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Original Research Article

Bacterial Yields of Air Samples in Hospital Environment

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ABSTRACT

Background: Hospitals and other healthcare facilities are complex environment that requires ventilation for comfort of the patients and control of hazardous pathogens. Airborne transmission occurs when pathogenic microorganisms are transferred from an infected to a susceptible individual via the air.
Objective: To detect the air borne bacteria in open plate technique.
Design: Descriptive design
Methods: A study on indoor air microbiological contamination in hospital wards were investigated and analysed in the period June-September 2014. Air samples were taken pre and post disinfection with 2% sodium hypochlorite.
Results: The results obtained by air investigation conducted in Shri Sathya Sai Medical College and Research Institute. The predominant bacteria isolated from collected air samples were: *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Citrobacter koseri* and *Tubercle bacilli*. According to IMA classes: pre disinfection, had 'C' grade and colony counts 32 $CFU.m^{-3}$ (26-50 $CFU.dm^{-2}.h^{-1}$) the performance was fair and for post disinfection with 2% Sodium hypochlorite the outcome was 'A' grade and colony counts 4 $CFU.m^{-3}$ (0-5 $CFU.dm^{-2}.h^{-1}$) the performance was very good.
Our study is the first in vitro analysis with the involvement of open bottle technique for Mycobacterium tuberculosis growth in LJ media.
Conclusion: From our study, it has been showed that the hospital air is dispersed with bacteria especially the most potent MDR Tuberculous bacilli. To make it healthy, safe for the diseased patients, disinfection is a must in the given scenario, 2% sodium hypochlorite was the ideal disinfectant and even TB bacilli was also reduced to zero.

Keywords: Indoor air, Hospital wards, Hospital personnel, Occupational diseases, Microbiological quality, airborne bacteria and Mycobacterium tuberculosis.

INTRODUCTION

Microorganisms are the primary sources of indoor air contamination. The indoor air environment can potentially place patients at greater risk than the outside environment because enclosed spaces can confine aerosols and allow them to build up to infectious levels. [(1)]

Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some

substances harmful for health. These are different kinds of toxic metabolism products, for example myco- toxins. [2]

Exposure to bio-aerosols, containing airborne micro- organisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions. [3]

In many human activities micro-organisms in the environment represent a hidden but dangerous risk factor. Concern has increased with the introduction of advanced technologies in hospitals, industry and agriculture.

In recent years, many studies have been carried out on this topic, and nowadays the evaluation of the level of air microbial contamination in places at risk is considered to be a basic step toward prevention. However, there are still problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination. [4]

Airborne infectious particles are a potential source of hospital infections. Control of airborne microorganisms depends on measures consistent with aseptic technique as well as contamination control connected with ventilation. Human-shed organisms can be controlled using ventilation; however, low infection rates in orthopedic surgery have resulted from contamination control efforts associated with body suit containment. [5] Control efforts are effective, but containment of human-source microbes is integral for infectious disease management. The focus for airborne infection transmission is on both the human-source and environmental-opportunist control.

The difficulty in monitoring human-source microbes involves complex sampling strategies for recovering the microbes from the air. Human-source microbes, especially *Staphylococcus* and *Enterococcus* species, are controlled with standard contact precautions and not ventilation. Ventilation is critical and can be used to control the spread of airborne human-source infectious agents such as *Mycobacterium tuberculosis*, *Varicella zoster* and *Rubella*.

