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Original Article

Microbiology Section

The Microbial Colonization Profile of Respiratory Devices and the Significance of the Role of Disinfection: A Blinded Study

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ABSTRACT

Introduction: Approximately 10-40% of all the nosocomial infections are pulmonary, which lead to grave complications. Elderly, debilitated, or critically ill patients are at a high risk. The respiratory care equipments which include ventilators, humidifiers, nebulizers may have been identified as the potential vehicles which cause major nosocomial infections if they are colonized by fungi or bacteria.

Aim: To determine the rate of colonization by bacteria and fungi of the oxygen humidifier chambers of the portable cylinders and central lines at our hospital. The Hudson's chambers of nebulizers were also studied for the same.

Methods: Swab samples were obtained from the equipments by using sterile cotton swabs on a tuesday, as these chambers were usually cleaned on every Saturday. Spot samples were taken from the ICUs, wards, the casualty and OPDs on a single day. Air samples were also obtained on the same day to determine whether the fungal spore load in the inhaled room air was normal or high. We performed a disinfection with 70% ethanol after cleaning these devices.

Results: 53/70 (75.71%) samples showed fungal growth; out of which, 23/33 (69.70%) were from the ICU, 24/30(80%) were from the wards and 6/7 (85.71%) were from the OPDs. 23/30 (76.66%) swabs from the central line humidifiers, 18/23(78.26%) swabs from the O_2 cylinder humidifiers and 8/17 (47.5%) swabs from the nebulizers grew bacteria. Of the total 61(87.14%) bacterial isolates, 42(68.85%) were gram negative bacteria and 19(31.14%) were gram positive cocci. Out of the 42 gram negative bacteria, 17 were multi-drug resistant like ESBL producers ie. *Pseudomonas spp.* (6) *Acinetobacter spp*. (4), *Klebseilla pneumoniae* (4), *E.coli* (2) and *Stenotrophomonas maltophila* (1). Our findings (before disinfection) showed that the colonization rate for fungi was 75% and that for bacteria, it was 87%. After the 70% ethanol disinfection and strict compliance with the hand hygiene, the colonization rates reduced significantly. The fungal colonization rate was reduced and only 15% fungi grew after the disinfection, while only 12% bacterial colonization rate was found.

Conclusion: This study indicates a potential in-hospital source of allergens and infections. The oxygen and nebulizer chambers need to be cleaned more frequently with disinfectants, to control the possible nosocomial infections.

Key Words: Microbial colonization, Respiratory devices, Aspergillus fumigates, *Acinetobacter spp*

INTRODUCTION

Respiratory infections are the commonest among nosocomial infections. *Nosocomial pneumonia* is the second most common nosocomial infection worldwide and the most common infection in intensive care units (ICUs). In the United States, The Center for Disease Control and prevention (CDC) roughly estimated 1.7 million hospital-associated infections from all types of microorganisms which included bacteria, fungi and viruses, which contributed to 99,000 deaths per year [1,2]. The National Nosocomial Infections Surveillance (NNIS) system report, the data summary from January 1992 through June 2004, which was issued in October 2004, reported a steady increase in the rate of nosocomial fungal infections, from 3.8 to 4.9 per 1,000 discharges [3]. The advances in the medical and surgical therapies over the past two decades have changed the types of patients care in the hospitals. The care in the special units makes use of invasive monitoring devices; parentral nutrition, broad- spectrum antimicrobial agents and assisted ventilation. These have helped in successfully treating the patients who had suffered from previously known to be devastating or fatal diseases [3,4]. This severely ill, immunocompromized, hospitalized patient population is highly susceptible to the nosocomial infections which are caused by a variety of bacteria and fungi. The resulting illness is often severe, rapidly progressive and difficult to diagnose or treat. Approximately 40% of all the nosocomial infections are pulmonary. The respiratory care equipments which include ventilators, humidifiers, and nebulizers have been identified as the potential vehicles which cause major nosocomial infections if these are colonized by fungi or bacteria. Nosocomial pathogens may also be acquired through the hands of the hospital personnel and contaminated intravenous lines or fluids [5,6,7].

