Measurement of air contamination in different wards of public sector hospital, Sukkur

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Abstract: The aim of this study was to evaluate and assess the index of bacterial contamination in different wards of the Public Sector Hospital of Sukkur (Teaching) Pakistan; whether or not the air contamination was statistically different from the acceptable level using active and passive sampling. In addition to this main hypothesis, other investigations included: occurrence of the most common bacteria, whether or not the bacterial contamination in the wards was a persistent problem and identification of the effective antibiotics against the identified bacteria. The evidence sought based on the One Sample T test suggests that there is a (statistically) significant difference between the observed (higher) than the acceptance level ($p<0.01$), the result based on One-Way ANOVA suggests that the contamination problem was persistent as there was no significant difference among observed contamination of all three visits at ($p>0.01$) and the result of antibiotic susceptibility test highlights sensitivity and resistance level of antibiotics for the identified bacteria.

Keywords: Airborne bacteria, air contamination, antibiotic sensitivity, active sampling, passive sampling.

INTRODUCTION

Predominant evidence suggests that the airborne microflora of indoor environments remain major causes of hospital infections (Setlhare G *et al*., 2014; Gould, 1970; Herman, 1980; Kelsen and McGuckin, 1980; Rainer *et al.,* 2001; Li and Hou, 2003). Many infections are spread by the direct contact, because healthcare workers do not wash their hands effectively before attending the patients (Ayliffe *et al*., 1988). It has been estimated that the airborne route of transmission accounts for between 10 to 20% of endemic nosocomial infections (Brachman, 1970). Most airborne microorganisms found in hospitals are disseminated within the building by the staff, patients and visitors. Only a minority of the microorganisms in the air, usually fungal spores, infiltrate from outside. Generally, the higher the occupancy level the greater the microbial burden in the air. Consequently, the air bioburden within hospitals tends to be very transient and can fluctuate widely, depending on occupancy levels and the tasks being performed (Meadow, *et al.,* 2014). Activities, such as bed making, release huge number of microorganisms into the atmosphere. According to study the total viable count in a patient room exceeded 6×10^3 colony forming units per cubic meter of air during vigorous bed making (Greene *et al*., 1960). Bacterial spores can remain viable for years and are very resistant to environmental stresses such as heat, cold and UV radiation (Nevalainen *et al*., 1993). Many species appear to lower their metabolic rate and reduce in size under conditions of nutrient starvation (Roszak and Colwell, 1987) and has also been observed by a number of investigators (Butkevich, 1938; Greene *et al*., 1960; Novitsky and Morita, 1977). The predominant mechanism that makes the pathogens airborne is the production of aerosol droplets by sneezing or coughing, and their subsequent loss of water, which allows them to float in the air over considerable distances and for a long time (Emmerson, 1995). Blessing-Moore *et al*. (1979) recovered *Ps. aeruginosa* from settle plates located near patients with cystic fibrosis and airborne *Pseudomonas* spp. were linked with an outbreak of nosocomial bacteraemia at a hospital in the USA (Grieble *et al*., 1974).

Nosocomial infections are a major threat to health for both developed and developing countries of the world. Although many researchers have reported epidemiological investigations to elucidate the source and transmission mechanism of healthcare-associated infections (HAI) over the past thirty years, no clear conclusion has been presented (Gaynes *et al*., 1996; Jarvis, 1996; Li and Hou, 2003). Most research journals are published from developed countries and conclusions on the basis of foreign observations are not appropriate here, as Sindh Province of Pakistan has a different climate, topography and different infrastructure of Public Sector Hospitals.

The objectives of this study are to evaluate and assess the index of bacterial contamination in different wards of the Public Sector Hospital of Sukkur (Teaching), Pakistan, and to investigate if the air contamination in the wards is statistically different (higher) than the acceptable level. Hence the null and alternative hypotheses are:
$H_{0:}$ "The observed air contamination in different wards of the public Sector Hospital of Sukkur is not significantly different from the acceptable level".

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H_1: "The observed air contamination in different wards of the Public Sector Hospital of Sukkur is significantly different (higher) from the acceptable level".