TB is an airborne disease caused by the bacterium *Mycobacterium tuberculosis*. *M. tuberculosis* is carried in airborne particles, called droplet nuclei, of 1– 5 microns in diameter. Infectious droplet nuclei are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing. Depending on the environment, these tiny particles can remain suspended in the air for several hours. *M. tuberculosis* is transmitted through the air, not by surface contact. Transmission occurs when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. [6]

Tuberculosis (TB) continues to be one of the major infectious diseases threatening millions of lives world- wide, mainly in developing (TB high-endemic) but also in developed (TB intermediate- and low-endemic) countries. It is estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (WHO, Global Tuberculosis Control, 2010). [7] Tuberculosis, which results from infection with *Mycobacterium tuberculosis* (MTB), continues to be one of the most important and dangerous of airborne infectious disease, especially in developing countries. [8]

Biological aerosols contain bacteria, viruses, yeasts and molds. Under special clinical circumstances, skin lesions may also be a source of airborne particles. Controlling airborne pathogens in healthcare facilities is not only important for the safety of the patient, but it is also important for hospital personnel. Various contamination control

procedures can limit exposure and risk of infection. Although it is not possible to eliminate all nosocomial infection (NI), their incidence can be significantly reduced by implementation of appropriate infection control policies. [9]

The Centers for Disease Control and Prevention and WHO have formulated TB infection-control guidelines for both resource-rich and resource- limited nations on the basis of a 3-tiered approach of controls: (1) administrative or work practice, (2) environmental, and (3) personal protection. [10]

Administrative controls: At the health care facility level, administrative control measures are the first line of defense against TB transmission and are intentioned to implement and monitor strategies to reduce generation of infectious particles to decrease staff and patient TB exposure. [11]

Environmental controls: Several strategies are available to reduce exposure to infectious particles, including natural ventilation, mechanical ventilation, and upper-room ultraviolet (UV) light. [12]

M. tuberculosis is susceptible to UV light, and the use of shielded fixtures that flood the upper room with high intensity UV light without endangering occupants in the lower room has long been advocated for TB infection control. [13]

Pulmonary tuberculosis is a true airborne disease, and exposure via aerosols is considered the primary mode of transmission. One commonly used method to control the spread of tuberculosis in clinical settings, is enhanced exhaust ventilation with exhaust air ducts fitted with High Efficiency Particulate Air (HEPA) Filters which trap virtually all MTB cells (Nicas, 1995). [14]

MATERIALS AND METHODS

The project was submitted to the institutional ethical committee & obtained NO: IEC 2014/163.

Study Area: The study was carried out at Shri Sathya Sai Medical College and Research Institute, Ammapettai, during the month of June - September 2014. The study was conducted in 21 different wards: which includes Male TBCD ward, Female TBCD ward, TBCD-OP, TBCD-isolation ward, Male surgical ward, Female surgical ward, Male medical ward, Female medical ward, Male ortho ward, Female ortho ward, Medical ICU, ENT-OP, OG ward, Casualty, Paediatrics ward, Laundry, Ophthalmology ward, Microbiology-central lab, Biochemistry-central lab, Pathology-central lab, Blood bank.

Open Plate Method: The samples were collected by open plate method by placing the petri plates in the targeted areas in the hospital wards according to 1/1/1 rule (petri dishes that will be left open for one hour, placed at a height of 1 meter from the floor and at a distance of 1 meter from the wall or any object) accepted by IMA index as the basis. According to this method, cfu (colony forming unit) index was used to determine the number of the colonies. [15] Air sampling was done by open plate technique, pre and post disinfection of the room.

We used Nutrient agar, Blood agar, Macconkey agar and LJ media for processing the samples. Blood agar was meant for identification and characterization of Gram positive bacteria and Macconkey agar for the further identification and characterization of Gram negative bacteria. LJ media were used for identification of Mycobacterium tuberculosis. The identification and colony counting of the microorganisms that were grown in the petri dishes at the specific area & time were tabulated & analysed. [16] Bacteria were identified by three arrays: 1. Macroscopic estimation (description of colony). 2.