The contaminated respiratory care equipment may lead to nosocomial infections by two routes. Firstly, the respiratory care equipment may serve as a reservoir for microorganisms, especially gram-negative bacilli. The fluid containing devices such as nebulizers and humidifiers may become heavily contaminated by bacteria and fungi which may be capable of multiplying in water. The pathogens may then spread to the patients by aerosolization

in the room. Secondly, the contaminated equipment may lead to a direct instillation or delivery of microorganisms to the airways, if the equipment is directly linked to a ventilator system or if contaminated medication is Instilated or aerosolized [8-10]. Many equipments such as oxygen masks and nebulizer chambers may be transferred from patient to patient several times daily but they may be seldom cleaned daily. The substantial clinical and financial impact of nosocomial pneumonia makes this an important subject matter for the hospital epidemiologists and microbiologists. Preventive measures may reduce the incidence of nosocomial pneumonia by preventing the transmission of highly pathogenic microorganisms to the patient by reducing the colonization of the reservoir site. The infection control activities should emphasize the establishment of appropriate preventive guidelines and policies and the continuing education of health care workers to maintain an optimal compliance with the preventive practices [11,12].

Another important aspect is an increasingly common finding of fungal infections in hospital settings. Although fungi are generally thought to be less pathogenic than bacteria to humans, such a prevalence has been found to be increasing in ill patients, especially in the ICUs. The usual explanation given is that fungi take the lead in infecting an immunocopmromized patient who is well covered with antibiotics. However, we thought that there may be another potential reason for the occurrence of such infections i.e. the fluid containing reusable respiratory care equipments such as nebulizers and oxygen humidifiers. A thorough knowledge and understanding about the colonization of respiratory devices by bacteria and fungi, are thus needed among clinicians and microbiologists to provide a better patient care. Continued epidemiologic and laboratory research is required to better characterize these pathogens and to thus improve the diagnosis and the therapeutic strategies in the future. Hence, we aimed to qualitatively evaluate the microbial colonization rate in the oxygen humidifier chambers (of the portable cylinders and central lines) and the Hudson's chambers of the nebulizers; which were being used in various wards and ICUs of our hospital. We also performed a study to determine the efficacy of the 70% ethanol wipe as a source of decontamination of the respiratory devices.

METHODS

Study period - Jan 2011 to April 2011. This study was approved by the Institutional ethical committee.

Sample collection methods - A total of 70 swabs samples were obtained from the inner surfaces of the oxygen humidifiers and the Hudson's chambers of the nebulizers by using sterile swabs on a Tuesday, as these chambers were usually cleaned on every Saturday at our institute. Spot samples were taken from ICUs (33), wards (30), the casualty and OPDs (7) on a single day. We performed a disinfection with 70% ethanol for all above mentioned equipment and after the disinfection, we collected swab samples from the inner surfaces of the equipments and followed the same protocol to determine the rate of colonization. Air samples were also obtained on the same day to determine whether the fungal spore load in the inhaled room air was normal or high.

Quality control- Ten swabs were collected from new, unused oxygen humidifiers and the Hudson's chambers of nebulizers as controls and the swabs were seeded on Sabouraud's dextrose agar (SDA) slants with antibiotics, on SDA slants without antibiotics and on blood agar to check the quality control.

Blinding- In order to maintain the blinding, the samples from various sites were coded. The pulmonologist investigators collected the samples and they were thus unblinded. The microbiology investigators were blinded to the information about the site as to which the swabs belonged to.

Bacteriological examination- The swabs were inoculated in glucose broth and they were incubated aerobically at 37°C in an incubator for 4 hours. The incubated glucose broth was then sub cultured on Mac Conkey's agar and blood agar and it was incubated at 37°C overnight. The growth after the overnight incubation were identified and confirmed by standard conventional methods.

