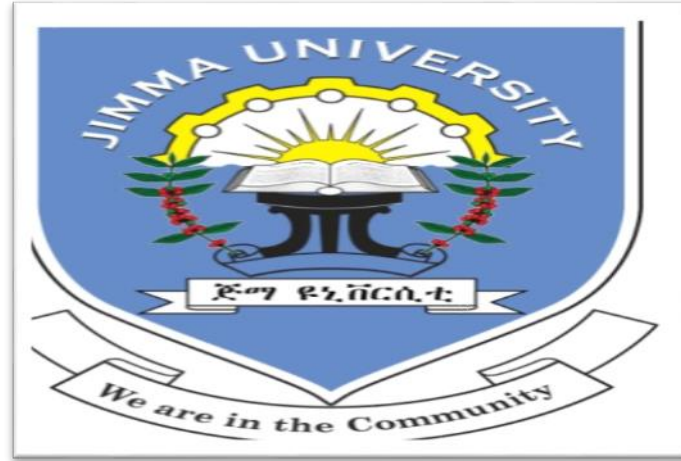ASSESSMENT OF MICROBIAL INDOOR AIR QUALITY AND DRUG SUSCEPTIBILITY TEST AGAINST BACTERIAL ISOLATES: IN CASE OF JUSH AND SHENEN GIBE HOSPITAL, JIMMA TOWN, SOUTHWEST ETHIOPIA

By

ANDUALEM GIRMA (BSc)

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ASSESSMENT OF MICROBIAL INDOOR AIR QUALITY AND DRUG SUSCEPTIBILITY TEST AGAINST BACTERIAL ISOLATES: IN CASE OF JUSH AND SHENEN GIBE HOSPITAL, JIMMA TOWN, SOUTHWEST ETHIOPIA

BY

ANDUALEM GIRMA (BSc)

ADVISORS:

1. SAMUEL FIKADU (MSc, Ass. professor)
2. HIGEMENGIST ASTATIKE (MSc)

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ABSTRACT

INTRODUCTION: Microorganisms are the primary source of air contamination in indoor environments. Indoor air has a greater potential to endanger patients health than outdoor air. Indoor aerosol types may have the ability to cause different levels of infection.

OBJECTIVE: Assessment of microbial indoor air quality and drug susceptibility test against bacterial isolates: in case of Jimma University Specialized Hospital and Shenen Gibe Hospital, Jimma town, southwest Ethiopia.

METHOD: A cross-sectional study was conducted. Bioaerosels sample were taken using passive sampling techniques with 1/1/1 schedule. The qualitative analyses of bacteria were carried out using biochemical and enzyme test. Fungal species were identified using Lactophenol blue cotton staining and macroscopically. The isolates of bacteria from both hospitals were tested against five commonly used antibiotic using Kirby-Bauer agar disk diffusions method on Mueller-Hinton agar.

RESULT: The bacterial population ranged from 280 cfu/m³ to 6369 cfu/m³ in Jimma University specialized hospital and 127 cfu/m³ to 3397 cfu/m³ in Shenen Gibe zonal Hospital. The fungal population were recorded from 159 cfu/m³ to 817 cfu/m³ in Jimma university specialized hospital and 74 to cfu/m³ to 818 cfu/m³ in Shenen Gibe zonal hospital. The microbial isolates included seven bacterial and six fungal isolates in both hospitals. They include, Staphylococcus aureus, coagulase negative Staphylococci, Klebsiella, Escherichia coli, Bacillus spp, Proteus spp and Streptococcus spp. while the fungal isolates includes Aspergillus spp, Alternaria spp, Fusarium spp, Penicillium spp Mucor spp, and Trychopton spp. The statistical analysis showed that the concentrations of bacteria and fungus in all studied wards were significantly different from each other (p-value=0.000) in both hospitals.

CONCLUSIONS: Jimma university specialized hospital was highly contaminated than Shenen Gibe hospitals. The degree of microbial load was highest in emergency outpatient in both hospitals. The lowest load recorded in minor operating theatre in Shenen Gibe hospital. Ampicillin was resistant drug under this study by all tested species. While Gentamicin was susceptible drug against all tested species in both studied hospitals

Key words: Susceptibility, Shenen Gibe, Bacterial isolates, JUSH

Acknowledgement

Acronyms

AMR Antimicrobial Resistance Agents

ASHRAE America Society of Heating and Air Conditioning Engineers

BA   Blood Agar

CFU Colony Forming Unit

DST Drug susceptibility test

HAI   Hospital Acquired Infection

HVAC Heating, ventilation, and air conditioning system.

IAQ   Indoor Air Quality

JUSH Jimma University Specialized Hospital

NA   Nutrient Agar

NI   Nosocomial Infection

PDA   Potato dextrose agar

SDA   Sabouraud Dextrose Agar

VOCs   volatile organic compound

WHO   World Health Organization

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CHAPTER ONE: INTRODUCTION

1.1 Background

Air pollution is introduction into the atmosphere of chemicals, particulate matter or biological materials that causes discomfort, disease or death to humans, damage to other living organisms. Both indoor and outdoor, is a major environmental health problem affecting developed and developing countries alike. It comes from sources of dust, gases and smoke, and is generated mainly by human activities but also naturally. While the problem regarding outdoor air pollution has been well publicized for several decades, it is indoor air pollution that is causing the most recent concerns for obvious reasons (1).

Indoor air quality is used to describe the quality of air in or around a particular building as it relates to the health of the people around that building and environment. Good air quality is very important especially in health care setting that involves a large number of people. Such settings that are of great concern because People spend a large part of their time each day indoors which include hospitals, schools, prisons, family houses, restaurants and banks (1–4).

Microbiological air quality is an important criterion and significant issue that must be taken in to account when indoor workplaces places are designed to provide a safe environment (5,6). Estimating density and diversity of microorganisms in the air of a hospitals can be an indicator of whether such environments are dirty or clean. In addition, it is considered as a source of hospital-associated infections. Microorganisms are the primary source of air contamination in indoor environments (7). Indoor air has a greater potential to endanger patients health than outdoor air. Indoor aerosol types may have the ability to cause different levels of infection. Although many present biological substances in inhaled air are not considered as pollution but if their amount increases by several folds of their ambient amount, they can stimulate or poison people once inhaled (8). This pollution type includes materials such as air-borne particles, large molecules or volatile compounds that are both alive and released by living creatures. Some bioaerosels such as bacteria and viruses can multiply. Some others, such as pollen of plants and mite droppings may just be irritating(7).

Among indoor environments where bio aerosols are considered a problem, hospitals are of major concern as in these environments there are a wide range of people such as hospital and medical staff, service users, patients and visitors who can contact bio aerosols and inhale them (9). Therefore, the presence of excess bio aerosol in hospitals air can be a serious health threat (10,11). In contrast, providing superior IAQ can

improve health, work performance and school performance, as well as reduce health care costs, and consequently be a source of substantial economic benefits.

Hospital acquired infections are an important cause of morbidity and mortality in hospitals in both the developing and developed worlds. The rate of this infection varies from 5-10% in the developed countries to 25% or more in developing countries. These infections are mostly caused by microorganisms or surfaces contaminated by the microorganisms or air currents and dust containing microbial infections nuclei (8).In the tropics, researchers have revealed that microorganisms such as: *Staphylococcus aureus, Escherichia, coli, Candida spp*, *Streptococcus spp, Klebsiella*, *Penicilliuim, Aspergillus* and *Bacillus* spp are some of the most commonly isolated microorganisms from hospital environments. Moulds are particularly important as a source of indoor air pollution because of their ability to produce harmful spores and mycotoxins (12). These pathogens are capable of causing hospital acquired infections that range from gastroenteritis, bedsores and urinary tract infection.

Healthcare facilities are complex settings, especially in developing countries, where factors such as overcrowding, improper design and ventilation can impact the growth and / or survival of microorganisms. Physical parameters such as temperature and humidity are known to influence the ability of microorganisms to survive and be airborne(13).under this study microbial indoor air quality and drug susceptibility test against bacterial isolates in Jimma University Specialized Hospital and Shenen Gibe Zonal Hospital were carried out.

1.2 Statement of the problem

Bioaerosels in health care settings are particulate matter of biological origin which include, living organisms such as bacteria, virus, fungi, their metabolites, toxins and fragments. Bioaerosels contribute to about 5-34 % of indoor air pollution and their role in healthcare settings has always been a topic of interest for researchers. Hospital environment contains a diverse range of bioaerosels population. The importance of estimation of quantity and type of these bioaerosol has been emphasized due to their effect on human health. They have been implicated in conditions ranging from allergies to disseminated infections in susceptible patients(11).

The complex hospital environment requires special attention to ensure healthful indoor air quality (IAQ) to protect patients and healthcare workers against hospital-acquired (nosocomial) infections and occupational diseases. Poor hospital IAQ may cause outbreaks of building-related illness such as headaches, fatigue, eye, and skin irritations, and other symptoms (6).The quality of air indoors is a problem in many buildings

in developed countries and this problem also existed in developing societies as well. It is fair to say that indoor environment problems still exist in many air- conditioned and mechanically ventilated buildings, even though existing standards may be met (14).

Several microbiological air contaminants are also of importance including moulds and fungi, viruses, bacteria, algae, pollen, spores and their derivatives. In airtight buildings especially (e.g. buildings which are energy efficient, but with poor ventilation), indoor air pollutants can accumulate, causing tight building syndrome(15).The microbial quality of indoor air in hospitals is as much of an issue any other type of buildings, with increased emphasis because of potential severity of the consequences of nosocomial infections. Many patients are actually at increased risk of infection while in the hospital. The problems of NI are generally largest in older hospitals which may have large wards and poor or no mechanical ventilation, and the situations even more difficult in developing countries (16).

The quality of air in hospitals in relation to microbial contamination at a given time period is determined by the quality of air entering into the building, the number of occupants in the building, their physical activities and resultant aerosol generation, human traffic and the efficiency of ventilation(17).
Nosocomial infections are infections acquired by patients when admitted into hospital wards for proper management of their ailment but while on admission, some patients acquire other ailments other than the one they were admitted for. This results from contact with a carrier of the pathogen directly or indirectly through inanimate objects. Improper/unhygienic ventilation system can continually be a source of nosocomial infection. Sneezing has been described as the most vigorous mechanisms of generating millions of air born(18).

Nosocomial infections transmitted by the airborne route, especially fungal infection such as aspergillosis, are a major source of morbidity and mortality in immune compromised patients. Bio-aerosols, of which fungal spores are one of the major types of microorganisms, can be present in all hospital environments, and may be transmitted through indoor and outdoor air, visitors, patients, and air conditioners (19).

Resistance to antimicrobial agents is a problem as well in health care facilities; in hospitals transmission of resistance bacteria is amplified because of the highly susceptible population (20). Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and

resistance has been documented for nosocomial infections (NIs) as well as for resistant community acquired infections. As resistance develops to first-line antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs. The development of antibiotic resistant bacterial strains is a serious threat to present hospital care practice (21).

In Africa especially in Nigeria at different health settings investigation on indoor microbial load determination and microbial diversity were conducted. Most of the results indicated that the health settings were highly contaminated. Here, In Ethiopia also some studies were conducted to show the status microbial indoor air quality. But it is so difficult to find standards in health care settings relating to indoor microbial concentration and their diversity. Many health facilities have an experience in exercising Infection prevention practice. However, these are not effective without considering the microbial indoor air quality. The present study was carried out to show the status of microbial pollutions and drug resistance pattern of bacterial isolates.

