucor, Candida and Fusarium species. The study presents evidence of increased concentration of indoor airborne bacteria due to human presence, movement and activities.

Keywords: Hospital Indoor Air; Correlation Analysis; Occupant Density; Bacteria; Mold; Spearman Coefficient.

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1. Introduction

Air represents a vehicle for movement of microbes from one habitat to another. The study of indoor microbiome is of critical importance since humans spend the majority of their time indoors and thus regularly encounter microbes in this habitat (THMP Consortium, 2012).

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The microbial loads in hospital indoor air are influenced by the number of occupants, their activities and the ventilation (Ayliffe, 1999). Occupants are a potential source of microorganisms or microbial vectors shaping the microbiome of indoor surfaces with which they come in contact with, as they shed microorganisms from the skin squames and the respiratory tract (Lax et al., 2014). Dressings and beddings also can act as the sources of airborne microorganisms. Sweeping of floors and changing of bed linens also can cause suspension of bioaerosols in air. Fungal spores gain entry into the hospital buildings through crevices in the walls and ventilation ducts with inadequate filtration.

Microorganisms are ubiquitous in the environment; not only do they cover virtually all surfaces we contact; they also cover our skin and are abundant in the air we breathe. More than 106 bacterial cells per dm3 are present in both outdoor and indoor air (Kembel et al., 2012). Sources of airborne bacteria in the environment are not well known but include humans, pets, soils and plants as direct sources and indirectly from dust perturbation (Bowers et al., 2012). Irrespective of the means by which they enter the air, they likely represent also an important component of the microbes to which we are exposed (Bhangar et al., 2015); given that humans inhale about 10-25m3 of air a day (Hinds, 1999). It would be expected that the contribution of various sources of bacteria to the composition and abundance of indoor air bacteria would be directly related to both their numbers in a given source habitat and the ease by which they enter the air. For example, the relative abundance of human associated microbes in indoor air increases with the number of residents (Hospodsky et al., 2012). The present study was therefore undertaken to evaluate the correlation between the bacterial load and human density in hospital indoor air setting.

2. Materials and Methods

2.1. Sample Collection and Procedure

A total of one hundred and sixty-two (162) air samples were collected from the nine units for three consecutive days using a BUCKBIO-CULTURE pump, which was coupled with six solid agar media plates (Nutrient agar, MacConkey agar, Mannitol salt agar, Eosin methylene-blue agar, Salmonella-Shigella agar, and Sabouraud dextrose agar). The machine actively sucked a flow rate of 240L per 2minutes at 4.8 pressure mm/H2O onto the solid culture media. Each day, the air samples were collected thrice: in the morning (8-9 am) after cleaning and disinfection and before influx of people, in the afternoon (1-2 pm) after influx of people and in the evening (4-6 pm). Walk-through exercise was conducted prior to every sampling to gather information on the number of occupants present (occupants includes; hospital personnel, patients and visitors), activities going on and the room conditions/characteristics. Upon collection, the culture media plates were immediately transported to the laboratory for incubation and microbiological analysis. The study sites were grouped as shown in Table 1:

Table 1: Grouping of Study Sites for the Three Days Sampling

Days	Sampling Sessions		
	MRN(8-9am)	AF(1-2pm)	EVE(46pm)
DAY 1	OT	CW	LU
	MW	NS	LR
	FW	OPD	A&E

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DAY 2	CW	LU	OT
	NS	LR	MW
	OPD	A&E	FW
DAY 3	LU	OT	CW
	LR	MW	NS
	A&E	FW	OPD

MW, Male Ward; FW, Female Ward; CW, Children's Ward; A&E, Accident and Emergency Ward; LR, Labour Room; LU, Laboratory Unit; OT, Operating Theatre; OPD, Out Patient Department and NS, Nurse's Station

2.2. Procedure

1) The 'ON' button was pressed to power the sampler.
2) The pump was calibrated using the specially made calibration head and an agar dish.
3) The head was unscrewed to remove the agar dish
4) For the sampling proper, the agar dish was placed firmly into the mouth of the bioculture pump and tightly screws on the sampling head.
5) The pump was turned on by pressing the 'ON' key.
6) Sampling was done by pressing one of the timing keys (1, 2, 5 or 10 minutes) and LED will light. The sampler will automatically run to the calibrated flow for the selected time.
7) At the end of sampling, the pump stops automatically. The 'COMPLETE' LED light up while the timer LED flashed to indicate which timing routine was selected
8) The head was unscrewed and the sampled agar dish removed.
9) The agar lid on the dish was replaced and secured with masking tape and labeled on the bottom of the dish.
10) The ager dish was taken immediately to the laboratory for incubation and microbiological analysis.