Antibiotic susceptibility tests - The Kirby- Bauer method which was recommended by the CLSI guidelines (2005) was used for the antimicrobial susceptibility testing [13,14].

Detection of the Extended Spectrum β-Lactamases- A screening Test (CLSI, 2010)

The initial screening test for the ESBL production was done as a part of the routine susceptibility testing. Two antibiotic discs, ceftazidime (30 µg) and cefotaxime (30 µg) were used for the screening for ESBLs. Plates with Mueller- Hinton Agar (MHA) were prepared and they were inoculated with the test organism (the turbidity corresponded to 0.5 McFarland's standard) to form a lawn culture. The above discs were placed on the surface of the agar. The plates were incubated at 37ºC overnight and the sensitivity pattern and the resistance pattern were recorded by reading the zone diameter of the test organism. If a zone diameter of ≤ 22mm for Ceftazidime and that of ≤27 mm for cefotaxime were recorded, these strains were considered as "Suspicious" for the ESBL production [15,16].

The Double Disk Approximation Test (DDAT)

A bacterial suspension which was equivalent to 0.5 McFarland's standard turbidity was prepared for the ESBL production test. A sterile swab was dipped into the standardized inoculum and the soaked swab was rotated against the upper inside wall of the tube to express the excess fluid. The entire surface of the MHA was swabbed to form a lawn culture and the inoculum was allowed to dry for a minute with the lid in place. With sterile forceps, a ceftazidime disk was placed on the agar plate, near the centre, giving a centre to centre distance of 15 mm with the Ceftazidime/clavulonic acid disc (30µg/10µg). The plates were inverted and they were incubated at 37ºC for 16-18 hours. Each plate was examined for the enhancement of the zone of inhibition for the ceftazidime disk, at the side which faced the Ceftazidime/clavulonic acid disk. If the strain was an ESBL producer, then the zone around the ceftazidime disk was extended towards the Ceftazidime/clavulonic acid disk. ATCC Escherichia coli -25922 were used as a negative control and ATCC K. pneumoniae -700603 was used as a positive control [15-18].

Mycological examination- The swabs were inoculated directly on Sabouraud's dextrose agar (SDA) plates with and without antibiotics i.e, Chloramphenicol (50 mg/ml) and Gentamicin (20 mg/ml) and the plates were incubated at 25°C and 37° C separately over a period of four weeks. The fungus identification was done, based on the growth rate, the colony morphology, the reverse and obverse surface colours of the SDA slant and the microscopic aspects such as the mycelia and the conidia types. Dematiaceous molds were considered when the colonies that could develop dark gray to black mycelia were particularly prominent when a black reverse

Swab Samples	ICUs	Wards	OPD	Total
Central line humidifiers	22	8	-	30
O_2 cylinder humidifiers	5	14	4	23
Nebulizer chambers	6	8	3	17
Total	**33**	**30**	**7**	**70**

[Table/Fig-1]: Distribution of swab sampling from various sites

(47.5%) swabs from the nebulizers grew bacteria.

NICU - neonatal intensive care unit, PICU - pediatric intensive care unit, MICU - medicine intensive care unit, SICU - surgical intensive care unit, O_2 –oxygen, OPD - out door patients.

Of the 51(75.71%) total fungal isolates, Aspergillus fumigatus 18 (33.96%) was predominantly isolated, followed by Aspergillus niger