1.3 Significance of the study

In indoor air quality in public health care facilities are given unsatisfactory attention. Few studies (in a few health care facilities) were studies conducted on determination of microbial indoor of air quality in Ethiopia. Most public health care facilities built and infection prevention practice are also practicing without considering microbial indoor air quality. The patients, visitors and health personals can be affected by poor indoor air quality since most pollutants released from health care facilities airborne pathogens and highly contribute for the occurrences of nosocomial infections. This study will significantly use to give insight on the quality of indoor air in health care facilities. The study will also identified potentials factors that enhance microbial load and take on improvements to produce good indoor air quality. The investigation is essentially provide information for health planners and policy makers in designing a strategy for improvements indoor air quality in health care facilities and to set standards. This study hopefully provide additional information on indoor air quality of health care facilities and fill research gaps and add to existing body of knowledge.

CHAPTER TWO: LITRATURE REVIEW

2.1 Air borne microbial load and compositions in health care settings

Health care facilities, indoor air contain a diverse range of microorganisms. Air borne microbes were detected in hospitals by different investigators (2). Two studies were conducted in Nigeria hospitals and health centers determined the microbial load of indoor air in different wards. Both of the studies were collected samples using the settle plate method for the enumeration of bacterial and fungal isolates. Results obtained from the study conducted in general hospital and a health center in Rivers State Nigeria showed that bacterial and fungal counts were varied morning and afternoon (11).

A quantitative and qualitative study of indoor air in two hospitals in Ekpoma, Edo State was carried out. According to this study the aim is at checking the microbial concentration of indoor air as it relates to hospital type, ward and sampling time. As the study revealed like that of other study the results is differ from hospitals to hospitals. In this study the bacterial and fungal population enumerated. The microbial flora also isolated included six bacteria and six fungal genera. The identified bacterial and fungal isolates were *Staphylococcus* aureus*, Klebsiella* spp*, Streptococcus* spp*, Bacillus* spp*, Pseudomonas spp, Escherichia coli*, *Aspergillus spp, Penicillium spp, Candida spp, Trichophyton spp, Microsporium spp and Rhizopus spp*. The degree of microbial distribution was highest in the waiting room and lowest in the theatre (2).

An investigation of the air quality and quantity of airborne microbes in selected hospitals of Zarqa City, Jordan, revealed that nine bacterial species were identified. The study was conducted in one private and governmental hospital. A microbial air sampler (PBI International, Milano, Italy) was used for sampling of airborne bacteria and fungi. In a governmental hospital, *Staphylococcus aureus* (16.2%) was found to be the most common organism, followed by *Micrococcus luteus*(13.3%) and *Coagulase-negative Staphylococcus* (17.2%), followed by *S. aureus* (16.8%) and *M. luteus*(10.7%) were found to be the most common in a private hospital. *Aspergillus* spp*., Penicillium spp.*, *Rhizopus spp*. and *Alternaria spp*. were isolated in both hospitals. As study finding showed indoor air of the governmental hospital was more contaminated than that of the private hospital in all units. Maximum bacterial rates were detected in the patient rooms, while minimum bacterial rates were detected in the operating rooms and neonatal wards. The time of visit showed higher microbial rates in governmental hospital, while the private hospital was not affected by this factor. Several explanations might be involved in these variations, that is, the age of hospital building, the number of beds, the number of visitors, disinfection procedures and ventilation systems. Finally the investigators were

concluded that the indoor air quality of hospitals in Zarqa city, especially the governmental hospital, needs more care and surveillance and should be given priority in Jordan (16).

Study was conducted in one private hospital (the Faith Medical Center) and one governmental hospital (Central hospital) in Benin City, Nigeria is aimed in determining air borne bacteria concentration and fungal isolates. As study indicated that the settle plate techniques were used for enumeration of bacteria and isolates of fungi. As the investigation revealed air samples from five wards of two hospitals were collected. Air samples were undertaken in three times a day. As the study mentioned that the highest bacterial population was recorded in the evening, ranging from 15 cfu/m^3 to 47 cfu/m^3 in the Faith Medical center and 17 cfu/m^3 to 52 cfu/m^3 in the Central hospital. The fungal concentration also determined with values ranging from 10 cfu/m^3 to 53 cfu/m^3 in both hospitals. The microbial isolates characterized and identified include six bacterial and four fungal genera, among which are the bacterial isolates: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteusmirabilis* and *Klebsiella aerogenes* and the fungal isolates include *Aspergillus, Penicillum, Mucor,and Fusarium* (23).

Study conducted in Olabisi Onabanjo University Teaching hospital in Nigeria, also revealed the quality of indoor air of different wards and units. During air sampling sedimentation techniques were used. The investigators were taken the sample after cleaning and before influx of people or patients into the wards. Three petri dishes were employed in each ward and samplings were done twice daily. As study indicated there was significant difference in the bacterial populations of the different sampling time. The bacteria isolates were *Staphylococcus aureus* , *Klebsiella sp, Bacillus cereus, Bacillus subtilis, Streptococcus pyogenesand Serratia marscences* while the fungi isolates included *Aspergillus flavus, Penicillium sp. ,Fusarium sp., Candida albicans and Alternaria sp. Staphylococcus aureus*was predominantly isolated bacterium while *penicillium sp*. Was the most isolated fungus (24).

Another study which was undertaken after two years in Teaching Hospitals in Nigeria, also determined microbiological load and composition of indoor air in different wards. The microbiological samples were collected from nine unites (25) , in the Teaching Hospital using the exposed prepared plate techniques. The air samples were collected thrice daily. The concentration of airborne bacteria and fungi in the nine different hospital units varied from wards to wards. The bacterial population ranges from 3.0 cfu/m^3 to 76.0 cfu/m^3, with the highest bacterial population recorded in the accident and emergency ward. The fungal population ranges from 6.0 cfu/m^3 to 44.7

cfu/m^3, while the highest fungal population was recorded in the accident and emergency ward. The micro flora characterized and identified, were representative of the normal microflora of the human body (skin, gastrointestinal tracts, respiratory tract) and the opportunistic pathogens. The microbial isolates included six bacterial genera, among which are, *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Bacillus* sp. and *Proteus mirabilis,* the fugal isolates included, *Aspergillus* sp, *Penicillium* sp., *Mucor* sp., *Candida* sp and *Verticillium* sp. The concentrations of airborne microflora recorded in the hospital environment, specifically in the accident and emergency ward was significantly different from other wards (26).

In the same fashion study was undertaken in determining air borne microflora load and biodiversity in two selected hospitals, India. The investigation was conducted in one governmental and one private hospital. Samples were collected using the settle plate techniques and "Hi Air" air sampler for enumeration bacterial and fungal isolates. Variety of bacteria and fungi were isolated using wide range of agar. Microbial aero biodiversity as well as microbial load enumerated in hospitals. Microbial load was highest in general ward and lowest in I.C.U. in both hospitals. *Aspergillus sp.* Were the most common isolate. *Staphylococcus* spp is common bacterial isolate (2).

An investigation conducted in the General Hospitals of Korea related to distribution characteristics of air borne bacteria and fungi revealed that the Mean concentrations of airborne bacteria and fungi were the highest in main lobby as followed by an order of surgical ward, ICU and biomedical laboratory. The study showed the predominant genera of airborne bacteria identified in the general hospital were *Staphylococcus* spp. (50%), *Micrococcus* spp. (15–20%), *Corynebacterium* spp. (5–20%), and *Bacillus* spp. (5–15%). On the other hand, the predominant genera of airborne fungi identified in the general hospital were *Cladosporium* spp.(30%), *Penicillium* spp. (20–25%), *Aspergillus* spp. (15–20%), and *Alternaria* spp. (10–20%). Under this study air sampling were done using active sampling method specifically impactor device (27).

As the results demonstrated in the study conducted in Iran evaluation bioaerosels among selected hospital wards, highest and lowest averages of bioaerosels density were obtained from Shahid Beheshti and Fatemieh Hospitals (36.18 cfu/m^{3}Vs. 24.03 cfu/m^3), respectively. Highest and lowest concentrations of bioaerosels were found in Women1 and operating room wards of Fatemiyeh Hospital, respectively (54.4cfu/m^3 VS. 13.3cfu/m^3). It appears that there had been no significant

correlation between concentration of bioaerosels in the hospitals and available guideline values (P = 0.3). The highest fungal populations were identified in the study included and more similar with other studies conducting in health care facilities, *Penicelium spp.* (32.06%), *Cladosporium spp.* (20.5%), *Aspergillus fumigatus* (14.61%) and *A. Niger* (7.43%), respectively. The highest bacterial population was *coagulase-negative staphylococci* (32.49%), *Bacillus spp.* (14.74%), Micrococcus spp. (13.68%) and *Staphylococcus aureus* (11.34%), respectively (10).

2.2 sources and influencing factors

The source and spread of microorganisms inside the hospital are important issues. The most potential source of airborne microbes inside the hospital is the infected patients. Airborne transmission occurs when pathogenic microorganisms are transferred from an infected to a susceptible individual via the air. Another source of microorganisms in the hospital are occupants of the building i.e. hospital personnel and visitors. Amount of materials brought from outside such as personal belongings, food and fruits are recognized as source of contamination. Bacteria in indoor air are mainly Gram positive cocci which arise from occupants. Generally they are not dangerous for human health but high viable counts are used as markers of crowded conditions and poor ventilation. A positive correlation between bacterial counts and occupant density in different hospitals was suggested. Dressings and bedding also can be the sources of airborne microorganisms. Sweeping of floors and changing of bed linens also can cause suspension of bio-aerosols in air. Various studies suggest that the distribution of microorganisms in the air varies among geographic areas and is also influenced by seasonal environmental and climatic factors such as temperature, humidity, time and wind speed. Significant monthly variation and daily fluctuation (time of sampling) in concentrations of airborne bacteria and fungi in a hospital ward was indicated (11). Kind of hospital along with the type of room and the time of sampling is a significant factor that influences the rate of indoor air microorganisms (19). Among all possible sources, outdoor air is thought to be the most important source of indoor microflora. Many studies have reported the role of outdoor microbial concentration through opened windows and doors in raising the microbial rates and homogenization of indoor air of building (12).

The indoor air quality can be compromised by improper maintained heating, ventilation and air conditioning system (HVAC). As different studies revealed that the moisture contents of building materials, relative humidity and temperature, outdoor concentrations, air exchange rates and number of people and pets significantly affect the load of indoor air bioaerosoles. In addition to these poor

and deficient hygienic conditions, low degree of cleanness and minimal disinfection procedures against airborne bacteria and fungi might raise the airborne bio contaminations (5).

According to several studies, there are factors affecting microbial load and indoor environment mainly include   the moisture content of building material, relative humidity and temperature, outdoor concentrations, air exchange rates, air movement, ventilation, particle pollutants, gaseous pollutants and number of people and pets. Generally higher concentrations of bio aerosols have been reported from warmer than cooler climates. Moreover, housing conditions, the activities and life style of occupants considerably contribute to the varying concentrations. Under normal conditions, bacteria and fungi do not notably grow in building materials or structures or on indoor surfaces, mainly because of lack of moisture (22).

In non-industrials indoor air, the most important sources of air borne bacteria is human being. Human activities like taking, sneezing, coughing, washing and toilet flushing are main sources for airborne bacteria. Various fungal spores release from Food stuffs, house plants and flower pots, house dust, pets and their beddings, textiles, carpets, wood material and furniture stuffing (13). In additions to these important sources of indoor air pollutants include outdoor air, the human body and human activities, emission from buildings materials, furnishings and appliances and use of consumer products.

The sources of indoor pollutants can be divided in to outdoor and indoor. The indoor pollutants are commonly emitted from indoor compartments, e.g. waxes, paints, furnishings, clothing, buildings materials and personal sources (28). Bacteria, fungi, pollen, viruses, rat droppings, mites, insect body parts or bird droppings can be sources of biological contamination of indoor air (29).

