Detection of Potential Pneumonia-Causing Bacteria Found in Nebulizers in Iligan City Hospitals: Some Implications on Infection Control

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Abstract

In the third national prevalence survey of infection in hospitals, pneumonia was the third most common (13.9 per cent) acquired infection in acute hospitals (Hospital Infection Society/Infection Control Nurses Association, 2007). Hospital acquired pneumonia is associated with crude mortality rates up to 70% and attributable mortality rates as high as 33% to 50%. Pneumonia is one of the top ten causes of deaths in the Philippines. Inhalation therapy equipment such as nebulizer may potentially act as a vector of hospital-acquired pneumonia. The major mechanism by which bacteria are disseminated is aerosolization of organisms in particle sizes that are sufficiently small to be deposited in terminal lung units. Nebulizers create aerosols of minute droplets that penetrate deeply into the narrowest airways and thus present a significant problem. This is especially so for small – volume medication nebulizers (Botman and de Krieger, 1987).

The main purpose of this study is to conduct a bacteriologic assessment of the different parts of the nebulizers in selected wards of Iligan City Hospitals. Acquisitions of samples were obtained through swabbing at different parts of the nebulizers specifically the air filter and compressor port from different wards of Iligan City Hospitals. Swab samples were subjected to the conventional method of identification of bacterial species.

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Bacillus sp., *Streptococcus* sp., and *Staphylococcus* sp. (pathogenic and nonpathogenic) were among the identified bacterial species. Between the air filter and compressor port, the latter yielded the highest number of bacterial species. Moreover, the parts of the nebulizer are not dependent on each other with respect to the total number or types of bacteria present. Bacteria are the most common causes of pneumonia. The most common organisms that cause pneumonia include *Streptococcus (S.) pneumoniae* (also called pneumococcus, *Staphylococcus (S.) aureus*, *Streptococcus pyogenes* or Group A streptococcus and to a lesser degree, *Bacillus anthracis* may also cause Typical pneumonia. Based on the findings of this study, it is recommended that proper care and maintenance should be observed to reduce if not, eliminate potential source of nosocomial pneumonia that may pose a threat to patient's health condition.

Keywords: pneumonia – causing bacteria, nebulizer, compressor port, air filter, nosocomial pneumonia

INTRODUCTION

Nosocomial infections, also called hospital-acquired infections, are those that are associated with the delivery of health care services. These can either develop during the client's stay in the health care facility, or after discharge. The most common settings where nosocomial infections develop are hospital surgical or medical intensive care units. The most common routes of transmission for Nosocomial infections are the urinary tract, the respiratory tract, bloodstream, and wounds, (Berman, Snyder, Kozier & Erb, 2008).

Nosocomial pneumonia is the second most common nosocomial infection worldwide but leads to the greatest number of nosocomial-related deaths (Rello, 2007). Based on an article written by de Guzman (2003) pneumonia is the most prevalent nosocomial infection in the Philippines of which is mostly from *Pseudomonas aeruginosa*. *P. aeruginosa* is a prevalent gram-negative opportunistic human pathogen most common in the nosocomial infection. This organism affects patients

who are immunocompromised and is hard to treat because it is antibiotic resistant. The other leading pathogen which is *Staphylococcus aureus* causes the same clinical manifestations with other bacterial pneumonia. However the illness is more severe and slow to respond to treatment (Archibald et al., 2008).

Nosocomial infections are multifactorial in origin. More factors for nosocomial infections are unknown and cannot be quantified. Freeman and McGowan (1981) as cited by Alora et al. (1990), stated that "Nosocomial infections were the only infections that regularly occurred under medical surveillance." It is imperative, therefore, that health care personnel should be more aware of and be vigilant on the detection and treatment of nosocomial infections.

Samuel et al. (2010) asserted that nosocomial infection is a recognized public health problem world-wide with a prevalence rate of 3.0-20.7% and an incidence rate of 5-10%. It has become increasingly obvious that infections acquired in the hospital lead to increased morbidity and mortality which has added noticeably to economic burden. Nosocomial infections are responsible for about 90,000 deaths in the U.S. per year and approximately 10% of American hospital approximately 10% of American hospital patients (about 2 million every year) acquired clinically significant nosocomial infections. In Italy in 2000s, about 6.7 % of hospitalized patients were infected; that means, between 450,000 and 700,000 patients had nosocomial infections out of which between 4,500 and 7000 died. In Switzerland, extrapolations assume about 70,000 hospitalized patients affected by nosocomial infections (between 2 and 14% of hospitalized patients). In Nigeria, nosocomial infection rate of 2.7 % was reported from Ife, while 3.8 %29 from Lagos and 4.2 % from Ilorin. The cause of nosocomial infections might be endogenous or exogenous. Endogenous infections are caused by organism present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel or medical devices endogenous or exogenous.