2.3. Microbiological Analysis

The bacterial culture plates were incubated at 37oC for 24-48 hours while the fungal culture plates were incubated at room temperature (28±02oC) for 3-7 days. The total colony forming units per cubic metre (cfu/m3) for the bacterial and fungal isolates were enumerated using the conversion table or formular devised by Feller (1950):

Pr= N[1/N + 1/N-1 + 1/N-2+....+1/N-r+1]

Where: N= 400 (number of holes in perforated lid of the sampler), r- Number of CFU Counted on Petri Dish, Pr - Statistically Corrected Total Count of Bacteria in TestedAir Volume.

2.4. Purification and Identification of Isolates

Different isolates from the primary plates were aseptically sub-cultured by streaking onto prepared nutrient agar plates. Plates were incubated at 37±20C for 24 hours. These gave pure culture of isolated organism. The pure culture of the isolates were streaked on prepared sterile set agar slant in stock bottles and kept in the refrigerator at 40C to 60C for further test and identification.

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Isolates were characterized using features such as size, edge, shape, elevation, colour, surface, consistency e.t.c. Microscopically, the bacterial isolates were identified using Gram's staining and biochemical reactions while the fungal isolates were identified using wet-mount preparation and fungi atlas.

2.5. Antibacterial Susceptibility Test

This was done by adopting the Kirby-Bauer disc diffusion method in accordance with McFarland standard prepared according to the National Committee for Clinical Laboratory Standards (NCCLS, 1998). Isolates were inoculated onto a nutrient broth medium and incubated at 37°C for 18-24hrs to obtain an equivalent of 0.5 McFarland turbidity standards. A sterile, non-toxic cotton swab was dipped into the standardized inocula and used to spread the entire surface of Mueller Hinton agar plates. Antibiotic discs (Oxoid) were placed aseptically on the surface of the agar plates using an antibiotics-disc dispensing machine and thereafter incubated at 370C for 24hours. The antibiotics screened include the following: Ciprofloxacin (5µg), Oxacillin (1µg), Meticillin (5µg), Streptomicin (10µg), Erythromycin (15µg), Ceftazidime (30µg), Vancomicin (30µg), Tetracyclin (30µg), Cefotaxime (30µg) and Gentamycin (120µg). Zones of inhibition produced after the incubated periods were measured and recorded to the nearest millimeters using transparent calibrated ruler (Cheesbrough, 2010).

2.6. Statistical Analysis

The data thus generated were analyzed using Microsoft Excel and Spearman's Rank Correlation Coefficient (r) to measure the strength of the relationship between the bacterial population and human population (Edwards, 1976). The formular is given as:

r=1- (6∈d2)/(n(n2-1))

Where: d=difference between corresponding ranks, n=number of samples.

3. Results and Discussions

3.1. Results

3.1.1. Microbial Load in Study Sites

The total heterotrophic microbial population in Cfu/m^3 from the different media used during the sampling is shown in Table 2. Higher microbial population was recorded in the afternoon and evening sessions between 12pm and 6pm in most of the wards as compared to the morning sessions that were low. Among the six media used, growth was observed on only four while Eosin Methylene Blue and Salmonella- Shigella agar showed no growth.