MATERIAL AND METHODS

An observation study on indoor air bacterial contamination in various wards of Public Sector Hospital (Teaching Hospital) Sukkur, Sindh Pakistan was carried by during May 2008 to July 2008. Air sampling was performed by both Passive (Pasquarella *et al.*, 2000) and Active methods (Oxoid manual). Three sets of triplicate sampling were carried out on one visit. Similarly, three visits (three samplings) were made and the interval between each visit was fifteen days. Samplings were carried out from various wards of hospital during May 2008 to July 2008.

Passive sampling

This was carried out by the 1/1/1 scheme (Fisher *et al.*, 1972). In this method, the nutrient agar (Oxoid) Petri plates (Sanaka) are kept with the lids open one meter above from floor, one meter away from walls, for one hour. Three sets of Petri plates were exposed to different wards on one visit. Similar visits were made to the hospital after 15 days. Plates were incubated for 24 hours at 37^0C.

Active sampling

A MAQS-90 (Oxoid) air sampler was used to collect air samples. The nutrient agar petri plates were fixed in the air sampler as per the manufacturer's instructions. The air flow rate was fixed at 1.5 L/S where the total volume of air sampled was 600 liters. After sampling, the plates were incubated at 37^oC for 24 hours.

The colony count was determined with an "Acolyte" (Fisher, UK) digital counter and the bacteria were identified by standard methods after Gram staining and recording colonial characteristics, according to Bergey's Manual of Determinative Bacteriology (1974) and the API system (Biomeriuex). The antibiotic sensitivity was performed using the standard Kirby-Bauer method (Cheesbrough, 2002) and various commercially available antibiotic disks (Oxoid) such as ciprofloxacin, amoxicillin-clavulanic acid, cephradine, levofloxacin, moxifloxacin, cafaclor, ofloxacin, clarithromicin were included in this study.

Data analysis

The observed (contamination) data was analyzed using One Sample T test. In order to conclude whether or not the observed contamination is (statistically) significantly different from the suggested acceptable level, the suggested acceptable value for each ward was defined in the one-Sample T test as a test value. To investigate if the contamination was a persistent problem in the wards, One-Way ANOVA was used to compare observed contamination on each visit (i.e. visit1 visti2 visit3). Finally the antibiotic sensitivity tests were performed by the standard Kirby-Bauer method to assess sensitivity and resistance of the antibiotics against the identified bacteria.

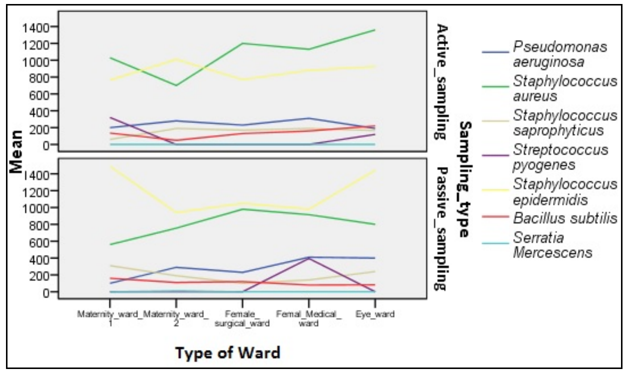

Fig. 1: All organisms: ward wise active passive sampling

RESULTS

Count: Active sampling of different wards

The mean CFU/m^3 of eye ward exhibited highest colony forming units (993 CFU/m^3 ± 25), followed by female medical ward (913 CFU/m^3 ± 37), Maternity ward 1 (836 CFU/m^3 ± 26), female surgical ward (833 CFU/m^3 ± 29) and maternity ward 2 (743 CFU/m^3 ± 26) respectively. The count, mean, standard deviations are given in table 1. The result of one way ANOVA for all wards suggests that on each visit the level of contamination was persistent as the observed contamination on each visit was not significantly different from other visits the result was consistent for all wards at ($p>0.01$) detailed result is shown in table 5.

Further the observed contamination in the wards was assessed against the accepted level using one sample T test. The table 2 shows that result of one sample T test: Maternity ward 1, t (8) =60.81, $p<0.01$; Maternity ward 2 t (8) =51.05, $p<0.01$; female surgical ward, t (8) = 53.46, $p<0.01$; female medical ward, t (8) =48.70, $p< 0.01$ and Eye ward, t (8) =60.81, $p<0.01$. Based on the t values and the significance level it can be concluded that the contamination in the wards is (statistically) significantly higher than the acceptable level. Therefore the null hypothesis is rejected.