Devices used on the respiratory tract for respiratory therapy (e.g., nebulizers) are potential reservoirs and vehicles for infectious microorganisms. The major mechanism by which bacteria are disseminated is aerosolization of organisms in particle sizes that are sufficiently small to be deposited in terminal lung units. Nebulizers create aerosols of minute droplets that penetrate deeply into the narrowest

airways and thus present a significant problem. Routes of transmission might be from device to patient, from one patient to another, or from one body site to the lower respiratory tract of the same patient via hand or device. Contaminated reservoirs of aerosol-producing devices (e.g., nebulizers) can allow the growth of hydrophilic bacteria that subsequently can be aerosolized during use of the device. Gram-negative bacilli (e.g., *Pseudomonas* sp., *Xanthomonas* sp., *Flavobacterium* sp., *Legionella* sp., and nontuberculous mycobacteria) can multiply to substantial concentrations in nebulizer fluid and increase the risk for pneumonia in patients using such devices (Centers for Disease Control and Prevention, 1997).

A nebulizer, also known as an atomizer, is a machine that vaporizes liquid medication into a fine mist to be inhaled into the lungs (Wallace, 2003). Nebulizer therapy is indicated for patients with asthma, acute bronchospasm, excessive mucus build-up, cystic fibrosis, pneumonia, and COPD, as this provides quick relief of symptoms. Nebulizer therapy also helps to loosen bronchial secretions in the respiratory tract thus providing relief from obstructions. It minimizes the risk of side effects of the medication, preventing the medication from being metabolized into a less effective form in the body.

Because inhaling and exhaling takes place when using a nebulizer, bacteria from the infected lungs go back into the aerosol. That is why regular cleaning is very important in between using a nebulizer to prevent inhaling bad bacteria that may cause lung infection the next time you use the device so for small-volume nebulizers (Botman & de Krieger, 1987). Meticulous use of medical and surgical asepsis is necessary to prevent transport of potentially infectious microorganisms. Many nosocomial infections can be prevented using proper hand washing techniques, environmental controls, sterile technique when warranted, and identification and management of clients at risk for infections (Berman et al., 2008).

The nosocomial infection that this study is most concerned about is nosocomial pneumonia. It occurs in 1% of hospitalized patients, including 10% of intensive care patients, and is the leading cause of hospital-related mortality. The disease may be caused by cross-infection between patients, usually carried by staff, or acquired from other colonized sites (Hough, 2001). According to a study by Ghazal et al. (2006) entitled "Outbreak of Burkholderia cepacia bacteremia in immunocompetent children caused by

contaminated nebulized salbutamol in Saudi Arabia," a total of 7 patients were reported to be positive with *B. cepacia* in their blood cultures. The patients were negative upon admission and positive after a few days of hospitalization. 5 inpatients admitted for respiratory problems were given nebulized salbutamol for at least 3 days, and developed fever and dry cough after a few days of hospitalization. The risk factor being studied is the concomitant use of nebulized budesonide with salbutamol. It was found out that concomitant use of the nebulizer is 26 times more likely associated with infection and is statistically significant.

As with any piece of medical equipment, a nebulizer should be sanitized after each use per manufacturer's instructions. However, such is not the case here in our local hospitals. In previous duties, the researchers have observed that some patients do not clean their nebulizer equipment. Some even just place them on top of their bedside table, instead of securing a clean container. Thus, this ignites the researcher's enthusiasm to conduct this study with the main purpose of surveying the presence of microorganism in the nebulizers which can be a potential source of nosocomial infection.

CONCEPTUAL FRAMEWORK

This study is anchored on the agent-host-environment model of health and illness by Leavel and Clark. The agent-host-environment model of health and illness by Leavel and Clark (1965) has been expanded into a general theory of the multiple causes of disease. The model has three dynamic elements, namely, the agent, the host, and the environment. The agent is defined as "a factor (biologic, chemical, physical, mechanical, psychosocial) that must be present or absent for an illness to occur," the host as "living beings (e.g., human or animal) capable of being infected or affected by the agent," and the environment as "everything external to the host that makes illness more or less likely." Because each factor constantly interacts with the others, health is an ever changing state. Health is maintained when the variables are in balance; otherwise, disease occurs. The microorganism present in the nebulizer serves as the agent that can cause infections, the nebulizers serve as the environment where the agent is found without the patient as the host

who may or may not require the infection associated with the microorganism.

In Florence Nightingale's Environmental Theory, health is linked to five environmental factors: (1) pure or fresh air, (2) pure water, (3) efficient drainage, (4) cleanliness and (5) light, especially direct sunlight. Deficiencies in these five factors produced lack of health or illness (Berman et al., 2008). Our study is focusing only on two factors namely fresh air and cleanliness. The presence or absence of microorganism in the nebulizer reflects cleanliness. Furthermore, ineffective cleaning of the nebulizer compromises the respiratory tract of the people using it. An environment wherein aseptic technique is practiced and maintained is one of the factors that can prevent nosocomial infection.

STATEMENT OF THE PROBLEM

The main purpose of this study is to conduct a bacteriologic assessment of the different parts of the nebulizer in selected wards of Hospital X and Hospital Y both in Iligan City. (For ethical purposes we have changed the names of the two hospitals into Hospital X and Hospital Y).