Sr. no	Name of the species	Frequency of isolation	Predominate site	Predominant equipment
1	*Aspergillusfumigatus*	18 (33.96%)	• TB & Chest OPD (5/18) • SICU(3/18) • PICU(3/18) • Female Surgery Ward(2/18) • Casualty (2/18) • MICU (1/18) • Male Med Ward(1/18) • Male Surgery Ward (1/18)	• Central line humidifier 10/18 • O_2 cylinder humidifier 6/18 • Nebulizer 2/18
2	*Aspergillusniger*	10 (18.86%)	• Pediatric Ward(3/10) • PICU(3/10) • Pediatric OPD (2/10) • Female MedicineWard(1/10) • Surgical ICU(1/10)	• CentralLine Humidifiers(4/10) • O_2 Cylinder Humidifiers(4/10) • Nebulizers (2/10)*
3	*Fusarium spp.*	8 (15.09%)	• Pediatric Ward(3/8) • Pediatric ICU(2/8) • Female MedicineWard(1/8) • Male TB Chest Ward (1/8) • Surgery OPD(1/8)	• Central Line • Humidifiers(4/8) • O_2 Cylinder Humidifiers(3/8) • Nebulizer(1/8)
4	*Alternaria spp.*	7 (13.20%)	• Male TB Chest Ward (2/7) • Surgical ICU(2/7) • Female Medicine Ward(1/7) • Female Surgery Ward(1/7) • Surgery OPD(1/7)	• O_2 Cylinder Humidifiers(5/7) • Central Line Humidifiers(2/7)
5	*Chaetomium spp.*	5 (9.4%)	• Female medicine Ward (1/5) • MICU(1/5) • Male TB Chest Ward(1/5) • SICU(1/5) • TB & Chest OPD(1/5)	• Central Lines(3/10) • Cylinders(1/5) • Nebulizer(1/5)
6	*Aspergillus flavus*	3 (5.6%)	• FemaleMedicine W(1/3) • PICU(1/3) • Casualty (1/3)	• O_2 Cylinders(3/3)
7	*Aspergillusgalucus*	3 (5.6%)	• MICU(2/3) • Medicine OPD(1/3)	• O_2Cylinders(2/3) • Central Line(1/3)
8	*Chrysosporium spp.*	3 (5.6%)	• MICU(1/3) • SICU(1/3) • Pediatric OPD (1/3)	• Central Lines(2/3) • O_2 Cylinders(1/3)
9	*Streptomyces spp.*	3 (5.6%)	• SICU(2/3) • TB & Chest OPD (1/3)	• Central Line(1/3) • O_2 humidifiers(1/3) • Nebulizers(1/3)
10	*Candida spp.*	2 (3.7%)	• Casualty(1/2) • MICU(1/2)	• Central Line(1/2) • Nebulizers(1/2)
11	*Trichoderma spp.*	2 (3.7%)	• MICU(2/2)	• Central Line(2/2)
12	*Penicillium spp.*	2 (3.7%)	• MICU(2/2)	• Central Line(2/2)
13	*Curvularia spp.*	1 (1.8%)	• MICU(1/2)	• Central Line(1/2)

[Table/Fig-2]: Frequency of Various Fungal Isolates in Swab Samples out of a total of 53 positive samples

of the colonies were observed. The species identification was done by lactophenol cotton blue (LPCB) staining of the culture positive fungi [19-23].

RESULTS AND OBSERVATIONS

A total of 70 swab samples were processed i.e. 33 from the ICUs, 30 from the wards and 7 from the OPDs. 53/70 (75.71%) samples showed fungal growth; out of these, 23 (69.70%) were from the ICUs, 24(80%) were from the wards and 6 (85.71%) were from the OPDs. 23/30 (76.66%) swabs from the central line humidifiers, 18/23(78.26%) swabs from the O_2 cylinder humidifiers and 8/17

10(18.86%) [Table/Fig-1 and 2].

Of the total 61(87.14%) bacterial isolates, 42(68.85%) were gram negative bacteria and 19(31.14%) were gram positive cocci. Out of the 42 Gram negative bacteria, 17 were multi-drug resistant i.e. ESBL producers ie. *Pseudomonas spp.* (6) *Acinetobacter spp.*(4), Klebseilla pneumoniae (4), E.coli (2), Stenotrpphomonas maltophila (1) [Table/Fig-3].