If ventilation and air conditioning system is not installed or properly maintained, indoor air pollution become more worsens. Biological contamination can also proliferate in moist components of the system throughout buildings (6).

2.3 exposures and health risks

Poor hospital IAQ may cause outbreaks of building-related illness such as headaches, fatigue, eye, and skin irritations, and other symptoms (30). Air borne fungi are not uncommon and for most individuals breathing typical ambient concentration of air borne fungi results in no adverse effects on health due to healthy immune system. However, hospitalized patients with immune system suppression are susceptible to infection from natural occurring air borne fungi that can grow at body temperatures. The incidence of infections caused by fungi that were once considered only

saprophytic has risen dramatically in recent years, for example hospitalized mortality caused by invasive *asperigllosis. Asperigllosis* is acquired by inhalation of air borne dusts particles that carry the spores. Pneumonia develops and the fungus disseminates through the blood stream to other organs. Mortality rates have been reported as high 95% in bone marrow transplant patients: 13-80% in leukemia patients: and 8-30% in kidney transplant patients. One study confirmed that about 9% of reported hospitals infections in the era of 1986 to 1990 were caused by fungi (25).

Some human diseases such as tuberculosis, Legionnaires' disease and different forms of bacterial pneumonia, coccidioidomycosis, influenza, measles, and gastrointestinal illnesses are the result of exposure to bioaerosels. In addition, they are associated with some noninfectious airway diseases, such as allergies and asthma (10).

Nosocomial infections transmitted by the airborne route, especially fungal infection such as aspergillosis, are a major source of morbidity and mortality in immune compromised patients. Bio-aerosols, of which fungal spores are one of the major types of microorganisms, can be present in all hospital environments, and may be transmitted through indoor and outdoor air, visitors, patients, and air conditioners (31).

Sufficient evidence exists to conclude that the exposure to specific types and concentrations of airborne mould or fungus in damp indoor environments is associated with increased respiratory irritation in some individuals. Signs and symptoms may include coughing, wheezing, and nasal congestion. Under severe mould exposure conditions, hypersensitivity pneumonitis has been reported in susceptible persons. Although a relationship between mould exposure and human health is not clearly defined, some of the occupants of damp or mouldy buildings have a greater risk of respiratory symptoms and asthma (11).

Airborne microflora of hospital environment has been a subject of numerous studies as it is a potential cause of hospital infection (27,29). Exposure to air borne bacteria and fungi causes potential biological hazard and have been associated with adverse health effects. Many species of bacteria as *Streptococcus pyogenes*, Mycobacterium tuberculosis, *Legionella pneumophila* and viruses may cause severe human infections. *Staphylococcus aureus* is a known opportunistic pathogen, which causes infection at sites of lowered host resistance, such as damaged skin and mucous membranes.

Prevalence of S. aureus and *Ps. aeruginosa*in almost all sampling sites, irrespective of season indicating their long term survival and consequent threat to hospitalized patients as well the working employees (32,33).Gram negative bacteria found in the air of the hospital ward could be a source of adverse endotoxin and *Acenetobacter* strains may be a potential cause of hospital infections transmitted by air. *Pseudomonas spp*. is difficult to eradicate from hospital as it is resistant to many of the disinfectant and antiseptics commonly used in hospitals (12).

2.4 microbial load standards

There is no uniform internationally accepted threshold limit value for airborne bacteria and fungi. Published values vary from country to country according to their sampling methods and climatic conditions. For the hospital environment 100 cfu/m^3 is the maximum limit for bacteria and 50 cfu/m^3 for fungi (34). Type of microbes should also be taken into consideration as microbial quality of indoor air is created not only by a total concentration of bacteria and fungi but by the presence of some particular species, which is very important for the health of people occupying the room (35). The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments mentioned that Overall Density of Total Bioaerosels Concentration (fungal and bacteria) should be not exceed 1000 CFU/m^3 (23). The sanitary standards of European Commission for non-industrial premises stated the level of contamination of indoor air by fungus and bacteria as follow 50 CFU/m3 as 'very low' bacterial load, 50–100 CFU/m3 as 'low', 100–500 CFU/m3 as 'intermediate', 500–2000 CFU/m3 as 'high' and above 2000 CFU/m3 as 'very high' load (36).

2.5 Antimicrobial susceptibility

There are numerous reported cases of emerging nosocomial infections caused by methicillin resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA) and other multi-drug (MDR) resistant strains (37).

Nosocomial infections in hospitals constitute major health concerns, for both patients and hospital employees, particularly in view of the spreading of antibiotic resistances among bacteria. Immune compromised patients are especially at risk for opportunistic microorganisms infections. Many bacteria are responsible for nosocomial infections (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa, and Clostridium difficile*) and countless cases are reported every day (38).

Study conducted at JUSH in 2011 and other similar studies showed the susceptibility patterns of isolates showed varying degrees of resistance to the antibiotics tested. *S. aureus* showed 100%

resistance to methicillin, 78% to ampicillin, 71.5% to penicillin and the least resistance which is 9.6% was observed for ciprofloxacin. On the other hand, *S. aureus* isolates were 100% sensitive for vancomycin (21,23).

Study undertaken in Hawassa Teaching and Referral Hospitals, The isolates showed different degree of susceptibility against tested antimicrobial agents. Gram positive isolates were sensitive to gentamicin 255 (80.3%) following by norfloxacin (63.6%), while they were resistant to amoxicillin (63.6%) and penicillin (57.6%) (39).

2.6. Control measures of microbes in health care settings

Control of airborne pathogens in hospitals is important for the safety of both the patient and hospital personnel. Although it is not possible to eliminate all nosocomial infections, their incidence can be significantly reduced by implementation of appropriate infection control policies. These are certain control measures which can be used to reduce microbial load in healthcare settings (11). The following points are important to control microbes in indoor air. These include appropriate ventilation, housekeeping activities, movement restriction, and quarantine, temperature and humidity maintenance.

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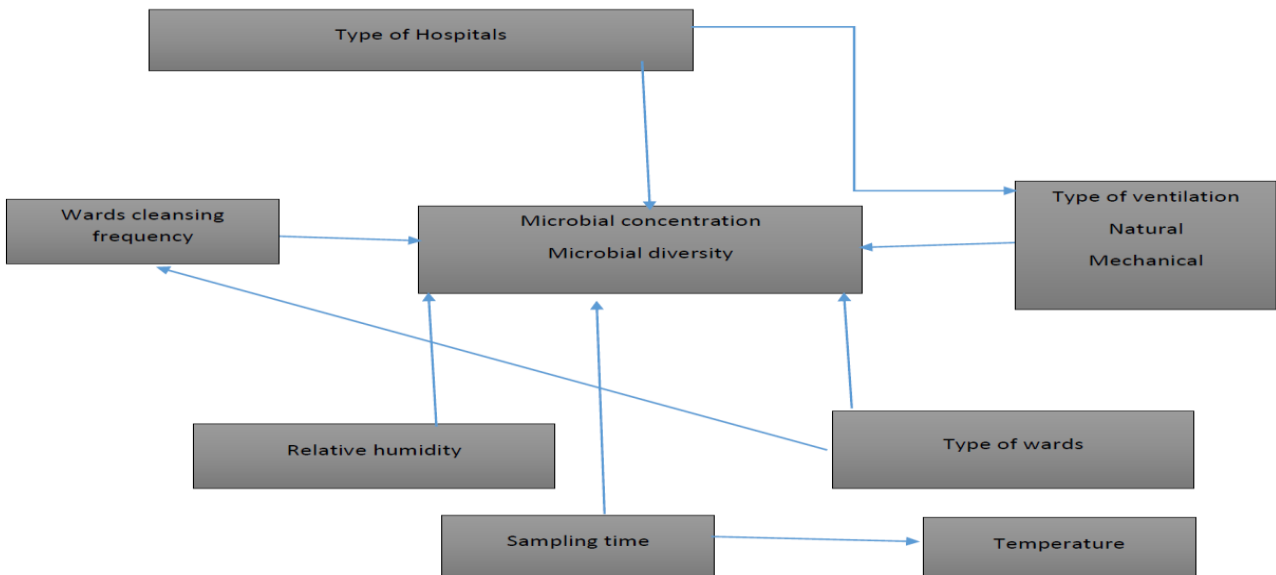
Figure 1. Schematic presentation of conceptual frame work for the study of microbial indoor air quality in JUSH and Shenen Gibe Hospital, 2016

CHAPTER THREE: OBJECTIVES

3.1 General objectives

✓ Assessment of microbial indoor air quality and drug susceptibility test against bacterial isolates: in case of JUSH and Shenen Gibe Hospital, Jimma town, southwest Ethiopia.

3.2 Specific objectives

✓ To evaluate microbial concentrations of indoor air in JUSH and Shenen Gibe hospital Southwest Jimma

✓ To characterize microorganisms of indoor air in JUSH and Shenen Gibe hospital, Southwest Jimma

✓ To conduct drug susceptibility test against bacterial isolates in JUSH and Shenen Gibe hospital, Southwest Jimma

✓ To assess factors influencing microbial indoor air quality in JUSH and Shenen Gibe hospital, Southwest Jimma

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Description of the study area

The study was conducted from May to October, 2016 in Jimma University Specialized Hospital (JUSH) and Shenen Gibe Zonal Hospital , located 352 Km away from Addis Ababa, south west Ethiopia those are found in jimma town. Jimma University Specialized Hospital is established in 1937 and the only teaching and referral hospital in the southwestern part of Ethiopia. It has a bed capacity of 450 and a total of more than 750 staffs of both supportive and professional. It provides services for approximately 9000 inpatient and 80000 outpatient attendances a year coming from the catchment population of about 15 million people. out of twenty wards ten wards were selected for sample collection from jimma University specialized hospital namely Operating Room, Intensive Care unit, Emergency OPD, Laboratory, Medical ward male and female, Surgical male and female, Pediatrics and maternity. Shenen Gibe hospital is established in 2012 and   also provides services for 1.5 million catchment area population. The hospital has 50 bed capacity and 192 staff s of both supportive and professionals.   Eight wards were selected from total of twelve wards.

4.2 Study period

The study was carried out during the period from May to October 2016 in Jimma town.

4.3 Variables

4.3.1 Dependent variables

- ✓ Bacterial and fungal load
- ✓ Microbial isolates

4.3.2 Independent variables

Time of sampling

Type of hospitals

Ventilation system

Temperature

Wards

Wards cleaning frequency

Relative humidity

4.4 Study design

Quantitative and qualitative laboratory based Cross-sectional study were conducted to determine microbial indoor air quality and drug susceptibility for bacteria isolates from two different hospitals in Jimma town.

4.5 Sampling technique and sample size

4.5.1 Sampling technique

Purposive sampling was used in selecting wards and then random sampling technique using lottery method were used to select rooms from selected wards of the hospitals.

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┌─────────────────────────┐                    ┌──────────────────────────────────────────┐
│   JUSH (20 wards)        │                    │  SHENEN GIBE HOSPITAL (12 wards)         │
└─────────────────────────┘                    └──────────────────────────────────────────┘
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JUSH (20 wards)

SHENEN GIBE HOSPITAL (12 wards)

8 wards are selected in convenience or purposive

1. Medical Female
2. Medical male
3. Gynecology
4. Laboratory
5. OPD
6. Minor OR
7. Pediatrics
8. Delivery

10 wards are selected in convenience or purposive

1. ICU
2. OR
3. Medical Female
4. Medical male
5. Surgical female
6. Surgical Male
7. Maternity
8. Laboratory
9. Pediatrics
10. Emergency OPD

10 rooms/units from JUSH and 8 rooms/units from Shenen Gibe hospital

Figure 2 Schematic presentation of sampling techniques in two selected hospital wards in assessment of indoor microbial air quality, Jimma Town

Different locations where most activities and tasks are performed inside the two hospitals were selected as sampling points for air pollutants. These locations were included medial ward A, surgical ward A, laboratory department, the emergency OPD, the intensive care unit (ICU), the Operation Room (OR), and the pediatric unit and maternity unit. Other factors studied included the type of

activity carried out at each selected location inside the hospital, time of sampling, and the effect of level of temperature and building condition were surveyed using checklist.