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Table 2: Microbial Counts in Cfu/M^3 from the Different Media Used During the Sampling (For 3 days)

SAMPLE SITES	THBC			TCC			TSC			THFC			TSSC& TCEMB
	MORN	AFTER	EVEN	MORN	AFTER	EVEN	MORN	AFTER	EVEN	MORN	AFTER	EVEN	NG
OT	26	29	24	14	14	12	6	10	7	9	23	12	
A&E	238	273	204	3	14	15	32	24	30	17	22	18	
LR	32	44	30	14	27	19	11	7	13	15	17	23	
MW	195	249	291	28	28	25	9	31	38	20	36	31	
FW	174	269	283	18	18	16	2	36	44	12	29	31	
CW	194	207	185	23	3	33	3	14	22	7	15	18	
NS	80	249	37	14	17	11	16	22	22	23	6	9	
OPD	230	292	76	10	15	10	17	30	19	19	28	15	
LU	163	294	86	16	22	7	8	33	21	21	17	12	

KEY: THBC=Total Heterotrophic Bacteria Count, TCC= Total Coliform Count, TSC= Total Staphylococcal Count, TSSC=Total Salmonella-Shigella Count, TCEMB=Total Count on Eosin Methylene-Blue Agar,THFC= Total Heterotrophic Fungal Count, OT= Operating Theatre, A&E= Accident and Emergency Ward, LR= Labour Room, MW= Male Ward, FW= Female Ward, CW= Children Ward, NS= Nurses Station, OPD= Out- Patients Department, LU= Laboratory Unit, NG=No Growth, Morn=morning, After=Afternoon, Even= evening.

3.1.2. Rank Correlation Coefficient of Bacterial and Human Population in Study Sites

The rank Correlation Coefficient table of the bacteria and human population with their room conditions are presented on Tables 3-8. The Spearman’s Correlation Coefficient for the three sessions showed positive direct linear correlations respectively (r=0.84, 0.88 and 0.93) with the following percentages 71%, 77% and 86%. More positive correlation was recorded in the afternoon and evening sessions than in the morning session.

Table 3: Rank Correlation Coefficient Table of Bacterial and Human Population During the Morning Sampling Session

STUDY SITES	X(BP)	Y(HP)	RANK X	RANK Y	D	D^2
OT	26	4	9	8	1	1
MW	224	48	1	1	0	0
FW	146	42	4	2	2	4
CW	169	35	3	4	-1	1
NS	72	9	7	7	0	0
OPD	133	37	5	3	2	4
LU	98	12	6	6	0	0
LR	32	3	8	9	-1	1
A&E	173	33	2	5	-3	9
TOTAL						19

r=1-(6∈d2)/(n(n2-1))
n=9, εd^2=19

$$r = 1- (6(19))/(9(81-1)) = 1- 114/(720) = 0.84$$

% correlation = r^2 x 100 = $(0.84)^2$ x 100 = 71%

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Table 4: Rank Correlation Coefficient Table of Bacterial and Human Population During the Afternoon Sampling Session

STUDY SITES	X(BP)	Y(HP)	RANK X	RANK Y	D	D^2
CW	250	37	4	4	0	0
NS	205	17	6	6	0	0
OPD	266	36	2	5	-3	9
LU	152	11	7	7	0	0
LR	42	3	8	8	0	0
A&E	257	46	3	2	1	1
OT	28	2	9	9	0	0
MW	287	58	1	1	0	0
FW	242	39	5	3	2	4
TOTAL						14

r=1-(6∈d2)/(n(n2-1))
n=9, εd^2=14
r = 1-(6(14))/(9(81-1)) = 1-84/(720) = 0.88
% correlation = r^2 x 100 = $(0.88)^2$ x 100 = 77%

Table 5: Rank Correlation Coefficient Table of Bacterial and Human Population During the Evening Sampling Session

STUDY SITES	X(BP)	Y(HP)	RANK X	RANK Y	D	D^2
LU	34	5	7	7	0	0
LR	29	4	8	8	0	0
A&E	276	40	2	4	-2	4
OT	23	3	9	9	0	0
MW	296	62	1	1	0	0
FW	260	48	4	2	2	4
CW	273	43	3	3	0	0
NS	35	10	6	6	0	0
OPD	66	14	5	5	0	0
TOTAL						8

r=1-(6∈d2)/(n(n2-1))
n=9, εd^2=8
r = 1-(6(8))/(9(81-1)) = 1-48/(720) = 0.93
% correlation = r^2 x 100 = $(0.93)^2$ x 100 = 86%

KEY: OT= Operating Theatre, A&E= Accident and Emergency Ward, LR= Labour Room, MW= Male Ward, FW= Female Ward, CW= Children Ward, NS= NursesStation, OPD= Out- Patients Department, LU= Laboratory Unit D=difference between corresponding ranks, BP= Bacterial population, HP= Human population.