DISCUSSION

One third of the nosocomial infections are considered as

Sr. no	Name of bacteria	Frequency of isolation	Predominate site	Predominant equipment
1	*Pseudomonas spp.*	15	• MICU(5/15) • SICU(5/15) • PICU(3/15) • OPD(2/15)	• Central Line(3/15) • O_2 humidifiers(8/3) • Nebulizers(4/3)
2	*Acinetobacter spp.*	10	• MICU(4/2) • PICU(4/10) • SICU(1/3) • OPD(1/3)	• Central Line(4/10) • O_2 humidifiers(4/10) • Nebulizers(2/10)
3	*E. coli*	8	• PICU(4/8) • MICU(2/8) • SICU(1/8) • OPD(1/8)	• Nebulizers(4/8) • Central Line(3/8) • O_2 humidifiers(1/8)
4	*Klebseilla spp.*	7	• PICU(4/7) • SICU(1/7) • MPICU(1/7) • OPD(1/7)	• Nebulizers(4/7) • Central Line(2/7) • O_2 humidifiers(1/7)
5	*Methicillinresistant Staphylococcus aureus (MRSA)*	5	• MICU(2/5) • PICU(1/5) • SICU(1/5) • OPD(1/5)	• Nebulizers(2/5) • Central Line(2/5) • O_2 humidifiers(1/5)
6	*Methicilin sensitive staphylococcus aureus (MSSA)*	6	• MICU(2/6) • SICU(2/6) • PICU(1/6) • OPD(1/6)	• Nebulizers(3/6) • Central Line(2/6) • O_2 humidifiers(1/6)
7	*Coagulase negative Staphylococcus aureus (CONS)*	8	• PICU(4/8) • MICU(2/8) • OPD(1/8) • S ICU(1/8)	• Central Line(4/10) • O_2 humidifiers(4/10) • Nebulizers(2/10)
8	*Stenotrophomonasmaltophila*	2	• SICU(1/2) • PICU(1/2)	• Central Line(1/2) • O_2 humidifiers(1/2)

[Table/Fig-3]: Frequency of Various bacterial Isolates in Swab Samples out of a total of 61 positive samples

preventable. The information on the potential risk of their transmission due to the colonization of respiratory equipment/ devices is insufficient. *Aspergillus spp.* is ubiquitous and it commonly Found in soil, water, decaying vegetation. The potential reservoirs in hospitals may include unfiltered air, ventilation systems, oxygen humidifiers, nebulizer chambers and tubings, the contaminated dust which is dislodged during hospital constructions, carpeting, food and ornamental plants [1,3,5,24]. *Aspergillus fumigatus, A. flavus, A. terreus* have become the common causes of nosocomial infections. Contaminated air or ventilation systems have been associated repeatedly with the outbreaks of nosocomial aspergillosis [25]. Construction and demolition activities near the hospital, renovation, cleanings and a moist environment within the ventilation system or the air filter have been commonly cited. During our study period, our hospital construction work was in progress and it was probably a reason for the isolation of *Aspergillus spp.* in higher proportions.

Hyalohyphomycosis i.e. nondematiaceous molds have recently been recognized as emerging nosocomial pathogens [24,25]. Our study finding showed colonization by *Fusarium spp.* in 8(15.09%) swab samples which were obtained, mainly from humidifiers. The mechanism of the infection may include inhalation into the lungs or the upper airways or breaks in the skin or the mucous membranes.

The patients who are hospitalized with exacerbations of obstructive airway disease such as asthma, often require nebulization and oxygen therapy. If the oxygen humidifier chambers or the nebulizer chambers are colonized by fungi, the clinicians may actually be directly delivering the fungal allergens to the patients' airways. We suspect that this may be the cause of an occasional delayed response to the asthma therapy.