COLONY	NUMBER OF ISOLATES	MORPHOLOGICAL CHARACTERISTICS	PHYSIOLOGICAL CHARACERISTICS		BACTERIAL GENUS
		GRAM STAINING	BLOOD AGAR	MANNITOL SALT AGAR	
A1	4	(+) cocci in cluster	Hemolytic, medium-sized colonies	Yellow (mannitol fermenter)	**Pathogenic *Staphylococcus***
	1	(+) cocci in cluster	Non-hemolytic, medium sized colonies	Pink (non-mannitol fermenter)	**Nonpathogenic *Staphylococcus***
	1	(+) cocci in chains	Hemolytic, small colonies	No growth (non-salt tolerant)	***Streptococcus* sp.**
	4	(+) rods	Hemolytic, large colonies	Pink (non-mannitol fermenter)	***Bacillus* sp.**

Specifically, this study aims to answer the following:

1. What are the possible pneumonia-causing bacteria found in the nebulizers, particularly in the air filter and compressor port, in selected wards of Hospital X and Hospital Y?
2. Is there a significant difference in terms of bacterial species of the samples isolated from the different parts of the nebulizer (air filter and compressor port)?

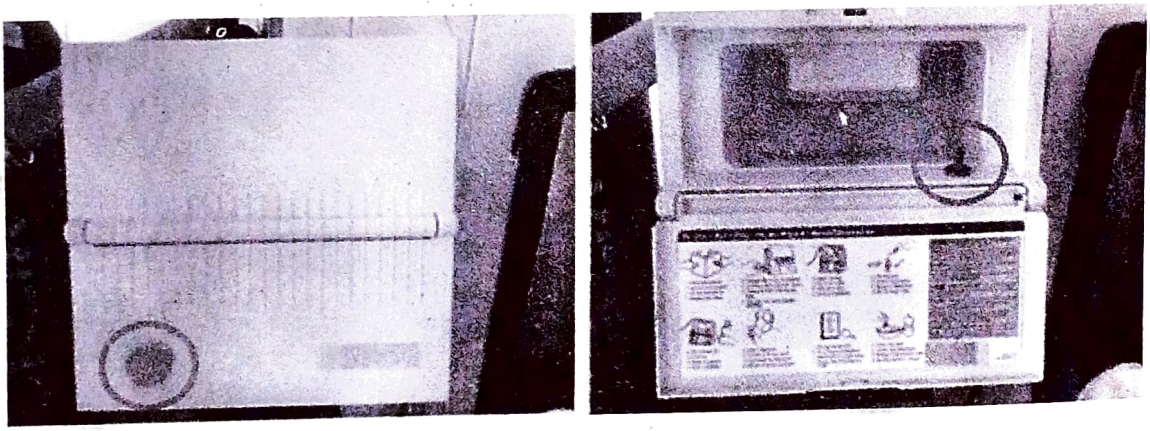
METHODOLOGY

This study employed a descriptive research design and aimed to identify bacteria present in the nebulizers that may cause respiratory nosocomial infection. Culture from the nebulizers was subjected to a series of tests to obtain description of the morphologic and physiologic

characteristics. The assessment of the microbiologic profiles of the culture swabs from the nebulizers was done at the microbiology laboratory of MSU-IIT College of Science and Mathematics.

Method of Data Collection

Acquisition of samples was obtained through swabbing at different parts of the nebulizers, specifically the **air filter** and **compressor port**, from different wards of Hospital X and Hospital Y namely, the Medicine ward, Surgery ward, and Pediatric ward. Swab samples were subjected to the conventional method of identification of bacterial species.