Table 6: Room Condition During Morning Sampling

Room condition	**Study sites**								
	OT	MW	FW	CW	NS	OPD	LU	LR	A&E
Fan on	N	N	Y	N	Y	Y	N	Y	Y
AC on	Y	N	N	N	N	N	N	N	N

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Windows open	N	N	Y	Y	N	Y	N	N	N
Activity on	N	Y	Y	Y	Y	Y	Y	N	Y
Water intrusion	N	N	N	N	N	N	N	N	N
Floor neat	Y	Y	Y	Y	Y	Y	Y	Y	Y
Bacterial population	26	224	146	169	72	133	98	32	173
Human population	4	48	42	35	9	37	12	3	33

Table 7: Room Condition During Afternoon Sampling

Room condition	**Study sites**								
	CW	NS	OPD	LU	LR	A&E	OT	MW	FW
Fan on	Y	N	Y	N	Y	Y	N	Y	Y
AC on	N	Y	N	N	Y	N	Y	N	N
Windows open	Y	N	Y	N	N	Y	N	N	Y
Activity on	Y	Y	Y	Y	N	Y	N	Y	Y
Water intrusion	N	N	N	N	N	N	N	N	N
Floor neat	Y	Y	N	Y	Y	Y	Y	N	Y
Bacterial population	250	205	266	152	42	257	28	287	242
Human population	37	17	36	11	3	46	2	58	39

Table 8: Room Condition During Evening Sampling

Room condition	**Study sites**								
	LU	LR	A&E	OT	MW	FW	CW	NS	OPD
Fan on	Y	Y	Y	N	Y	Y	Y	Y	Y
AC on	N	Y	N	Y	N	N	N	Y	N
Windows open	Y	N	Y	N	N	Y	Y	N	Y
Activity on	N	N	Y	N	Y	Y	Y	Y	Y
Water intrusion	N	N	N	N	N	N	N	N	N
Floor neat	Y	Y	Y	Y	Y	Y	Y	Y	N
Bacterial population	34	29	276	23	296	260	273	35	66
Human population	5	4	40	3	62	48	43	10	14

KEY: N=no, Y= yes, OT= Operating Theatre, A&E= Accident and Emergency Ward, LR= Labour Room, MW= Male Ward, FW= Female Ward, CW= Children Ward, NS= NursesStation, OPD= Out- Patients Department, LU= Laboratory Unit.

3.1.3. Mean of Bacterial Count and Occupant Population

Figure1. Shows the mean of the bacterial load and human population from the study sites. The total bacterial load and human population of the nine units varied during the sampling sessions with the male ward and operating theatre recording the highest and lowest bacteria and occupant population respectively. The bacterial population increased with increase in human population. The mean bacterial load of the male ward and operating theatre were 269.0 Cfu/m3 and 26.0 Cfu/m3 with 52 and 3 human occupants respectively.