4.5.2 Total plate required for fungal and Bacteria
Sample size was determined on convenience.   For JUSH ten selected wards 80 blood agar plates and 80 potato dextrose agar plates were exposed for air sampling four petri plates were used for air sampling including control in one session. In the same fashion 64 blood agar plates and potato 64 dextrose agar plates for eight Shenen Gibe hospitals selected were surveyed. Eight settle plates were exposed in each studied ward. Therefore, a total of 288 settle plates were used for air sampling in both hospitals.

4.5.2 Sampling time
Samples were collected for twice a day (in the morning between 8:00-11:00 Am and 2:00- 5:00 PM and in the afternoon between.

4.6 Air sampling and microbiological examination
Laboratory procedure and reagents required were well described in Annex I.

4.6.1 Air sampling
4.6.1.1 Air borne bacterial sampling using blood agar
Air sampling was performed with settle plate method. Settle plate samples of indoor air from the selected rooms of the hospitals were collected without controlling any indoor environmental condition. The air samples were collected by exposing 10 cm diameter blood agar plates, in the air labeled with room number, time and date of sample collection and then transported to selected rooms and placed lid open at 1metre above the ground for 1 hour and then the plates were covered with their lids and taken to Environmental Health Science and Technology   laboratory and incubated aerobically for 24-48 hour at 37^0 C to allow the growth of bacteria.

4.6.2 Air borne bacteria examination
All blood agar plates were incubated at 37^0C for 24-48 hours. The total numbers of bacterial colony forming units per cubic meter were counted and recorded The isolated micro-organisms were characterized morphologically and based on its colony size, shape, margin, opacity, elevation, pigment production and Gram's character and identical colonies was sub cultured into nutrient agar and MacConkey agar plates incubated at 37^0C for 24 hours and stored for further examination. Bacterial isolates were Characterized and identified according to the methods of Buchanan and Gibbons (40,41).

4.6.2.1 Gram stain

A drop of normal saline was placed on clean and dry slide. The smear was fixed by dry heat, covered by crystal violet for 1 minute and then the stain was washed with clean water. Lugol's iodine was added for 30-60 seconds and then washed by clean water. The smear was decolorized by 70% alcohol for few seconds, and then washed by clean water. Saffaranine was added for 2 minutes, and then washed by water. Smear preparations were air-dried and examined by light microscope using high-resolution objective power with oil immersions ($\times$100) (40,41). The gram positive rods shape bacteria were reported as bacillus. Gram positive cocci clusters and chains were further examined using enzyme test such as catalase and coagulase test. Gram negative bacteria were further analyzed using different media and test to isolates the species (ANNEX I).

Figure 3. Gram staining procedure were conducted in Environmental health biology laboratory

4.6.2.4 Manitol fermentation

Test organism was inoculated into manitol salt agar, incubated at 37°C and examined after 24 hours for manitol fermentation; it was indicated by formation of yellow color around the growth (40,41).

4.7. Biochemical tests
4.7.1. Indole test

The test is based on bacteria that break down the amino acid tryptophan with release of indole. The test was done by inoculating the tested organism on normal saline water and then incubated at 37°C for 24 hours. Indole production was detected by adding Kovac's reagent. The result was observed by formation of red ring in the surface of the tube (40,41).

4.7.2 Motility test

This test is used for performing the detection of motility of gram-negative enteric bacilli. The test was done by inoculating the tested organism on normal saline water and then incubated at 37°C for 24 hours.

Bacterial motility was observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of nonmotile organisms only occurs along the stab line (40,41).

4.7.3 Simmon Citrate utilization test

This test is based on the ability of bacteria to utilize citrate as source of carbon and ammonium as source of nitrogen in the presence of bromothymol blue as an indicator. Inoculation was done by a sterile loop into broth medium and incubated at 37°C for 18-24 hours. Positive result detected by changing of the indicator's color from green to blue (40,41).

Figure 4. Displayed prepared simmon citrate agar in test tube for biochemical test

4.7.4 Urea hydrolysis test

This test is used to identify bacteria, particularly those growing naturally in an environment which produce urease enzyme to break down the urea into ammonia and carbon dioxide, which lead to change the pH to alkaline in presence of phenol red indicator. The test was done by inoculating the test organism in urea agar and then incubated at 37 °C for 24 hours. Positive result appeared in the changing of indicator's color from yellow to pink (42).

4.7.5 Lysine iron agar test

This test is used for the differentiation of microorganisms on the basis of lysine decarboxylase and hydrogen sulfide production. The test was done by inculcating the test organisms in LIA then incubated at 37 °C for 24 hours. A positive lysine deaminase reaction is a red slant. A negative reaction is a purple slant. A negative reaction is a purple slant. A positive hydrogen sulfide reaction is blackened medium at the apex of the slant (42).

4.7.6 Triple iron sugar agar test

The test is used to differentiation of microorganisms on the basis of dextrose and lactose fermentation and none lactose fermenter and hydrogen sulfide production. The test was done by inoculating the test organism in TIS agar and then incubated at 37 °C for 24 hours.

An alkaline Slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose. An alkaline slant-alkaline butt (red/red)

indicates dextrose and lactose did not ferment (non-fermenter). Cracks, splits, or bubbles in the medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production (42).

Figure 5. Showing biochemical test media in test tube motility, urea, simmion citrate agar, triple sugar iron agar and lysine iron agar

4.7.8 Catalase test

This test is used to differentiate between staphylococci (which produce catalase enzyme) from streptococci (which cannot produce catalase enzyme). One drop of hydrogen peroxide (H_2O_2) solution was placed on slide, and small amount of bacterial growth was added by wood stick. The formation of air bubbles indicated positive result (42).

4.7.9 Coagulase test

This test is used to identify *S. aureus* which produces coagulase enzyme. This enzyme can clot the plasma by converting fibrinogen to fibrin. The test was done by placing drop of plasma on slide and then the organism under test was added and mixed gently. Positive was detected by the clumping of bacterial cells within 10 seconds (42).

4.8 Air borne fungal sampling

Air samples were collected using 10 cm Petri dishes plate exposure or passive method and count method. Culture plates containing potato dexstrose agar with supplemented of 10% tartaric acid was exposed on a stool and allowed the 1/1/1 sampling scheme to settle plates are positioned one meter off the floor, one meter from the walls or any obstacle and left open for one hour . The Petri dishes were labeled with room number, time and date of sample collection and transported to selected rooms and placed lid open at 1metre above the ground which is human breath zone and located at the centers of rooms for 1 hour and then the plates were covered with their lids and the four replicates one control of each media was   used for

isolations of fungi. Thereafter, the plates were   covered and transferred immediately to the laboratory for incubation for 25°C to $28\,^{\circ}$C for 3 to 4 days.

Figure 6. Displayed from right to left sealed media container, labeling on lid of petri dishes air sampling using a stool allowed the 1/1/1 for measuring the microbial air contamination in hospital environments

4.8.1 macroscopically and microscope examination of fungal

The fungal colonies were enumerated after which morphological and colonial characteristics of each colony was identified according to the manual of Barnett and Hunter (43).

The fungal morphological studies consisted of mycelium growth, colour, and characters of fruiting bodies of fungi. Macroscopic characters like fungal colony growth, colour, texture, shape, reverse colour, exudates, and margin of the colony were noted for fungal identification. Microscopic examination of the fungal hyphal characteristics was carried out. A portion of the obtained culture was placed and teased out into a clean glass slide upon a drop of lactophenol cotton blue using sterile inoculating needles and covered with 22 mm x22 mm clean coverslip(ANNX I). Then sealed the edges of coverslip attached with nail polish or per mount to preserve as references (43).

Microscopic characterization of all the fungal isolates was done by making the slides of different fungal species. The documentation of isolates i.e. size of head, vesicle shape, phailides, matulae, conidiophores and conidia characters like:, wall, shape, surface and conidia attachment with condiophore was done microscopically. Identification was done by comparing the data with the synoptic keys published by Barnett & Hunter and identification keys for *Aspergillus* species (44,45).

Microscopic examination of the fungal hyphal characteristics was carried out. This was done by taking a tiny portion of the fungal colony using an inoculating needle and macerating it on a clean slide. Lactophenol cotton blue was then added to them microscopic slide after which covers slip was placed on the emulsion before observing microscopically.

4.8.2 Colony enumeration and conversion

The results were described in both colony forming unit and colony forming unit per cubic meter of air. The total number of colony forming unit (cfu) were enumerated and converted to organisms per cubic meter air. The numbers of microorganisms as CFU/M^3 were estimated using Koch sedimentation Method according to Polish standards (46):

$$N = 5a * 10^4 (bt)^{-1}$$

Where

N=microbial CFU/m^3 of indoor air;

a= number of colonies per Petri dish;

b=dish surface, cm^2;

t= exposure time, minutes

4.9 Antimicrobial sensitivity test

Kirby-Bauer agar disk diffusions method on Mueller-Hinton agar (MHA) media was used to determine antimicrobial susceptibility of identified microbes (**Annex I**). Antimicrobial sensitivity tests were performed on Mueller-Hinton agar (MHA) media for bacteria isolates with five commonly used antibiotics (vancomycin (VA, 30μg), gentamycin (CN, 10μg), Ampicillin (AM, 10μg), ceftriaxone (CRO, 30μg) and norfloxacin (NOR, 10μg) by Kirby-Bauer agar disk diffusions method matching tests to 0.5 McFarland turbidity standards. Then after the antimicrobial sensitivity result were interpreted according to the principles established by clinical and laboratory Institute (41,47) by measuring the zone diameter of inhibition .The results were reported as susceptible, intermediate or resistance according to interpretative chart of complete growth inhibition zone diameter sizes for bacteria using the modified Kirby–Bauer disk diffusion technique (42). Controls were used for each test and the control was *E. coli* ATCC 25922 which is obtained from Ethiopian Health and Nutrition Research institute laboratory.

Figure7. Kirby-Bauer disks diffusions susceptibility test, placement of antibiotic disks, inoculation of suspensions

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┌─────────────────────────────────────────────────────────────────────────────────┐
│  Prepared Blood agar plates (144 plates for both hospital) and Potato dextrose    │
│  agar plates (144 plates for both hospital)                                       │
│                  Transported to the studied wards                                 │
└─────────────────────────────────────────────────────────────────────────────────┘
                                      │
                                      ▼
┌─────────────────────────────────────────────────────────────────────────────────┐
│      Air sampling were collected using passive sampling techniques (1/1/1         │
│      schedule)                                                                    │
│   Immediately transported to Environmental Health science and technology          │
│   laboratory                                                                      │
└─────────────────────────────────────────────────────────────────────────────────┘
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Potato dextrose agar plate were incubated at 28° C for 3 to 4 days

Blood agar plate were incubated 35° to for 24-48 hours

Colony forming unit were counted from all positive potato dextrose agar

Colony forming unit were counted from all positive blood agar

Fungal characterization were done macroscopically an d microscopically (Barnett, 1974)

The numbers of microorganisms as CFU/M³ were estimated using Koch sedimentation Method

$$N = 5a \cdot 10^4 (bt)^{-1},$$

Characterization of bacteria according to Buchman (Buchman, 1974)

Fungal isolates were identified

DST was performed for bacterial isolates with five commonly used antibiotic (WHO, 2003)

Fig showing schematic presentation of the study in hospitals, Jimma town, 2016

Figure 8. Showing schematic representation of the method of the study microbial indoor air quality in hospital

4.10 Data analysis and processing

Calculated CFU/m^3 bacterial and fungal concentrations were statistically analysed using SPSS version 20 Statistical software and Microsoft Excel. Analysis of variance (ANOVA) at $\alpha=0.05$ was conducted to determine sampling time, type of hospital and type of wards significance, and separated using Tukey Multiple Comparisons Test at 0.05 level test were conducted to compare the difference of individuals mean.