The mean scores along with standard deviation of different bacteria observed in this study stood as follows: *Staphylococcus aureus* (1084±246), *Staphylococcus epidermidis* (869±104), *Streptococcus pyogenes* (88±139), *Pseudomonas aeruginosa* (242±59), *Bacillus subtilis* (139±61) and *Staphylococcus saprophyticus* (155±54). The overall ward wise and the patterns of all bacteria are shown in fig. 1.

Table 1: Mean and standard deviation (Active Sampling)

Wards	N	Mean	Std. Deviation	Std. Error Mean
Active Sampling				
Maternity Ward 1	9	836.33	26.458	8.819
Maternity Ward 2	9	743.33	26.053	8.684
Female Surgical Ward	9	833.33	29.925	9.975
Female Medical ward	9	913.33	37.782	12.594
Eye Ward	9	993.33	25.495	8.498

Table 2: One Sample T test Significance and the acceptable levels (Active Sampling)

Wards	t-value	df	Sig. (2-tailed)	Mean difference	Test value/ Acceptance level	95% Confidence Interval	
						Lower	Upper
Active Sampling							
Maternity Ward 1	60.814	8	.000	536.333	300 CFU/m^3	516.00	556.67
Maternity Ward 2	51.050	8	.000	443.333	300 CFU/m^3	423.31	463.36
Female Surgical Ward	53.467	8	.000	533.333	300 CFU/m^3	510.33	556.34
Female Medical ward	48.700	8	.000	613.333	300 CFU/m^3	584.29	642.38
Eye Ward	81.584	8	.000	693.333	300 CFU/m^3	673.74	712.93

Table 3: Mean and standard deviation (Passive Sampling)

Wards	N	Mean	Std. Deviation	Std. Error Mean
Passive Sampling				
Maternity Ward 1	9	870.11	15.878	5.293
Maternity Ward 2	9	765.00	19.242	6.414
Female Surgical Ward	9	826.67	20.261	6.754
Female Medical ward	9	973.33	46.869	15.623
Eye Ward	9	988.00	20.724	6.908

Table 4: One Sample T test Significance and the acceptable levels (Passive Sampling)

Wards	t-value	df	Sig. (2-tailed)	Mean difference	Test value/ Acceptance level	95% Confidence Interval of the Difference	
						Lower	Upper
Passive Sampling							
Maternity Ward 1	130.39	8	.000	690.11	180 CFU/m^3	677.91	702.32
Maternity Ward 2	91.20	8	.000	585.00	180 CFU/m^3	570.21	599.79
Female Surgical Ward	55.77	8	.000	376.66	450 CFU/m^3	361.09	392.24
Female Medical ward	14.29	8	.000	223.33	750 CFU/m^3	187.31	259.36
Eye Ward	116.96	8	.000	808.00	180 CFU/m^3	792.07	823.93

Table 5: One way ANOVA Active & passive sampling visits

Wards	Active Sampling		Passive Sampling	
	F	Sig	F	Sig
Maternity Ward 1	.149	.865; p > .01	3.342	.106; p > .01
Maternity Ward 2	2.42	.169; p > .01	1.727	.256; p > .01
Female Surgical Ward	.612	.573; p > .01	.585	.586; p > .01
Female Medical ward	.833	.479; p > .01	.320	.738; p > .01
Eye Ward	.554	.601; p > .01	1.401	.317; p > .01

Table 6: Antibiotic Sensitivity and Resistance offered *by Streptococcus pyogenes*:

Name of antibiotic	Potency	Standard zone of inhibition (mm)		Mean	Remarks
		Sensitive	Resistant		
Ciprofloxacin	05ug	>21	<15	30	S
Amoxicillin-claulanic acid	03ug	>18	<13	32	S
Cephradine	30ug	>14	<12	8	R
Levfloxacin	05ug	>17	<13	40	S
Moxifloxacin	05ug	>18	<13	32	S
Cafaclor	30ug	>18	<13	28	S
Ofloxacin	05ug	>16	<12	35	S
Clarithromicin	15ug	>18	<13	38	S