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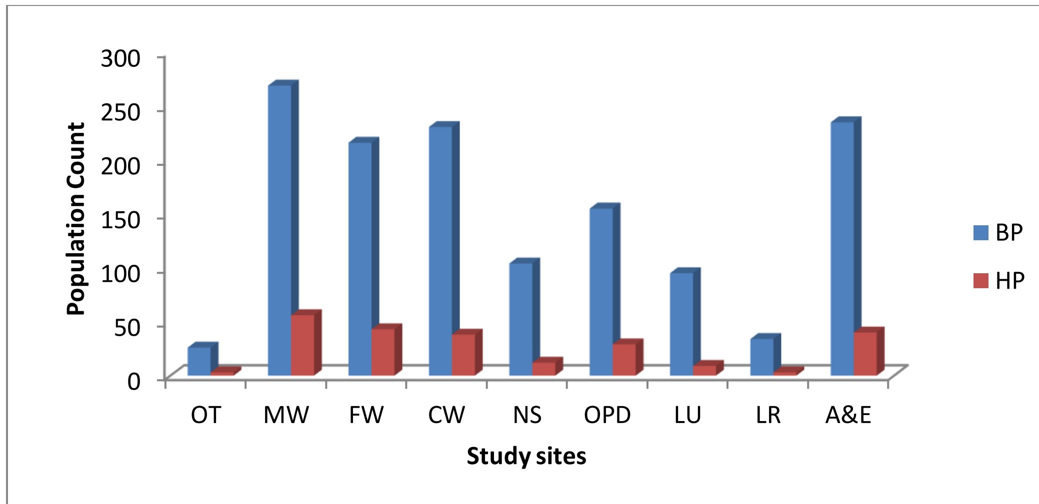

Figure 1: Mean of Bacterial and Human population in study areas

KEY: OT= Operating Theatre, A&E= Accident and Emergency Ward, LR= Labour Room, MW= Male Ward, FW= Female Ward, CW= Children Ward, NS= Nurses Station, OPD= Out- Patients Department, LU= Laboratory Unit, BP= Bacterial population, HP= Human population.

3.1.4. Distribution and Prevalence of Microorganisms from Study Site

The frequency of occurrence of isolates from study sites is represented on Figure 2. Six (6) airborne bacterial and five (5) fungal isolates were consistently isolated and characterized from study units. The microbial isolates include; Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Micrococcus roseus, Proteus mirabilis, Klebsiella pneumoniae, Aspergillus, Penicillium, Fusarium, Candida and Mucor species. Staphylococcus aureus and Aspergillus species were predominantly isolated at 100% prevalence, being isolated in all the nine wards/units of the hospital.

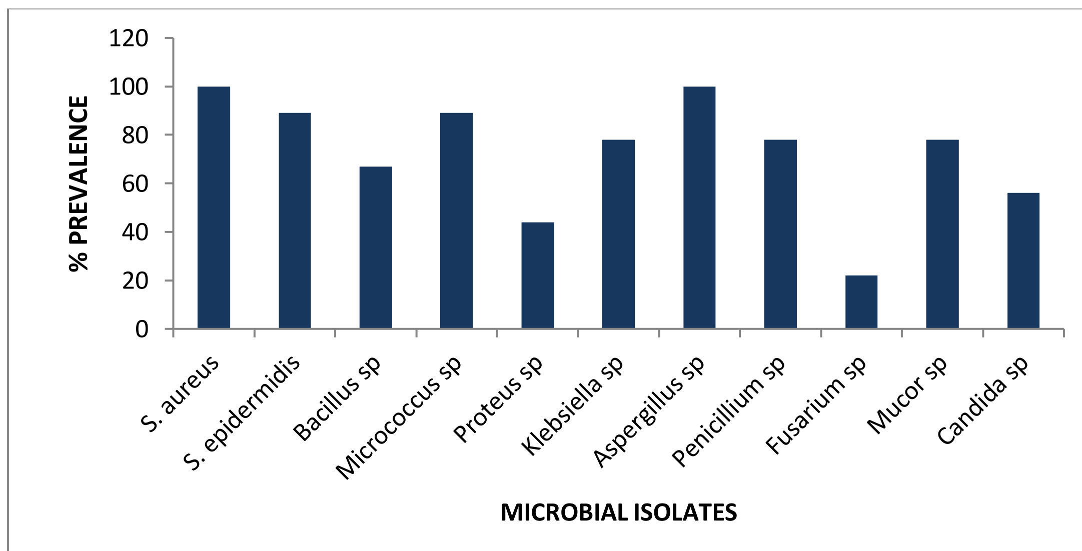

Figure 2: Percentage Prevalence of Microbial Isolates from Study Sites

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4. Discussion

Healthcare facilities are complex settings, especially in developing countries, where factors such as overcrowding, improper design and ventilation can impact the growth and/or survival of microorganisms. Indoor air quality in hospital is a concern due to presence of airborne microorganisms that may cause nosocomial infections (Beggs, 2003). Airborne particles are a major cause of respiratory ailments of humans causing allergies, asthma and pathogenic infections of the respiratory tracts.