4.11 Data Quality Management

Data quality were assured during media preparation, air sampling, coding, transporting, sample processing entry and analysis. The appropriate media were prepared based on the standards method before air sampling. Aseptic techniques like utilization of safety clothes; sterilization of sampling utensils; cold storage and handling of serialized utensils; proper incubation of samples were applied. Controls were also used to check the presence of cross contamination.

Figure 9. From right to left showing sterilization of petri dish in dry oven, in autoclave and handling of utensils in safety cabinet

4.12 Ethical Clearance

Ethical clearance was obtained from ethical committee of Jimma University, college of public health and medical science. Permission paper was obtained from Administration of JUSH and Shene Gibe Hospital.

4.13 Dissemination plan

The findings of this study will be disseminated to college of public health and medical science and department Environmental Health Sciences and Technology, JUSH and Shene Gibe Hospitals. Finally effort will be made to present in various seminars and workshops and for publication in international journal.

CHAPTER FIVE: RESULTS

This study was conducted to elucidate the distribution pattern of airborne fungi and bacteria and drug susceptibility test against bacterial isolates over five months of the year in 2016. Relations to the present study 10 wards from Jimma University Specialized Hospital and 8 wards from Shenen Gibe Hospital i.e. 288 air samples evaluated for Bioaerosels composition analysis and microbial concentration were reported in terms of cfu per plate and cfu/m^3. For each air sample air borne fungal and bacteria were identified. Samples were taken simultaneously from both hospitals in order to compare the load and bioaerosels composition.

Eighty (80) air samples from Jimma University Specialized Hospital and sixty four (64) from Shenen Gibe Hospital totally 136 air samples were collected for examination of air borne fungal and equal number of air samples were also taken for enumeration and characterization of air borne bacteria. Over all density and average bioaeroaols concentration were measured based on each hospital and studied wards. The fungal samples were collected on potato dextrose agar supplemented with 10% tartaric acid and all PDA and blood agar samples were yield fungal colony and bacterial growth respectively. In the same manner, bacteria were collected using blood agar and all samples were yield bacterial growth. All controls were not yield growth.

5.1 Environmental parameters and building condition of studied hospitals

Relative humidity and room temperature also were measured at the time of sampling. In both hospitals these parameters were measured using smart phone applications. The mean of room temperature was $23.3C^0$ and relative humidity was 76.35 respectively for both hospitals. The temperature (20–28 °C) and humidity (66–85 %) range were recorded and temperature was in the ranges which inhibit microbial multiplication (10). Most of the studied wards were characterized by poor waste management and unhygienic housekeeping. Almost in all wards in both hospitals were used dry sweeping frequently which facilities bioaerosels to spread in to air and compromised the quality. Based on the observation the hospitals were practicing housekeeping twice per day regularly. Some of the wards were used disinfectant like chlorine solution for cleaning the contaminated floor bun not regularly.

5.2 Fungal concentration or load in JUSH

Fungal load were determined in estimated in colony forming unit per cubic meter of air. The range of microbial distribution were found between 176.87 and 817.14 cfu/m^3 the mean fungal concentration of all studied wards were also recorded which accounts 427.81 $\pm$ 176.87 cfu/m^3. Figure 10. Highlights the level of fungal concentration in each investigated wards during morning and afternoon. The highest load was detected in emergency OPD (i.e., 711 cfu/m^3) medical ward a male and surgical ward male. These areas were characterized by patient numbers and average occupants. Relatively the lowest concentration also detected in intensive unit care (which was 223 cfu/m^3) of the air.
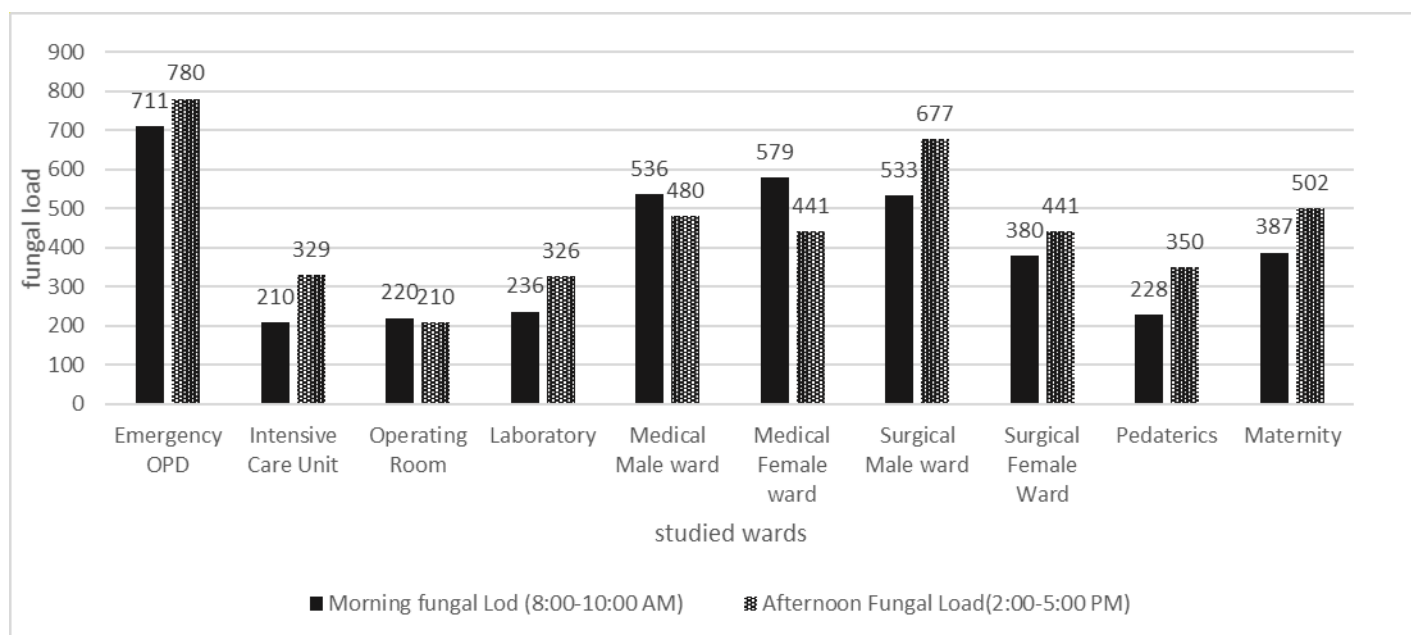
Figure 10. Fungal concentration of indoor air of Jimma University specialized hospital after 60 minutes exposure in the morning and afternoon

5.4 Statistical significance test for mean fungal concentration among different wards

Airborne fungal loads obtained by passive method with an exposure for 60 minutes were found to vary with each other and found to be statistically significant. One way ANOVA test was conducted to obtain the mean fungal concentration of wards as presented below. The highest mean fungal concentration (817.41 cfu/m^3) was found in Emergency OPD and the least (214.97 cfu/m^3) concentration was found in operating room and intensive care unit and the fungal population range from159.24 cfu/m^3 to 743.10 cfu/m^3. The grand total average concentration was 427.18 cfu/m^3. And table 6 showed that there were significant mean difference among the studied wards (p-value= 0.000).

Table 1. ANOVA test result on mean fungal concentration difference of wards in Jimma University Specialized Hospital during both session

Morning sessions					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1161934.099	9	129103.789	30.476	.000
Within Groups	127088.977	30	4236.299		
Total	1289023.077	39			
Afternoon sessions					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1046840.203	9	116315.578	42.258	.000
Within Groups	82575.134	30	2752.504		
Total	1129415.337	39			

Tukey honest significance difference test were conducted to compare the difference of individuals mean. As (table 2) highlights that emergency OPD has significant different mean fungal load compared with all others fungal density (p-value=0.000).the others studied wards also compared and the result attached to ANNEX III.

Table 2. Showed the difference of individual mean fungal concentration of ten wards and their statistical significance in Jimma University Specialized Hospital, 2016

(I) WARDS	(J) WARDS	Mean Difference (I-J)	Std. Error	Sig	Confidence interval	
					Upper	Lower
Emergency OPD	Intensive care unit	476.38125*	38.40128	.000	350.8930	601.8695
	operating Room	530.78500*	38.40128	.000	405.2968	656.2732
	Medical laboratory	464.43750*	38.40128	.000	338.9493	589.9257
	Medical Ward male	237.52625*	38.40128	.000	112.0380	363.0145
	Medical Ward female	236.20000*	38.40128	.000	110.7118	361.6882
	Surgical Ward male	140.65750*	38.40128	.016	15.1693	266.1457
	Surgical ward female	335.72125*	38.40128	.000	210.2330	461.2095
	Pediatrics	456.47625*	38.40128	.000	330.9880	581.9645
	Maternity	301.22125*	38.40128	.000	175.7330	426.7095

5.4 Level of fungal pollution in JUSH

according to sanitary standards of European commission for none – industrial premises pollution level were determined (36). Tables 3 and 4 showed the pollution status of JUSH studied wards in the morning session. Most of the wards were found in the status of intermediate and high pollution of fungal population.

Table 3. . Fungal pollution level in the morning session for each investigated ward of Jimma University Specialized Hospital, Jimma town, Southwest Ethiopia, 2016

Bioaerosols	Range of values in CFU/M³	Pollution degree	EOPD	ICU	OR	LAB	MEM	MEF	SURM	SURF	PED	MAT
Fungal	<25	Very low										
	25-100	low										
	100-500	Intermediate		√	√	√	√	√		√	√	
	500-2000	High	√						√			√
	>2000	Very high										

EOPD= Emergency OPD   LAB= laboratory   MEM= Medical male ward   MEF= Medical female ward SURM= Surgical Male Ward PED= Pediatrics   MAT= Maternity   ICU= Intensive care unit OR= Operating Theatre

Table 4. . Fungal pollution level in the afternoon session for each investigated ward of Jimma University Specialized Hospital, Jimma town, Southwest Ethiopia, 2016

Bioaerosols	Range of values in CFU/M³	Pollution degree	EOPD	ICU	OR	LAB	MEM	MEF	SURM	SURF	PED	MAT
Fungal	<25	Very low										
	25-100	low										
	100-500	Intermediate		√	√	√	√	√		√	√	
	500-2000	High	√						√			√
	>2000	Very high										

EOPD= Emergency OPD   LAB= laboratory   MEM= Medical male ward   MEF= Medical female ward SURM= Surgical Male Ward PED= Pediatrics   MAT= Maternity   ICU= Intensive care unit OR= Operating Theatre

5.5 Association of fungal isolation with time of sampling

Time of sampling was not statistically significance ($p > 0.05$) associated with fungal loads of the hospital indoor air. Of the plates set, in the morning the mean fungal load were 403.39 cfu/m³ and 456.47 cfu/m³ plates set in the afternoon respectively.