Microscopic estimation (staining by Gram stain method for Gram positive and Gram negative bacteria and Ziehl Neelsen staining technique for Acid fast bacilli. 3. Biochemical tests according to bacterial classification by Bergey. [17] Along with this, Niacin test were done for TB as per the standard protocol. [18]

The index of microbial air contamination (IMA): IMA classes and maximum acceptable levels of IMA have been defined empirically. (Table 1)

Table1: Index of Microbial Air Contamination (IMA) Classes and their application

IMA VALUES	CFU.dm^{-2}.h^{-1}	PERFORMANCES	PLACES AT RISK
0-5	0-9	Very good	Very high
6-25	10-39	Good	High
26-50	40-84	Fair	Medium
51-75	85-124	Poor	-
≥ 76	≥ 125	Very poor	-

This has been possible, as large amounts of data are available in many different types of closed environments and in the open air, over a number of years. The measurement of the IMA is meaningful in places where there is an infection or contamination risk. Therefore, the lower levels of contamination have been taken into account. Five classes of IMA have been devised: 0–5 very good; 6–25 good; 26–50 fair; 51–75 poor; >76 very poor. IMA classes have been also normalized to cfu/dm^2. Each class represents a different increasing level of contamination. Maximum acceptable values of IMA have been established, related to different infection or contamination risks. [19] Following the studies of Fisher, the IMA was devised in 1978 with the aim of unifying and standardizing the technique of air sampling by settle plates. The 1/1/1 scheme was adopted. The IMA classes and the maximum acceptable IMA levels for each environment at risk were empirically defined by performing a large number of tests in different environments. Samplings were done as followed by our previous study. [20]

RESULT

A total of 21 hospital wards were screened and the organisms isolated were *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Citrobacter koseri* as shown in the Table 2.

According to IMA classes, for bacteria, the results obtained pre disinfection, the performance was fair with 'C' grade and colony counts 32 CFU.m^{-3}(26-50 CFU.dm-^{2}.h^{-1}) and for post disinfection with 2% sodium hypochlorite the performance was very good with 'A' grade and colony counts 4 CFU.m^{-3}(0-5 CFU.dm-^{2}.h^{-1}) as shown in the Table 2 & 3.

Along with bacterial cultures, isolation for *Mycobacterium tuberculosis* was also done by using Lowenstein Jensen medium (LJ medium) in 21 wards. The results were positive for TB bacilli in TBCD isolation ward and Male TBCD ward; few bacilli were seen in Microbiology central lab as shown in Table 4 and Figure 1.

As a preventive measure for these microorganisms, an effective disinfectant 2% Sodium hypochlorite was used. Bacterial isolates were reduced in much higher quantity and proved to be an effective infection control practice as *Mycobacterium tuberculosis* were also reduced.

In our study the use of 2% sodium hypochlorite in areas where we had *Mycobacterium tuberculosis* proved to be highly effective. Pre disinfectant collection of sample was positive, while post disinfectant collection did not grow the potent pathogen as shown in the Table 5 and Figure 2.

Table 2: Colony identification for indoor air open plate technique - pre disinfection

S. No.	Ward Name	No: of colonies	Microorganisms	IMA Value	Performance	Grade
1	Male TBCD ward	32	*Klebsiella pneumoniae,*	26-50	Fair	C
2	Female TBCD ward	26	*Streptococcuspyogenes, Staphylococcus aureus*	26-50	Fair	C
3	TBCD-OP	28	*Staphylococcus aureus, Klebsiella pneumoniae*	26-50	Fair	C
4	TBCD-isolation ward	28	*Staphylococcus aureus, Klebsiella pneumoniae*	26-50	Fair	C
5	Male surgical ward	26	*Staphylococcus aureus, Pseudomonas aeruginosa*	26-50	Fair	C
6	Female surgical ward	27	*Staphylococcus aureus, Streptococcus pyogenes*	26-50	Fair	C
7	Male medical ward	28	*Staphylococcus aureus, E.coli*	26-50	Fair	C
8	Female medical ward	29	*Proteus mirabilis, Citrobacterkoseri*	26-50	Fair	C
9	Male ortho ward	30	*Staphylococcus aureus, E.coli*	26-50	Fair	C
10	Female ortho ward	27	*Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*	26-50	Fair	C
11	Medical ICU	26	*Staphylococcus aureus, Klebsiella pneumoniae*	26-50	Fair	C
12	ENT-OP	26	*Streptococcus pyogenes, Citrobacterkoseri*	26-50	Fair	C
13	OG ward	28	*Klebsiella pneumoniae, Proteus mirabilis, E.coli*	26-50	Fair	C
14	Casualty	29	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Citrobacterkoseri*	26-50	Fair	C
15	Paediatrics ward	31	*Klebsiella pneumoniae, Citrobacterkoseri, Streptococcus pyogenes*	26-50	Fair	C
16	Laundry	29	*Staphylococcus aureus, Pseudomonas aeruginosa, E.coli*	26-50	Fair	C
17	Ophthalmology ward	30	*Staphylococcus aureus, Pseudomonas aeruginosa*	26-50	Fair	C
18	Microbiology-central lab	29	*Staphylococcus aureus, Proteus mirabilis, Citrobacterkoseri*	26-50	Fair	C
19	Biochemistry-central lab	28	*Staphylococcus aureus, Klebsiella pneumoniae*	26-50	Fair	C
20	Pathology-central lab	27	*Staphylococcus aureus, Acinetobacterbaumannii*	26-50	Fair	C
21	Blood bank	26	*Proteus vulgaris, E.coli*	26-50	Fair	C