The bacterial isolates from this study include; *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Micrococcus roseus, Proteus mirabilis and Klebsiella pneumoniae.* Some of these clinical isolates have been reported by earlier researchers (Lateef, 2003). *Staphylococcus aureus* as the most frequently isolated bacterium from the study has been incriminated from various diseases such post operative infections, urinary tract infections, skin infections, respiratory infections, pneumonia and food poisoning (Jaffal *et al*., 1997).

The high prevalence of *Staphylococcus* species may be attributed to the presence of increased number of occupants beyond room capacity as *Staphylococcus* species are constantly shed from skin, clothes and hair of humans, improper ventilation and lower susceptibility of the organisms to the environmental stress (Borriello *et al.,* 2005).

Bacteria in indoor air are mainly gram positive cocci which arise from occupants. Generally, they are not dangerous for human health but high viable counts are used as markers of crowded conditions and poor ventilation (Nevalainen, 1989). The highest and lowest mean microbial concentration was observed in Male ward and Operating theatre respectively. At the time of this study, the male ward was at its maximum capacity with patients (i.e. overcrowded), as well as presence of some non sterile devices such as personal belongings; this invariably will attract more patient relatives/visitors in and out of the ward thereby increasing the shedding of microbes and agitation of air. Jabbari *et al*, 2009 reported that overcrowding of patients and their activities might be the main cause of microbial contamination in indoor air. In addition, there was no cross ventilation as most of the windows were closed during the time of sampling. The structural design, high sanitary standards, air purification systems such UV light application and the restriction of movement in and out of the Operating theatre could be responsible for the low microbial burden observed. However, in a similar study by Ekhaise *et al.* 2010, Operating theatre and Nurses station recorded the highest microbial population; this is at variance with this study in which Operating theatre and Nurses station were the least burdened units of the hospital.

The high microbial loads recorded in the afternoon and evening sessions could be as a result of the activities going on (as was working hours) as well as the hospital's visitation time which is from 4-6pm, where there are much influx of people into the wards as compared to the morning session that was done immediately after disinfection and cleaning where there were no influx of people yet. This agrees with the study carried out by Choobineh *et al.,* (2009), that human presence, unsuitable disinfection as well as ventilation defects were some of the causes of high bioaerosol density of hospital wards.

Information on the characteristics of the wards are the time of sampling which include: number of people present, activities going on, time of sampling, ventilation etc could influence the microbial load as the mode of ventilation and conditions of a building would influence the composition of

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airborne bacteria communities indoors. The results obtained are consistent with a recent study by Meadow *et al.* (2014) that showed the importance of the ventilation strategy in shaping the microbial community inside a university office building especially in rooms that were naturally ventilated overnight.

The correlation model gives an insight on the measure of the strength of the relationship between the microbial load and human population. From the study, there is a perfect direct linear relationship between the two. Hence, the occupants could be a source of microbial load. A positive correlation between bacterial counts and occupant density in different hospitals was suggested by Warner and Glassco (1963), Kaur and Hans (2007). Previous research has indicated that human occupancy increases the airborne bacterial load (Hospodky *et al.*, 2012).During a sneeze, millions of tiny droplets of water and mucus are expelled at about 100metres per second. Some patients' activities such as talking, walking in wards, sneezing and coughing can cause an increase in emission or shedding of microbes in hospital wards (Marcelou, 1977). This result also confirms with report of Okhuaya and Okaraedge (1992) that human population and activities such as talking, walking, laughing, sweeping all contribute to microbial load in hospital.

5. Conclusions & Recommendations

Indoor air harbours a diverse collection of bacteria originating from both indoor and outdoor sources. While humans themselves and their activities can be substantial sources of bacteria in hospital indoor air, their contribution is influenced by the number and activities of the people present. Measures such as monitoring and regulating the number of patients/staff/visitors per ward and ensuring adequate ventilation should be practiced to avoid air pollution and to keep microorganisms at bay.

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