Table 5. ANOVA test result on mean fungal concentration at different sampling time in Jimma University Specialized Hospital, 2016

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53016.679	1	53016.679	1.710	.195
Within Groups	2418438.414	78	31005.621		
Total	2471455.093	79			

5.6 Frequency of Fungal isolates Contamination in indoor air of JUSH

Regarding the isolated fungi from indoor air samples collected from different studied wards during afternoon and morning were 409. The identified isolates were included *as A. alteranata, Aspergillus spp, Fusarium spp, Pencilluim spp, Trichophyton spp and* some of *Rhodotorula mucilaginosa* and other unidentified yeast (figure 11).

The prevalence of specific fungal genera was determined of indoor air by taking sample contain diverse colony from each wards and sum up and divided by total identified colonies species. The top four identified fungal included *A. alteranata 121(29.6%), Aspergillus spp 111(27.13%), Trichophyton spp 80(19.6%)*

F. oxysporum 55(13.4%),Pencillium spp 30(7.31%) .
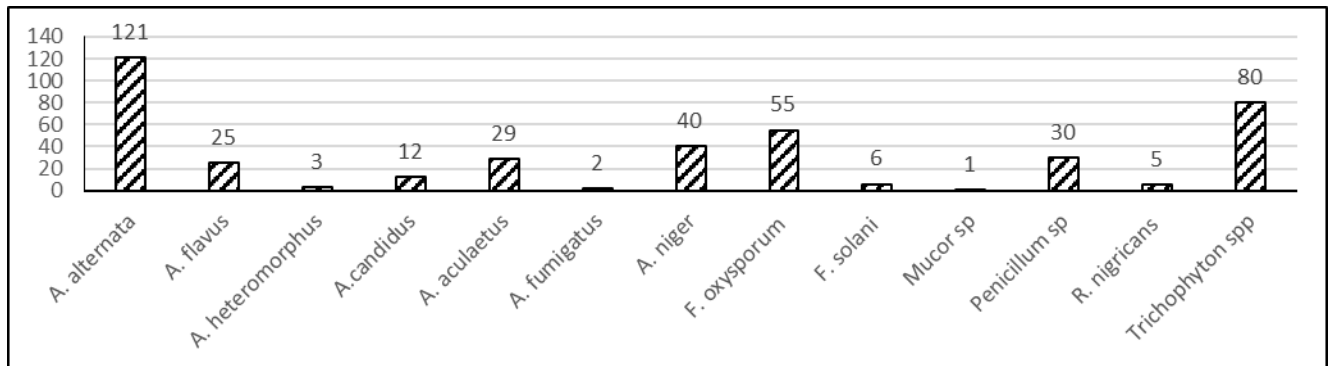
Figure 11. Percentage of detected fungal isolates of indoor air of Jimma University Specialized Hospital, Jimma town, Southwest, Ethiopia, 2016

From this study noticed that different growth density and diversity of fungi. The following figures are describing growth and diversity of fungi in different studied wards with negative control.

Figure 12. Plates showed that fungal colonies were grown on PDA from emergency OPD and ICU in Jimma University Specialized Hospital with control
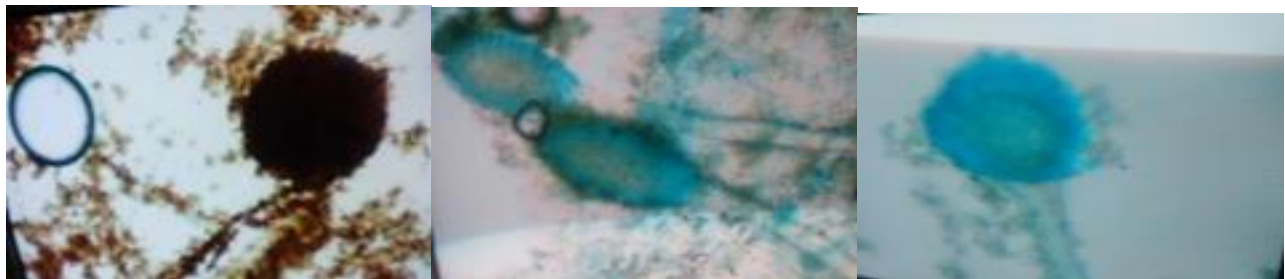
Figure 13. Identified Aspergillus Spp isolates under microscopic examination from Jimma university specialized indoor air

Figure 14 identified fungal isolates from Jimma University Specialized Hospital indoor air *Rhizopus spp, Fusarium spp, and Trichopyton spp* from left to right

Figure 15. Identified fungal isolates from Jimma University Specialized Hospital indoor air *A. alteranata* and *Pencillium spp* from left to right

5.7 Bacterial load and prevalence in JUSH

The result of this study revealed that wards had variation in cfu/m^3 bacterial concentration of Indoor air. The highest level bacterial load were detected in Pediatrics (5228 cfu/m^3) followed by emergency outpatient department(5080 cfu/m^3) to have characteristics of high populated area, while the lowest level were detected in intensive care unit and operating room to have a characteristics of less populated area. The level of bacterial concentration in each investigated wards during morning and afternoon also varied (figure 16).
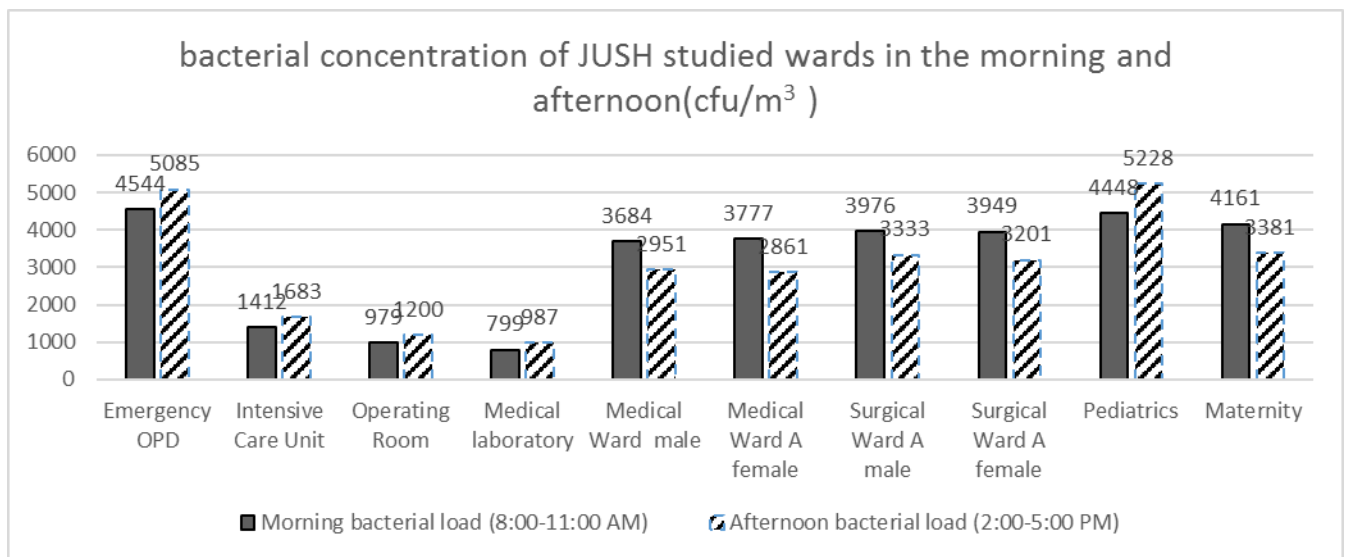
Figure 16. Bacteriological concentration of indoor air of Jimma University Specialized Hospital after 60 minute time exposure, 2016

5.8 Statistical significance test for mean Bacterial concentration in the studied wards

One way ANOVA test was conducted to obtain the mean bacterial concentration of wards as presented below. The highest mean bacterial concentration in (4814.22 and 4838.11 cfu/m$^{3)}$ was found in emergency OPD and pediatrics respectively. And the minimum (895.05 cfu/m^3) concentration was found in

laboratory. The grand total average concentration was 3081.87 CFU/m^3. Types of wards was significantly ($P > 0.05$) associated with bacteriological load of the hospital air (table 6).

Table 6. ANOVA test result on mean bacterial concentration difference of wards of wards in Jimma University Specialized Hospital during morning session, 2016

Morning sessions					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	79610726.278	9	8845636.253	289.455	.000
Within Groups	916788.429	30	30559.614		
Total	80527514.707	39			
Afternoon sessions					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	74630719.458	9	8292302.162	34.283	.000
Within Groups	7256252.847	30	241875.095		
Total	81886972.305	39			

5.9 Association of bacterial load with time of sampling

Time of sample collection was not statistical significantly (p>0.05) associated with bacterial load of the hospital indoor air in jimma university specialized hospital studied wards.

Table 7.ANOVA test result on mean bacterial concentration at different sampling time in Jimma University Specialized Hospital, 2016

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	660984.805	1	660984.805	.317	.575
Within Groups	162414487.012	78	2082237.013		
Total	163075471.816	79			

5.10 Level of pollution of indoor air in JUSH

The level of pollution based determined on each selected wards according to sanitary standards of European commission for none – industrial premises (36). Table 8 showed the pollution degree of JUSH studied wards in the morning and afternoon session which was similar. All wards had the status of poor indoor air the quality.

Table 8. Bacterial pollution level in the morning session for each investigated ward of Jimma University Specialized Hospital, Jimma town, Southwest Ethiopia, 2016

Bioaerosols	Range of values in CFU/M³	Pollution degree	ICU	OR	EOPD	LAB	MEM	MEF	SURM	SURF	PED	MAT
Bacteria	<50	Very low										
	50-100	low										
	100-500	Intermediate										
	500-2000	High	√	√								
	>2000	Very high			√	√	√	√	√	√	√	√

EOPD= Emergency OPD   ICU= Intensive Care unit   OR= Operating theatre   LAB= laboratory   MEM= Medical male ward   MEF= Medical female ward   SURM= Surgical Male Ward PED= Pediatrics   MAT= Maternity

5. 11 Bacterial isolates in JUSH

 After morphological and colony characterization gram stain were conducted. Ten gram positive rods, 42 gram negative rods and 112 gram positive cocci were isolated.