Table 3: Colony identification for indoor air open plate technique post disinfection with 2% Sodium hypochlorite

S.No	Ward Name	No.Of Colonies	Microorganisms	IMA Value	Performance	Grade
1	Male TBCD ward	2	Micrococci	0-5	Very Good	A
2	Female TBCD ward	1	Micrococci	0-5	Very Good	A
3	TBCD-OP	No growth	Sterile	0-5	Very Good	A
4	TBCD-isolation ward	4	Micrococci	0-5	Very Good	A
5	Male surgical ward	No growth	Sterile	0-5	Very Good	A
6	Female surgical ward	No growth	Sterile	0-5	Very Good	A
7	Male medical ward	No growth	Sterile	0-5	Very Good	A
8	Female medical ward	3	Micrococci	0-5	Very Good	A
9	Male ortho ward	4	Micrococci	0-5	Very Good	A
10	Female ortho ward	3	Micrococci	0-5	Very Good	A
11	Medical ICU	No growth	Sterile	0-5	Very Good	A
12	ENT-OP	1	Micrococci	0-5	Very Good	A
14	Casualty	No growth	Sterile	0-5	Very Good	A
15	Paediatrics ward	2	Micrococci	0-5	Very Good	A
16	Laundry	4	Micrococci	0-5	Very Good	A
17	Ophthalmology ward	2	Micrococci	0-5	Very Good	A
18	Microbiology-central lab	3	Micrococci	0-5	Very Good	A
19	Biochemistry-central lab	4	Micrococci	0-5	Very Good	A
20	Pathology-central lab	3	Micrococci	0-5	Very Good	A
21	Blood bank	No growth	Sterile	0-5	Very Good	A

Figure 1: shows pre disinfection -Growth in LJ Media

Table 4: Sample screening for indoor air open bottle technique - pre disinfection

S.NO	WARD NAME	NO.OF.AFB BACILLI
1	Male TBCD ward	Positive for AFB
2	Female TBCD ward	Negative for AFB
3	TBCD-OP	Negative for AFB
4	TBCD-isolation ward	Positive for AFB
5	Male surgical ward	Negative for AFB
6	Female surgical ward	Negative for AFB
7	Male medical ward	Negative for AFB
8	Female medical ward	Negative for AFB
9	Male ortho ward	Negative for AFB
10	Female ortho ward	Negative for AFB
11	Medical ICU	Negative for AFB
12	ENT-OP	Negative for AFB
13	OG ward	Negative for AFB
14	Casualty	Negative for AFB
15	Paediatrics ward	Negative for AFB
16	Laundry	Negative for AFB
17	Ophthalmology ward	Negative for AFB
18	Microbiology-central lab	Positive for AFB
19	Biochemistry-central lab	Negative for AFB
20	Pathology-central lab	Negative for AFB
21	Blood bank	Negative for AFB