Table 7: *Staphylococcus saprophyticus*: Antibiotic Sensitivity and Resistance

Name of antibiotic	Potency	Standard zone of inhibition (mm)		Mean	Remarks
		Sensitive	Resistant		
Ciprofloxacin	05ug	>21	<15	22	S
Amoxicillin-clvulanic acid	03ug	>18	<13	20	S
Cephradine	30ug	>14	<12	5	R
Levfloxacin	05ug	>17	<13	22	S
Moxifloxacin	05ug	>18	<13	30	S
Cafaclor	30ug	>18	<13	28	S
Ofloxacin	05ug	>16	<12	18	S
Clarithromicin	15ug	>18	<13	38	S

Table 8: *Bacillus subtillis*: Antibiotic Sensitivity and Resistance

Name of antibiotic	Potency	Standard zone of inhibition (mm)		Mean	Remarks
		Sensitive	Resistant		
Ciprofloxacin	05ug	>21	<15	35	S
Amoxicillin-clavulanic acid	03ug	>18	<13	8	R
Cephradine	30ug	>14	<12	8	R
Levfloxacin	05ug	>17	<13	35	S
Moxifloxacin	05ug	>18	<13	38	S
Cafaclor	30ug	>18	<13	38	S
Ofloxacin	05ug	>16	<12	30	S
Clarithromicin	15ug	>18	<13	38	S

Table 9: *Pseudomonas aeruginosa*: Antibiotic Sensitivity and Resistance

Name of antibiotic	Potency	Standard zone of inhibition (mm)		Mean	Remarks
		Sensitive	Resistant		
Ciprofloxacin	05ug	>21	<15	38	S
Amoxicillin-clavulanic acid	03ug	>18	<13	8	R
Cephradine	30ug	>14	<12	10	R
Levfloxacin	05ug	>17	<13	42	S
Moxifloxacin	05ug	>18	<13	40	S
Cafaclor	30ug	>18	<13	20	R
Ofloxacin	05ug	>16	<12	35	S
Clarithromicin	15ug	>18	<13	18	S

Count: Passive sampling of different wards

In the passive sampling the eye ward also exhibited highest colony forming units (988 CFU/m^3 ±20), followed by female medical ward (973 CFU/m^3 ±46), Maternity ward 1 (870 CFU/m^3 ±15), female surgical ward (826 CFU/m^3±20) and maternity ward 2 (765 CFU/m^3 ±19) respectively. The count, mean, standard deviations are given in table 3. The result of one way ANOVA for all wards suggests that on each visit the level of contamination was persistent as the observed contamination on each visit was not significantly different from other visits the result was consistent for all wards at (p>0.01) detailed result is shown in table V.

Further the observed contamination in the wards was assessed against the accepted level using one sample T test. The table 4 shows that result of one sample T test: Maternity ward 1, t (8) =130.39, p<0.01; Maternity ward 2 t (8) =92.20, p<0.01; female surgical ward, t (8) =55.77, p<0.01; female medical ward, t (8) =14.29, p<0.01 and Eye ward, t (8) =116.96, p<0.01. Based on the t values and the significance level it can be concluded that the contamination in the wards is (statistically) significantly higher than the acceptable level. Therefore the null hypothesis is rejected.

The mean scores along with standard deviation of different bacteria observed in this study stood as follows: *Staphylococcus aureus* (802±162), *Staphylococcus epidermidis* (1180±264), *Streptococcus pyogenes* (79 ±176), *Pseudomonas aeruginosa* (286±128), *Bacillus subtilis* (110±32), *Staphylococcus saprophyticus* (196±82) and *Serratia mercescens* (2±4). The overall ward wise and the patterns of all bacteria are shown in Fig 1; also it compares the pattern of both active and passive sampling.