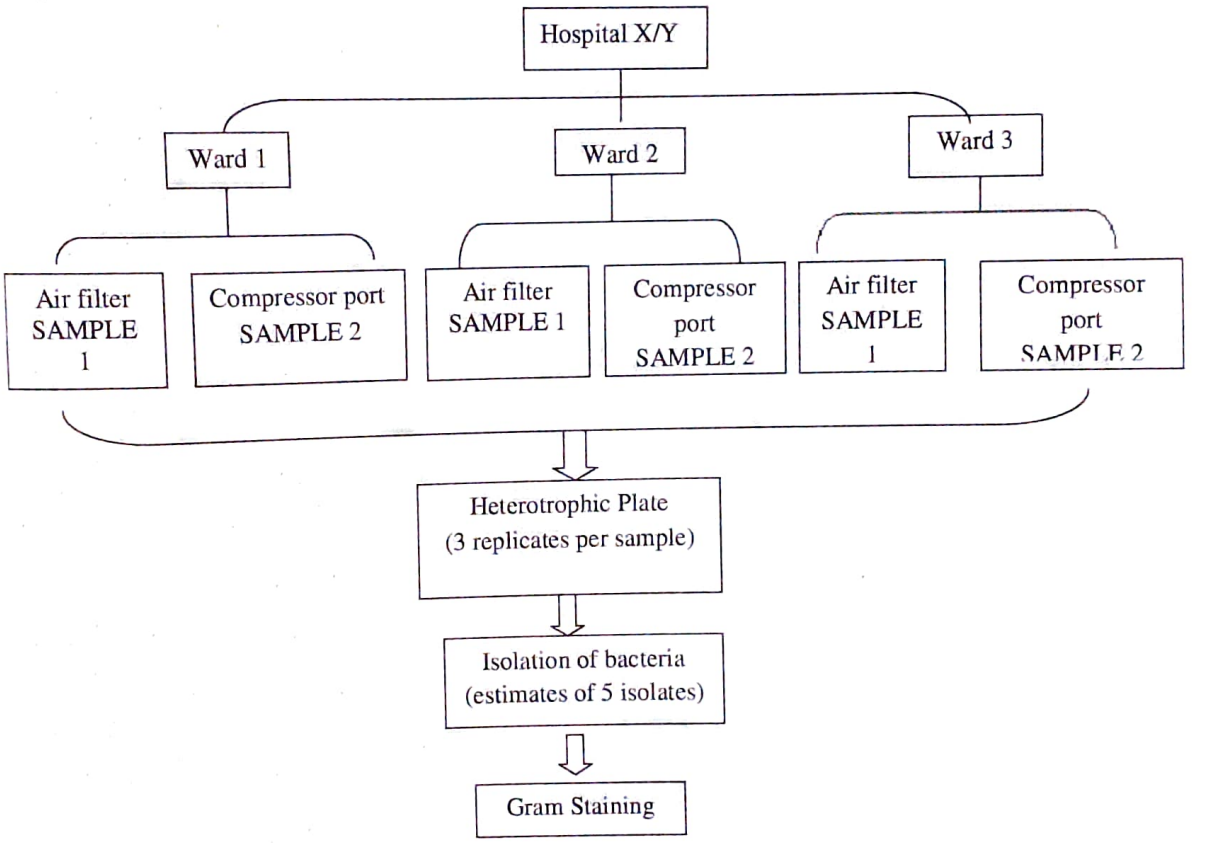


Figure 1. A prototype of a nebulizer: (left) closed, with air filter; (right) open, with compressor port

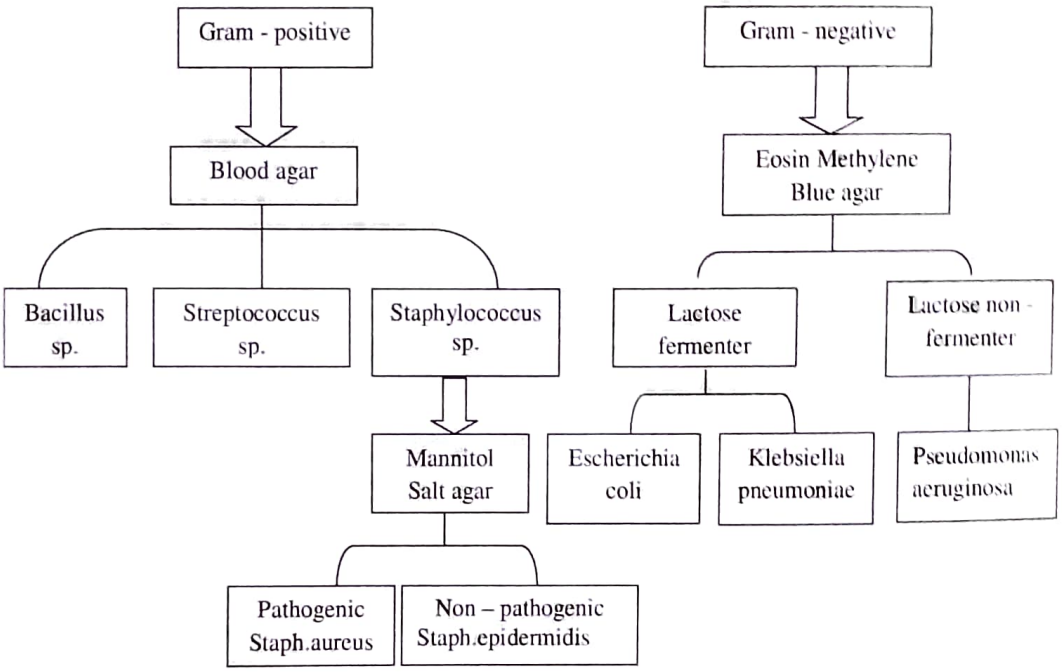


Figure 2. Schematic diagram of the procedure used

Collection of Samples

Collection of samples from the air filter and compressor port was done in each sampling site in Hospital X and Hospital Y, with only one sampling per hospital in three wards, namely, the Medicine ward, Surgery ward, and Pediatric ward. The sampling date was determined randomly, as there can be no way of predicting the peak hours of nebulizer use.