Table 5: Area screened for air analysis post disinfection with 2 % Sodium hypochlorite

S.NO	WARD NAME	NO.OF.AFB BACILLI
1	Male TBCD ward	Negative for AFB
2	Female TBCD ward	Negative for AFB
3	TBCD-OP	Negative for AFB
4	TBCD-isolation ward	Negative for AFB
5	Male surgical ward	Negative for AFB
6	Female surgical ward	Negative for AFB
7	Male medical ward	Negative for AFB
8	Female medical ward	Negative for AFB
9	Male ortho ward	Negative for AFB
10	Female ortho ward	Negative for AFB
11	Medical ICU	Negative for AFB
12	ENT-OP	Negative for AFB
13	OG ward	Negative for AFB
14	Casualty	Negative for AFB
15	Paediatrics ward	Negative for AFB
16	Laundry	Negative for AFB
17	Ophthalmology ward	Negative for AFB
18	Microbiology-central lab	Negative for AFB
19	Biochemistry-central lab	Negative for AFB
20	Pathology-central lab	Negative for AFB
21	Blood bank	Negative for AFB

Figure 2: shows post disinfection with 2% sodium hypochlorite- no growth in LJ media

DISCUSSION

The study of airborne microorganisms in indoor environments is important to understand the dissemination of airborne infection particularly in health care area like hospital. [(21)] It is believed that the environment where patients are treated has an important influence on the prospect of such patient's recovery or acquiring infection that may complicate their health. [(22)] It is therefore, important to evaluate the quality of the indoor air in the hospital environments. The number and type of airborne microorganisms can be used to determine the degree of cleanliness.

In this study, the most frequently isolated bacterial isolates were *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Citrobacter koseri.*

The screening of Male TB ward ,TB isolation ward and Microbiology central lab gave positive isolation for *Mycobacterium tuberculosis* bacteria in pre disinfectant sampling. While the post disinfection sampling was sterile for the dreadful pathogen. This proves beyond doubt the importance of infection control policies in which disinfection has a pivotal role, which not only cleanses the area but removes microbial agents also. Further the detection

of positive growth for the Tubercle bacilli in these wards is to be correlated with infected person's aerosols getting deposited in different areas of the ward by way of coughing, sneezing & talking.

This has to be controlled by the provision of Exhaust in the ward & control of air circulation. The fitting of UV light is an added facility which will further reduce the circulation of the pathogen.

But the boon we had in our study was the effective disinfectant: 2% sodium hypochlorite, which was highly effective in controlling & combating the *Mycobacterium* bacilli, therefore it is a mandate to practice meticulously the hospital infection control policies that includes the thorough disinfection of the patient care area.

The presence of Mycobacteria in Microbiology lab also indicates a high risk area, where the infection samples are handled for smear & culture inoculation, the sample to be handled in Type II b biosafety cabinet, [(23)] so that there is air control & reduces the mycobacterial aerosol in the lab. The second most steps are adherence to the Standard precautions in the form of proper use of mask, gloves while processing these samples. This will ensure the reduction of this bacterium. The final & vital step is proper disinfection using sodium hypochlorite, this will make the area totally devoid of the microbe as proved by our study.

This is in contrast to the study 5.25% sodium hypochlorite Effective against most bacteria and some viruses and is registered as effective against HIV, HBV, H1N1 (Influenza A), MRSA and TB. [(24)] Thus 2% sodium hypochlorite can be used as an effective disinfectant to reduce the quantity of TB bacilli.

CONCLUSION

From our study, it has been showed that the hospital air is dispersed with bacteria especially the most potent MDR tubercle bacilli. To make it healthy, safe for the diseased patients, disinfection is a must in the given scenario, 2% sodium hypochlorite was the ideal disinfectant and even TB bacilli was also reduced to zero.

Adherence to hospital infection control policies & proper use of ideal disinfection in patient care area will reduce the morbidity & mortality of the hospitalized patient, in addition to the prevention of nosocomial infections!!!

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