The antibiotic sensitivity

The antibiotics used in this study included ciprofloxacin, amoxicillin- clavulanic acid, levofloxacin, cephradine, moxifloxacin, cafaclor, clarithromycin and ofloxacin. The evidence of the antibiotic sensitivity tests using standard Kirby-Bauer method suggests that Gram-positive bacteria were predominant as compared to gram-negative bacteria. Ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin and ofloxacin were the most effective antibiotics against most bacteria identified. Results of antibiotic sensitivity test are given in tables 6 to 9.

DISCUSSION

The current findings of elevated CFUs in Public Sector Hospital Sukkur were very high compared to the acceptable levels as revealed by Pasquarella *et al.* (2000) and Daniel, (2009). High contamination (CFU 988/dm^2 /h and 993 CFU/$m^3$was observed in eye ward both through passive and active methods, respectively. Currently findings of only five wards are presented in this paper. Generally congestive, overcrowding and lack of ventilation were observed in the wards under study especially in the eye ward. The contaminated beds of the patients were placed at near distance; open dirty dust bins and contaminated instruments were observed. It may be possible that shedding from skin, and clothes of patients, attendants, visitors and other hospital staff might transfer bacteria among individuals (cross-infections). It is generally accepted that shedders can disperse large number of coccal bacteria into environment resulting in high concentrations of staphylococci, which remain viable for long periods of time (Sands and Goldmann, 1998).

Very little information is available regarding the acceptable levels for the active sampling method. In this paper 300 CFU//m^3 has been taken as a standard acceptable level as shown by Fung 2009 and exceeding this is considered a very serious and hence unacceptable level in an indoor environment.

The female medical ward also exhibited unacceptably high CFU both through active and passive methods. This could also be attributed to overcrowding patients, attendants, visitors, open yet dirty dustbins, and sneezing that may result in cross-infections. All wards showed elevated CFU.

The data presented in this paper showed the Gram-positive bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus pyogenes* were dominant as compared to Gram negative bacteria (*Pseudomonas aeruginosa*). These findings coincided with the observations of (Tambekar *et al.*, 2007; Kaur and Hans, 2007; Jaffal *et al.*, 1997; Adnan *et al.*, 2004; Kim *et al.*, 2011).

In the current study, all the hospital wards showed raised CFUs indicating high air contamination and variations in bacterial CFUs observed in different wards, which could be related to comings and goings (frequent visits) of many individuals (patients, attendants, visitors and healthcare personnel). Flies, mosquitoes and occasional presence of cats were seen in the different wards under study. It is already accepted that there exists a relationship between occupants, density, human activities and microbial concentrations in the indoor air. The microbial load inside hospitals is highly influenced by the number of occupants, their activity and ventilation.

It was also observed during air sampling(s) that there were no nosocomial guidelines for patients, visitors, attendants and health care personnel. Rampant visitors and attendants (with unchecked shoes or without foot covers) seen moving from one ward to another. Moreover, visitors and attendants were seen sitting on the patients' beds and sharing the same utensils (plate, spoon, glass and cups). It was also observed that patients, attendants and visitors were coughing and spitting in the corridors and wards of the Hospital freely.

The current study also showed that ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin were effective against most of the airborne bacteria. These finding are not surprising as these antibiotics are reportedly broad spectrum (Miño *et al*., 2005; Drago *et al*., 2001; Cunha *et al*., 1997). The present findings showing increased levels of bacterial count correlate with unhygienic conditions, unchecked visitors, sneezing, coughing, over crowd, unfiltered conditioners, unfiltered ventilation, cross infection, high level of dusting, building design and so on. This systematic survey study should act as food for thought for concerned authorities.

CONCLUSIONS

The evidence sought in this study based on both active and passive sampling suggests that the observed air contamination in the wards was significantly higher than the acceptable levels. Hence it can be deduced that the public sector hospital wards under study exhibited high level of air contamination that could be linked to unhygienic conditions and lack of commitment. It was also observed that the air contamination in the wards under observation was persistent as there was no significant difference in the observed air contamination on each visit; hence the contamination was persistently higher. Moreover, ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin and ofloxacin were the most effective antibiotics against most of the identified bacteria. The airborne infections are a serious problem(s) for developing countries such as Pakistan and it is essential to take preventive measures against this problem because it is directly concerned with human health and economy. The public sector hospital wards under study showed high level of contamination due to unhygienic conditions and lack of commitment.

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