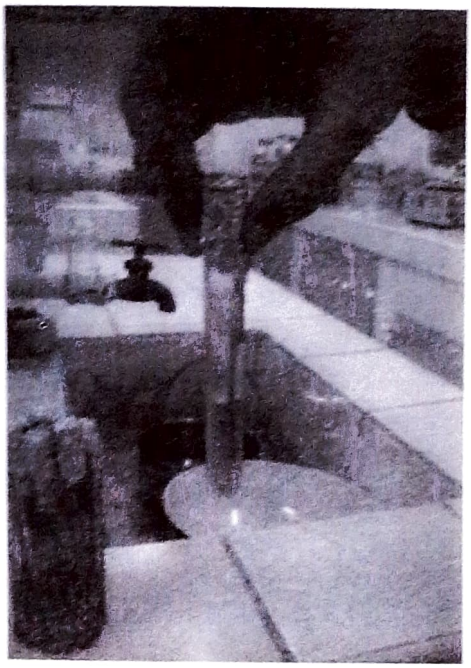


Figure 3. Test tubes with nutrient broth

Before going to the sampling site, test tubes covered with cotton plugs and filled with 3-mL nutrient broth were sterilized. The sterile test tubes were labeled accordingly and kept in vertical position during transport to the sampling site to prevent the broth from being tilted to the edge of the tube and becoming contaminated. They were then kept inside an ice bucket, submerged in ice and wrapped in sterile plastic, still in vertical position. Each sample was swabbed with a wet sterile cotton applicator (previously soaked in sterile water), observing aseptic technique to eliminate unnecessary contaminants. After every swabbing, the swabbed cotton applicator was put into its respective sterile test tube, dipping it into the 3-mL nutrient broth. The tube was then submerged into the ice to inhibit further proliferation of bacteria. The tubes with the swabs were incubated for 24 hours, then transported carefully back to the laboratory for the bacteria to be grown, cultured, observed, and identified.

Figure 4. Swabbing of the air filter and compressor port

The samples collected were then streaked for isolation onto a nutrient agar plate using Multiple Interrupted Streak method (as seen in figure 5). It was then allowed to incubate for 24 hours at room temperature. The isolated colonies that formed were streaked and isolated twice over to ensure that the bacterial isolates were not contaminated. Modified sterile vial slants with nutrient agar were utilized for stocking the pure bacterial isolates for further testing.

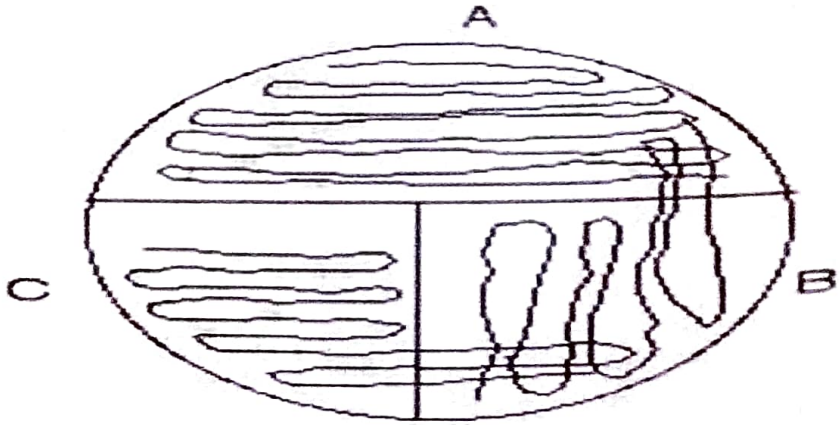


Figure 5. Multiple Interrupted Streak Method

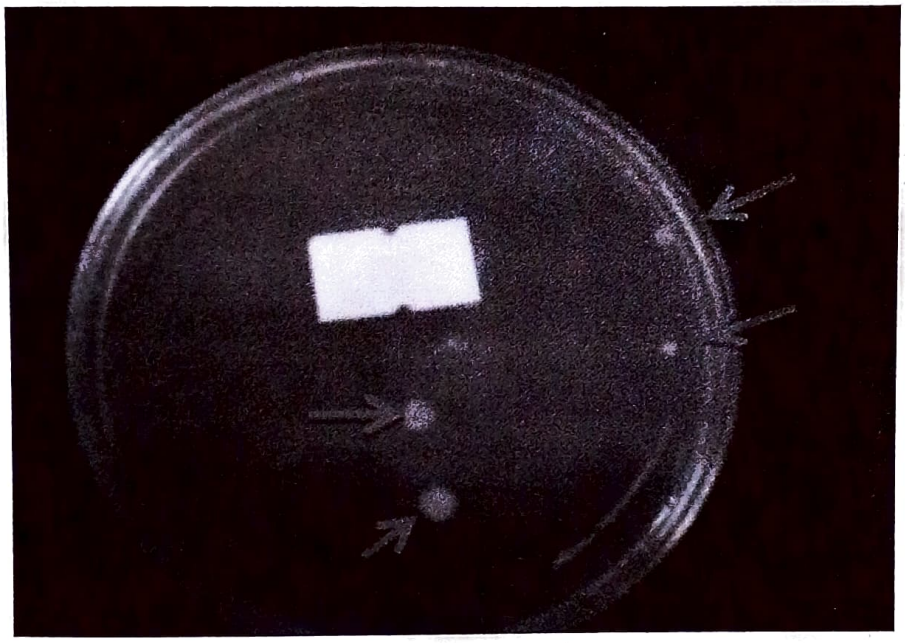


Figure 6. Bacterial growth (red arrow) on nutrient agar

After the isolation of the different bacterial isolates, they were then characterized according to the whole shape of the colony, size in millimeters, edge/margin, elevation, color, opacity, surface, and consistency as shown in Figure 7.