Total of 80 settle plates were analysed for biochemical tests. And seven species/genera of bacteria were isolated from the tests. *Staphylococcus aures* and *coagulase negative species* were the most frequent isolated bacteria, 41.46% and 22.56% of the exposed plates being positive for them respectively. Figure 18 highlights the shape of gram stained colony. Streptococcus 4.3 % and E. coli were the least prevalent species in the studied wards. *Staphylococcus aures* and CNS were consistently isolated from the ten different hospital investigated wards.
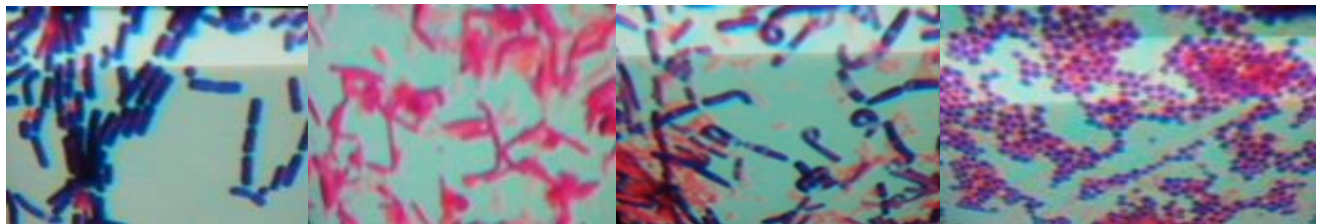
Figure 17. The gram stain result of indoor air of Jimma University Specialized Hospital Gram positive rods, Gram negative rods Gram positive rods and Gram positive cocci from left to the right

The proportion of bacteria which isolated from indoor air samples were displayed in figure 18. From 80 settle plates air samples at JUSH studied wards out 164 isolates *S. aures* 68(41.46%) was the dominate isolates followed by CNS 37(22.56%).
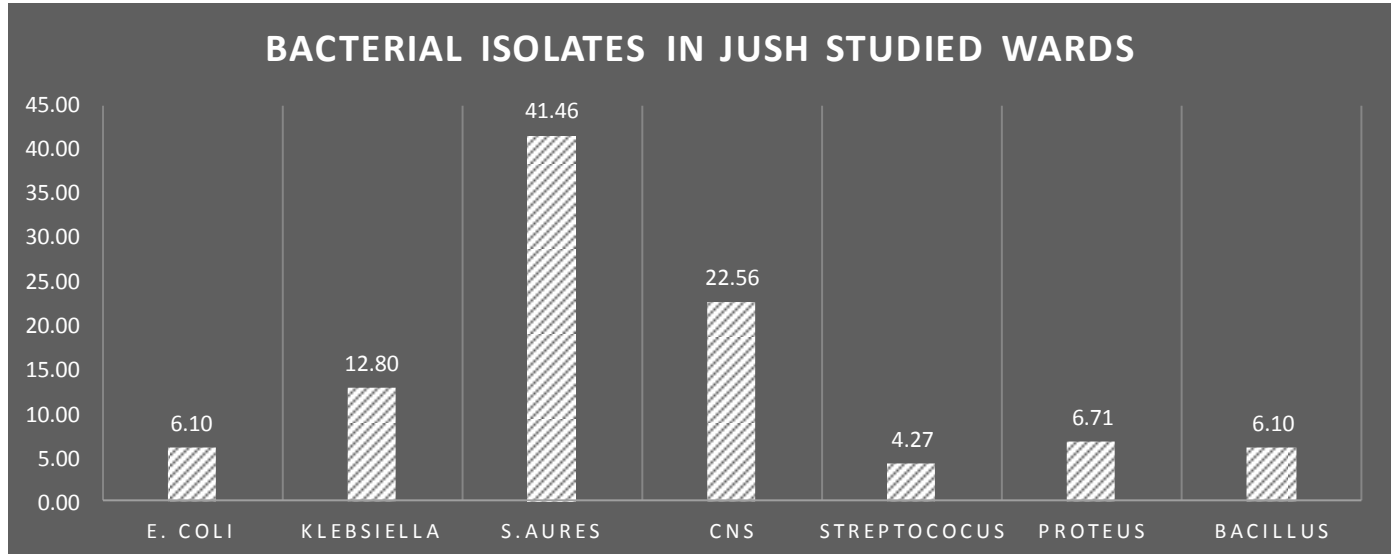
Figure 18.percentage of detected bacteria in bioaerosels for Jimma university Specialized hospital studied wards in jimma town, 2016

5.12 Fungal concentration or load in Shenen Gibe Hospital

Quantified of fungal load were presented in both morning and afternoon sessions. The lowest concentration of fungal populations were recorded in Minor OR and the highest level were recorded in OPD which were 111 cfu/m^{3}and 746 cfu/m^3 respectively. The mean fungal loads were recorded 309.18 cfu/m^3 $\pm$172.36 cfu/m^3 and the range distribution were found between 74.31 cfu/m^3 and 745.76 cfu/m$^{3.}$
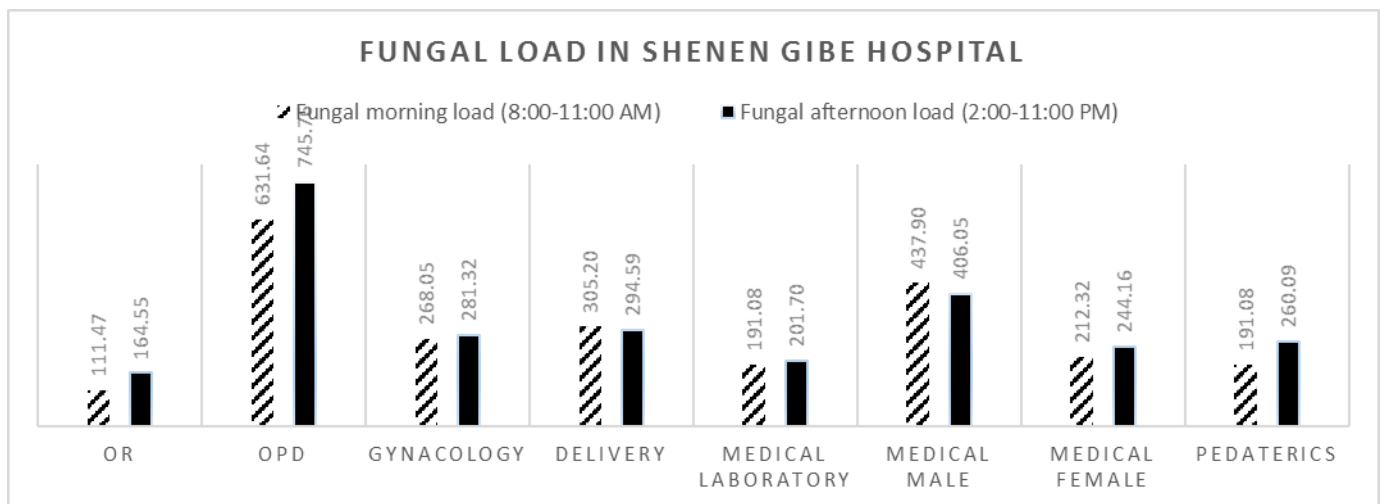
OR= Operating Theatre OPD= outpatient department   GYNA= Gynecology ward DEL= Delivery ward MEM= Medical male ward MEF= Medical Female war PED= pediatrics

Figure 19. Fungal concentration (cfu/m^3) of indoor air of Shenen Gibe Hospital after 60 minute time exposure in cfu/m^3, 2016

5.13 Association between mean fungal concentration and studied wards

One way ANOVA test was conducted to obtain the mean fungal concentration of wards as presented below. The highest mean fungal concentration at both sessions were detected (688.69 cfu/m^3) were found in OPD and (138.00 cfu/m^3) concentration were found in OR . Mean fungal concentration difference of wards were also calculated and there was statistical significant difference both in the morning and afternoon among wards (p-value=0.000) (table 9).

Table 9. ANOVA test result on mean fungal concentration difference among studied wards in Shenen Gibe hospital during morning session and afternoon, 2016

Morning sessions					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	786704.607	7	112386.372	48.233	.000
Within Groups	55921.638	24	2330.068		
Total	842626.245	31			
Afternoon session					
Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	952537.837	7	136076.834	53.544	.000
Within Groups	60993.591	24	2541.400		
Total	1013531.428	31			

5.18 Level of pollution of Fungal in Shenen Gibe Hospital

The status of pollution was determined on each studied wards according to sanitary standards of European commission for none – industrial premises (36). Table 10 showed the pollution degree of Shenen Gibe Hospital studied wards in the morning and afternoon sessions. All of the studied wards were found in the status of intermediate pollution of fungal load.

Table 10. Evaluation of air quality in the designated areas of Shenen Gibe Hospital according to the sanitary standards for non-industrial premises, 2016

Bioaerosels	Range of values in CFU/M^3	Pollution degree	OR	OPD	GYN	DEL	LAB	MEM	MEF	PED
Fungal	<25	Very low								
	25-100	low								
	100-500	Intermediate	√	√	√	√	√	√	√	√
	500-2000	High								
	>2000	Very high								

OR= Operating Theatre OPD= outpatient department GYNA= Gynecology ward DEL= Delivery ward MEM= Medical male ward MEF= Medical Female war PED= pediatrics

Tukey honest significance difference test were conducted to compare the difference of individuals mean. As table highlights that OPD has significant different with gynecology, OR, delivery medical male ward and no significant difference with medical laboratory of fungal load. OPD and medical male ward had significant different mean fungal load compared with all others fungal density (table 11). The remaining wards were also compared (ANNEX III).

Table 11.showed the difference of individual mean fungal concentration of eight wards and their statistical significance in Shenen Gibe hospital, 2016

(I) WARDS	(J) WARDS	Mean Difference (I-J)	Std. Error	Sig	Confidence interval	
					Upper	Lower
Outpatient department	OR	550.68875*	26.97378	.000	465.7677	635.6098
	GYNACOLOGY	414.01250*	26.97378	.000	329.0914	498.9336
	DELIVERY	388.80125*	26.97378	.000	303.8802	473.7223
	MEDICAL LABORATORY	492.30375*	26.97378	.000	407.3827	577.2248
	MEDICAL MALE	266.72125*	26.97378	.000	181.8002	351.6423
	MEDICAL FEMALE	460.45750*	26.97378	.000	375.5364	545.3786
	PEDIATRICS	463.11125*	26.97378	.000	378.1902	548.0323

5.14 Isolates of in indoor air of Shenen Gibe Hospital

Regarding the isolated fungi from indoor air samples collected from different studied wards during afternoon and morning were identified *as A. alteranata, Aspergillus spp, Fusarium spp, Pencilluim spp, Trychophyton spp , Mucor spp* and some of *Rhodotorula mucilaginos* and other unidentified yeast (figure 22). The prevalence of specific fungal genera was determined of indoor air by taking sample contain diverse colony from each wards and sum up and divided by total identified colonies species. The top four identified fungal included *A.alteranata (33.01%), Trychopton spp (11.65%), F. oxysporum(14.56%),Pencillium spp(7.31%)* and *Aspergillus spp(30.2%)*
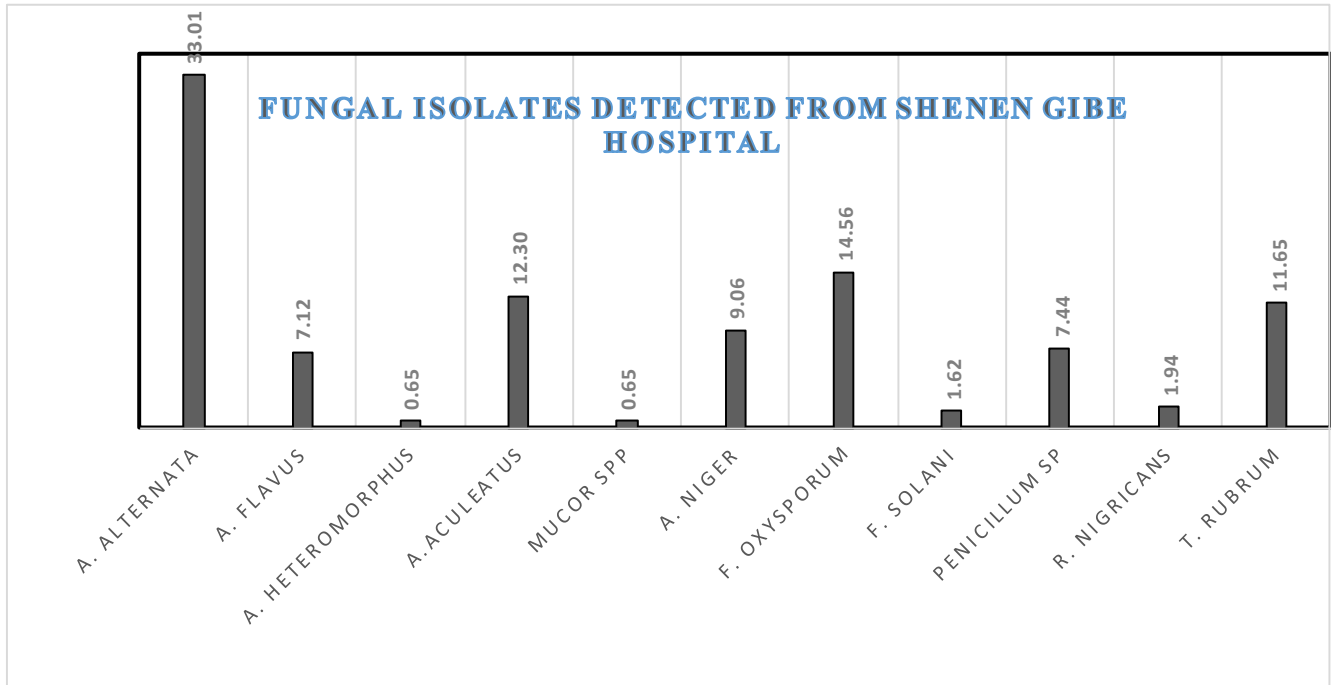
Figure 20. Percentage of detected fungal isolates of indoor air of Shenen Gibe hospital, Jimma town, Southwest, Ethiopia, 2016

From this study noticed that different growth density and diversity of fungi. The following figures are describing growth and diversity of fungi in different studied wards with negative control.