Finding fungi or yeast cells in the sputum of the patients who receive corticosteroid or antibiotic therapy is not uncommon. A study showed that fungi may be found in the sputa in 42.4% of the patients who had received prior antibiotics and in those of 64.2% of the patients who had received prior inhaled steroids [26]. A majority of the nosocomial infections are caused by *Candida spp.* [27]. In our study, Candida tropicalis was isolated from two central line nebulizers. There are several reports on the growing prevalence of non-albicans Candida among hospitalized patients [28,29]. Cross-infections from the staff to the patients may be common, even in the ICUs. Rangel-Frausto et al., reported that the hands of the health care workers were reservoirs for the *Candida spp.* and that 85% of the Candida were non- albicans Candida [27]. An outbreak of Candida tropicalis fungaemia in the neonatal intensive care unit (NICU) was reported in neonates who were receiving total parenteral nutrition and antimicrobial agents [29,30]. The same study showed that Candida tropicalis was isolated from two NICU workers and not from the environment. Washing hands as promptly and thoroughly as possible between the patient contacts and after contacts with blood, body fluids, secretions, excretions and equipment or the articles which are contaminated by them, is an important means of infection control and an isolation safety measure.

The aetiology of bacterial nosocomial pneumonia depends on the duration of the hospitalization before the pneumonia develops. Early onset nosocomial pneumonia occurs during the first four or five days of the hospital stay. It is more commonly caused by community acquired pathogens such as Streptococcus pneumoniae, Methicillin-susceptible Staphylococcus aureus, H. influenza and Morexella catahalis [31-33]. In contrast, late onset nosocomial pneumonia (which usually occurs after five to six days of

hospitalization) is commonly caused by *Pseudomonas aeruginosa*, *Acinetobacter spp*., Methicillin resistant *Staphylococcus aureus*, etc. [34]. After 10 or more days in the hospital, *Enterobacteriaceae* and *P. aeruginosa* are the most common pathogens which are responsible for nosocomial infections. Several studies have reported the aetiology of nosocomial pneumonia in the long-term care settings. The present study showed high colonization rates of *Pseudomonas spp.* and *Acinetobacter spp*., followed by those of *E. coli* and *Klebseilla spp. Klebseilla pneumoniae* was associated with 2%-5% of the nosocomial infections, particularly those from the lower respiratory and the urinary tracts. The nosocomial outbreaks which are caused by gram negative bacteria Have been associated with their drug resistance to the third-generation cephalosporins and aminoglycosides [30-35]. The present study showed a total of 17/61 (27.86%) extended spectrum β-lactamases (ESBL) species.

We performed a disinfection with 70% ethanol after cleaning these devices (oxygen humidifiers and nebulizer chambers) with distilled water and soap. The health care workers were educated on hand hygiene with alcohol-based hand rubs before and after each patient contact. We collected swabs again from the same wards and ICUs after the 70% ethanol disinfection and determined the colonization rate by using the same methodology. Our finding (before the disinfection) showed the colonization rate for fungi to be 75% and that for bacteria to be 87%. After the 70% ethanol disinfection and the strict compliance of hand hygiene, the colonization rates reduced significantly. The fungal colonization rate reduced and only 15% fungi grew after the disinfection, while only a 12% bacterial colonization rate was found.

The limitation of this present study was that we did not take into consideration any contamination with viruses or Mycobacteria. This is because the prevalence of the nosocomial viral or Mycobacterial infection is very low. We assessed only the 70% ethanol wipe disinfection and we did not compare its results with other high level disinfectants; the reason being the cost of the various disinfecting agents and the practicability of their use.

CONCLUSIONS

We consider it prudent to perform a periodic surveillance for the bacterial and fungal colonization in the respiratory equipment, particularly in water-sealed devices. Proper cleaning and sterilization or a high level disinfection of the reusable equipments is essential, to prevent the infections which are associated with the respiratory therapies such as oxygen therapy, nebulization, etc. Devices or parts of the devices need to be rinsed in water after they have been chemically disinfected. Sterile water has been recommended because tap or locally prepared distilled water may harbour microorganisms that can potentially cause pneumonia. The implementation of new and regular hygiene measures for the maintenance of such equipments is desirable.

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