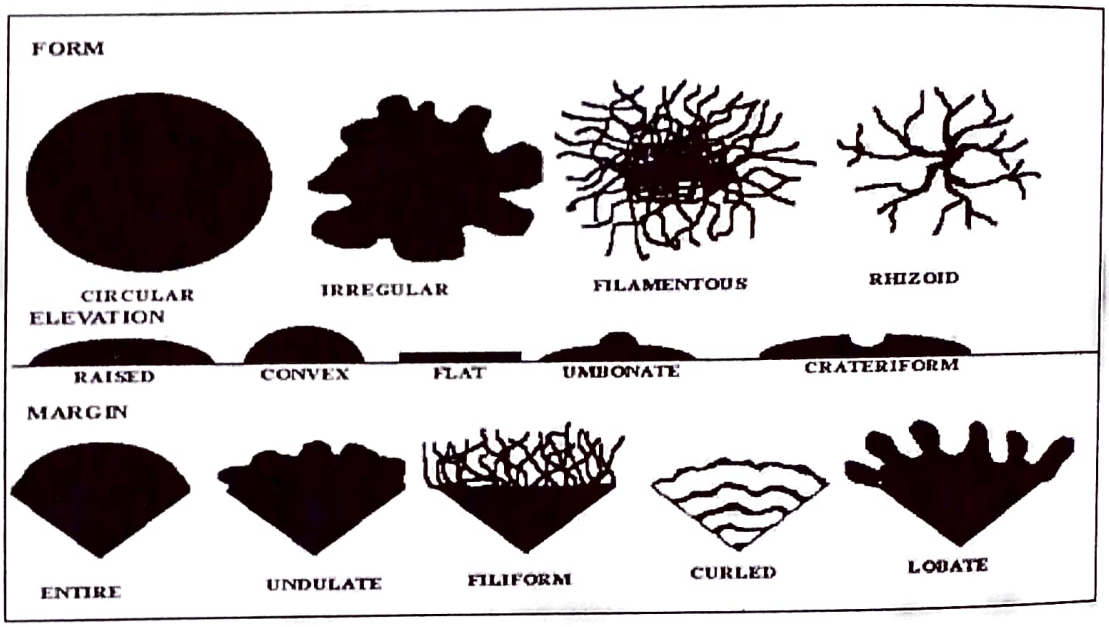


Figure 7. Different types of colonial morphologies, regarding the shape, edge, and elevation of the colony (Hurlbert, 1999)

The shape of the colony refers to the overall figure exhibited by the bacterial colony: round, irregular, filamentous, rhizoid and curled. The edge of the bacterial isolate is a colony morphology characteristic likewise considered in such forms as: entire, filamentous, undulate and lobate. Another cultural characteristic of bacterial isolates on nutrient agar growth is the elevation which assumes the forms as: raised, flat, convex and umbonate.

Bacteria are characterized and classified mostly by their enzyme or biochemical reactions. As they grow on various types of media, they produce certain types of metabolites that are detected by their interaction with test reagents which may result in a color change (Enriquez, 1995). Thus, identification of the isolates was based from morphological and biochemical tests for bacteria.

The Gram stain, which is used to classify bacteria on the basis of their forms, sizes, cellular morphologies, and Gram reactions and which divides most clinically significant bacteria into 2 main groups, is the first

step in bacterial identification. A drop of distilled water was placed on a clean glass slide. Using aseptic techniques, an inoculum from 24-hour-old culture was removed and smeared onto the clean glass slide. This was allowed to dry, and heat-fixation was conducted by gently and thoroughly heating the glass slide through a flame three times. The bacteria smear was first stained with the basic dye crystal violet for 1 minute and washed thoroughly with running water. The smear was treated with Gram's iodine solution for 1 minute and then rinsed with ethanol. Finally, the counterstain safranin was applied for 1 minute, and rinsed with running water. The slide was set to dry for 1 minute, then microscopy followed and the shape, arrangement, and gram reaction were noted. All stained slides were then examined in a microscope under low power objective (LPO) and immersion oil objective (OIO). Results from the gram staining reaction were differentiated by the color of bacterial cells in which the gram negative appeared pink while those that were gram positive appeared purple (Bruckner, 2008, as cited by Acuzar et al., 2010).

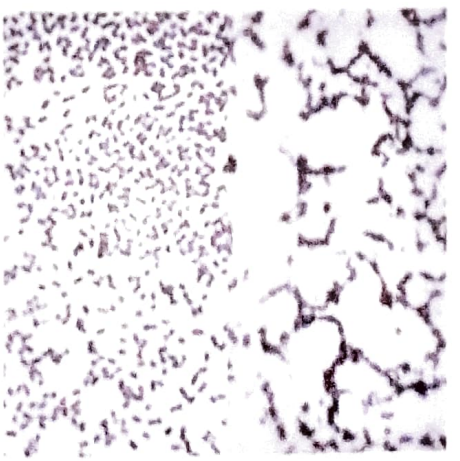


Figure 8. Gram stain reactions: (left) negative, (right) positive

For any bacterium to be propagated for any purpose it is necessary provide the appropriate biochemical and biophysical environment. A iety of specialized media are designed to facilitate determining the :hemical reactions of bacteria (Todar, 2008).

Blood agar is a bacterial growth medium that can distinguish normal from pathogenic bacteria based on the effect of bacterial hemolytic exotoxins on red blood cells. Alpha hemolysis (α-hemolysis) means that the bacteria generate chemicals that only partially break down the blood cells. This results in the media showing a yellowish/greenish/brownish discoloration (like a bruise) around the colony, indicating incomplete hemolysis. Beta hemolysis (ß-hemolysis) means that the bacteria's hemolytic exotoxins completely beak down the blood cells. The ß-hemolysis pattern results in the media displaying clear halos around bacterial colonies. Gamma hemolysis is essentially no hemolysis at all, and there is no change to the color of the medium (Port, 2008). All Gram-positive bacteria have undergone this test. The selected colonies were streaked in Blood Agar Plates. The plates were then allowed for incubation of the microorganisms for 48 hours within room temperature.

Mannitol Salt agar is used as a selective media for the isolation of pathogenic *Staphylococci*. *Staphylococcus aureus* grows on this medium and ferments mannitol to produce yellow colonies. Most coagulase negative species of *Staphylococci* and *Micrococci* do not ferment mannitol and grow as small red colonies. (Sigma-Aldrich Inc., 2011) Mannitol Salt agar was prepared and was then poured in petri dishes. Using aseptic technique, an inoculum from the mother culture was then streaked onto the petri dishes. It was then allowed to incubate at room temperature for 48 hours.

On the other part of the test, Eosin Methylene Blue agar, a slightly selective and differential plating medium is being utilized for the isolation of gram-negative enteric bacteria. The use of Eosin and Methylene Blue enable differentiation between lactose-fermenting and non-fermenting organisms. When the bacteria ferment the lactose, the pH decreases in the medium and the medium changes to a purple color. Gram-negative bacteria that ferment lactose appear with a green metallic sheen. Those that cannot ferment lactose appear clear on the medium (Jett et al., 1994, as cited by Castillo et al., 2010). Eosin Methylene Blue (EMB) agar was used as culture medium. Gram-negative bacilli were streaked on this agar and incubated at room temperature for 24-48 hours. Purple growth on this agar suggests *Klebsiella* sp. while colorless growth in this agar implies *Pseudomonas* sp. Positive growth with green metallic color suggests *Escherichia coli*.

RESULTS AND DISCUSSION

Twelve samples were collected from the two hospitals. Since each sample has three replicates, there were thirty-six samples all in all. Among the thirty-six samples that were streaked in petri plates, only nine plates yielded isolates. These were the samples from the pediatric, medicine, and surgery wards of Hospital X.

Table 1. Morphological and physiological characteristics of bacterial isolates

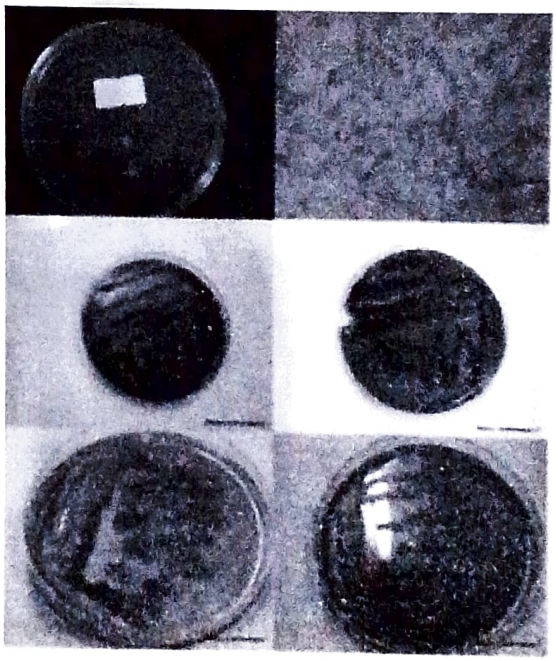


Figure 9. Staphylococcus sp.

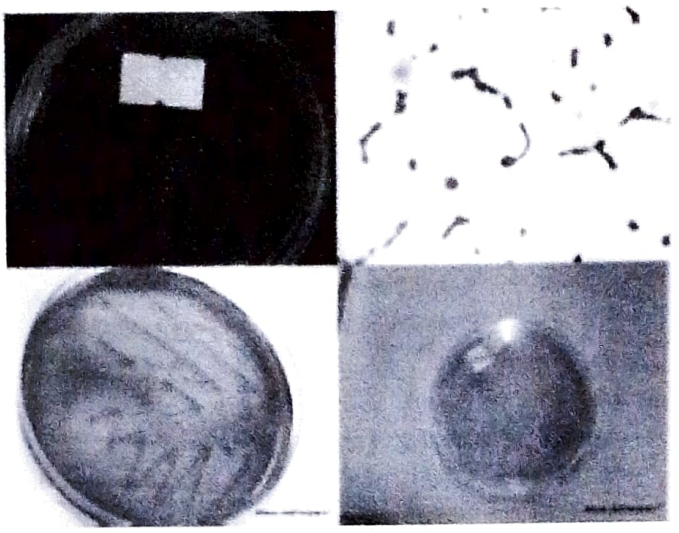


Figure 10. Streptococcus sp.

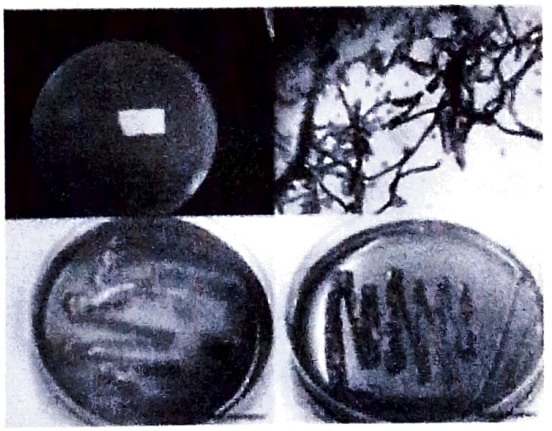


Figure 11. Bacillus sp.

Findings show that *Bacillus* sp., *Streptococcus* sp., and *Staphylococcus* sp. (pathogenic and nonpathogenic) were among the identified bacterial species.

According to Harris (2002), the *Staphylococcus* genus includes at least 40 species. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms (Madigan & Martinko, 2005). Among the *Staphylococcus* species, *S. aureus* is considered the most serious pathogen. This species is considered the most

resistant of all non-spore-forming pathogens, with well-developed capacities to withstand high salt, extremes in pH, and high temperatures. It also remains viable after months of drying, and resists the effects of many disinfectants and antibiotics (Talaro, 2011). It is a frequent cause of both hospital acquired pneumonia and community acquired pneumonia including ventilator-associated pneumonia (Haessler & Brown, 2009). "*Staphylococcus* species were the most common bacteria isolated in suction machines in the study done by Acuzar et al. (2010) in hospitals in Iligan City. Due to its ability to resist multidrug treatment S. aureus is hard to treat even with antibiotics and intensive care, which causes increased morbidity and mortality in children, elderly, and patients with medical conditions.

Most members of the *Streptococcus* genus are quite sensitive to drying, heat, and disinfectants, and seldom develop drug resistance, though pneumococci (*S. pneumoniae*) and enterococci are exceptions. Despite the large number of streptococcal species, human disease is most often associated with *S. pyogenes* and *S. pneumoniae* (Talaro, 2011). *Streptococcus pneumoniae* is a normal inhabitant of the human upper respiratory tract. The bacterium can cause pneumonia, usually of the lobar type, paranasal sinusitis and otitis media, or meningitis, which is usually secondary to one of the former infections. *S. pneumoniae* is currently the leading cause of invasive bacterial disease in children and the elderly. *S. pneumoniae* is known in medical microbiology as the pneumococcus, referring to its morphology and its consistent involvement in pneumococcal pneumonia (Todar, 2008). Although infection is often acquired endogenously from one's own flora, it may occur after direct contact with respiratory secretions or droplets from carriers, possibly from hospital staff. *S. pneumoniae* is very delicate and does not survive long out of its habitat (Talaro, 2011). This study was conducted at random, so there is a probability that *S. pneumonia* was there as a transient bacteria at the time of swabbing. This will explain why, among all fastidious bacteria, only *S. pneumonia* was found.

Among the different *Bacillus* species, *B. subtilis* and *B. licheniformis* have been implicated in food poisoning and in various infections, including septicemia, pneumonia, wound infection, and peritonitis (Baron et al., 1994).

Table 2. Number of bacterial isolates from the compressor port and air filter of nebulizers in different wards in two selected hospitals in Iligan City

Number of bacterial isolates	Hospital X(Public Tertiary Hospital)			Hospital Y(Private Tertiary Hospital)			TOTAL
	Pediatric	Medicine	Surgery	Pediatric	Medicine	Surgery	
Air filter	0	1	0	0	0	0	1
Compressor port	4	3	2	0	0	0	9

Hospital Y is a tertiary level **accreditation** of **hospital** and a training **hospital** and its Quality Management System is in compliance with **ISO** 9001:2008 requirements. Several factors have been identified as the reasons why there were no isolates found in the said hospital, and these include, implementation of regular disinfection schedule of all the hospital equipments including nebulizers by the hospital personnel per shift or every eight (8 hours), the Infection Control Committee regularly and religiously do a quarterly culture of hospital equipments to identify isolates and corrective measures were done immediately if isolates will be found out, and lastly, decontamination or fumigation of the hospital rooms will be done if there will be suspected patient/s who had communicable disease/s .

For Hospital X, it was found out that between the air filter and compressor port, the latter yielded the highest number of bacterial species.

Conclusion and Recommendations

Among the identified bacterial species were *Staphylococcus* sp, *Streptococcus* sp., and *Bacillus sp.*

Between the air filter and compressor port, the latter yielded the highest number of bacterial species. This is potentially dangerous since the compressor port is where the air from the nebulizer goes out and into the patient's respiratory tract. Therefore, compressor ports must be properly cleaned after every use to prevent cross-contamination. If the compressor port is not being used it should be covered with a clean cloth or kept clean by wiping it with a clean cloth. Not only the compressor port

but other parts of the nebulizer must be cleaned as well. After each treatment, the nebulizer cup, and mask or mouthpiece must be washed in warm soapy water, rinsed carefully and air dried. Equipment must be disinfected either with vinegar, water, or disinfectant solution every third day of using and air dried. Air filters must be changed every 6 months or sooner if filter turns completely gray in color. It is important for the health care workers to observe proper infection control through cleaning and disinfecting equipment, such as nebulizers, every after use in order to prevent respiratory nosocomial infection. Infection-control committees in hospitals must determine general and specific control measures. Given the prominence of cross-infection, hand hygiene is the single most important preventive measure in hospitals. Hospitals can also collaborate with biologists in periodic bacteriologic assessment of their own equipment, and chemists for manufacturing of disinfectants that target these types of bacteria.

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