Figure 21.Plates showed that fungal colonies were grown on PDA from emergency OPD and ICU in shenen Gibe Hospital

Figure 22. Identified fungal isolates from Shenen Gibe hospital indoor air *Mocur spp*, *A. alteranata spp*, and *A. niger* from left to right
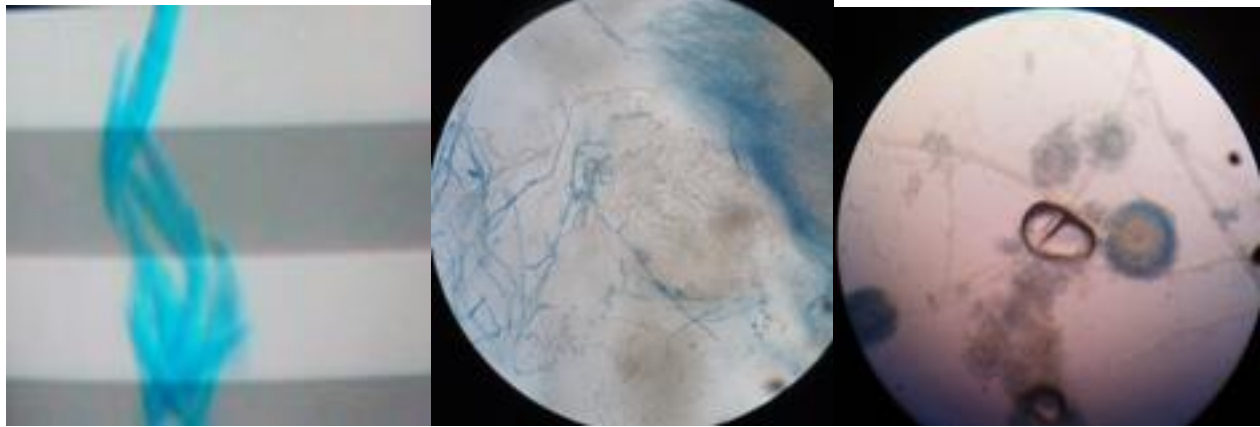
Figure 23. Identified fungal isolates from Shenen Gibe hospital indoor air *Fusarium spp, Trychopton spp, and Aspergillus spp* from left to right

5.16 Bacterial load in Shenen Gibe Hospital

The result of this study highlights studied ward variation in Cfu/m^3 bacterial Indoor air. It shows the highest level were detected in outpatient department 3593 Cfu/m^3 to have characteristics of high populated area, followed by gynecology ward 3275 Cfu/m^3 , while the lowest level were detected in minor OR 149 Cfu/m^3 to have a characteristics of less populated area.
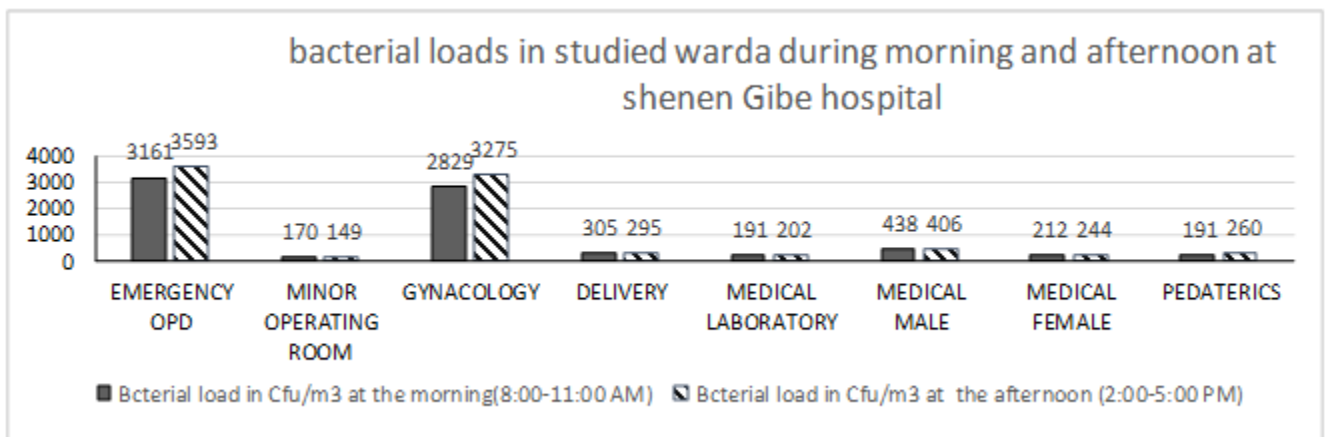
Figure 24. Fig the bacterial concentration of Shenen Gibe Hospital during morning and afternoon, jimma town, 2016

5.17 Statistical significance test for mean bacterial concentration

Airborne bacterial loads obtained by passive method with an exposure for 60 minutes were found to vary with each other and found to be statistically significant (table 12). One way ANOVA test was conducted to obtain the mean bacterial concentration of wards as presented below. The highest mean bacterial concentration (377.12 cfu/m^3) was found in outpatient department ward and the least (159.23 cfu/m^3) concentration was found in Minor OR. The grand total average concentration was 995.05 cfu/m^3. Mean bacterial concentration difference of wards were also calculated and there was statistical significant difference both in the morning and afternoon among wards (p-value=0.000).

Table 12. ANOVA test result on mean fungal concentration difference of wards of wards in Shenen Gibe hospital during afternoon session, 2016

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45600593.293	7	6514370.470	711.747	.000
Within Groups	219663.693	24	9152.654		
Total	45820256.985	31			

Tukey honest significance difference test were conducted to compare the difference of individuals mean. As table highlights that OPD has significant different with Minor OR, gynecology, delivery medical male ward and no significant difference with medical laboratory fungal load. OPD has significant different mean fungal load compared with all others fungal density (ANNEX III).

Table 13. Showed the difference of individual mean Bacterial concentration of eight wards and their statistical significance in Shenen Gibe hospital, 2016

(I) WARDS	(J) WARDS	Mean Difference (I-J)	Std. Error	Sig	Confidence interval	
					Upper	Lower
Outpatient department	MINOR OPERATING ROOM	3217.88875*	69.38459	.000	2999.4464	3436.3311
	GYNACOLOGY	325.10875*	69.38459	.000	106.6664	543.5511
	DELIVERY	3077.22625*	69.38459	.000	2858.7839	3295.6686
	MEDICAL LABORATORY	3180.73500*	69.38459	.000	2962.2927	3399.1773
	MEDICAL MALE	2955.14125*	69.38459	.000	2736.6989	3173.5836
	MEDICAL FEMALE	3148.88500*	69.38459	.000	2930.4427	3367.3273
	PEDATERICS	3151.53625*	69.38459	.000	2933.0939	3369.9786

5.18 Level of pollution of bacteria in Shenen Gibe Hospital

The level of pollution was determined on each selected wards according to sanitary standards of European commission for none – industrial premises (36). Table 14 showed the pollution degree of Shenen Gibe Hospital studied wards in the morning in both sessions. Most of the wards were found in the status of intermediate and high pollution of bacterial load.

Table 14. Bacterial pollution level in the afternoon session for each investigated ward of Shenen Gibe zonal Hospital, Jimma town, Southwest Ethiopia, 2016

Bioaerosels	Range of values in CFU/M^3	Pollution degree	OR	OPD	GYN	DEL	LAB	MEM	MEF	PED
Fungal	<50	Very low								
	50-100	low								
	100-500	Intermediate	√			√	√	√	√	√
	500-2000	High								
	>2000	Very high		√	√					

OPD= outpatient department   LAB= laboratory   MEM= Medical male ward   MEF= Medical female

Table 15. Bacterial pollution level in the afternoon session for each investigated ward of Shenen Gibe zonal Hospital, Jimma town, Southwest Ethiopia, 2016

Bioaerosels	Range of values in CFU/M^3	Pollution degree	OR	OPD	GYN	DEL	LAB	MEM	MEF	PED
Fungal	<50	Very low								
	5-100	low								
	100-500	Intermediate	√	√			√	√	√	√
	500-2000	High								
	>2000	Very high			√	√				

OPD= outpatient department   LAB= laboratory   MEM= Medical male ward   MEF= Medical female

PED = Pediatrics   GYN= Gynecology   DEL= Delivery   OR= Minor operating theatre

5.19 Bacterial isolates in Shenen Gibe zonal Hospital

Nine gram positive bacillus species, 46 gram negative bacillus and 55 gram positive cocci were isolated. Seven species/genera of bacteria were isolated from the total of 64 settle plate. The collected air samples were examined for identification of species using biochemical test. *Staphylococcus aures and Klebsiella* species were the most frequent isolated bacteria, **27.3 % and 18.2%** of the exposed plates being positive for them respectively. Fig highlights the shape of gram stained colony. Streptococcus 9.1 % and *bacillus* 8.2% were the least prevalent species in the studied wards. Similarly *Staphylococcus aureus* and *CNS* were consistently isolated from all studied wards.
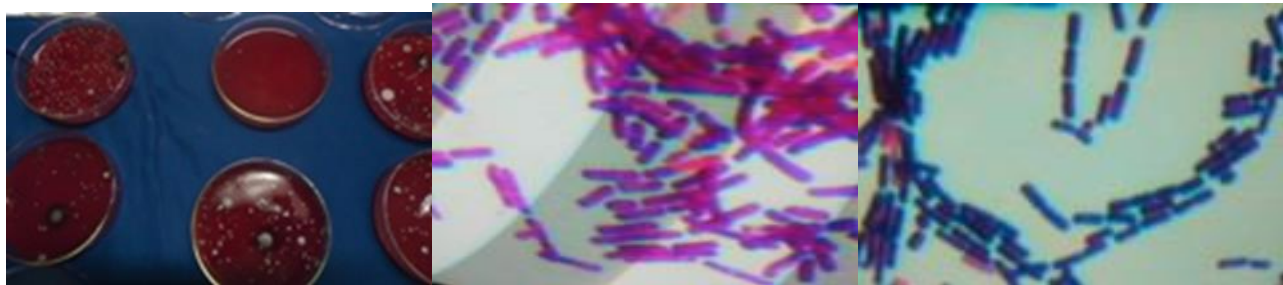
Figure 25. Bacterial colony on blood agar and rod shape gram negative and gram positive from shenen gibe hospital

The proportion of bacteria which isolated from indoor air samples were displayed in figure(28), from 64 settle plates air samples at Shenen Gibe Hospital studied wards out 110 isolates *S. aureus* 30(27.3%) was the dominate isolates followed by *Klebsiella spp* 20(18.1%).
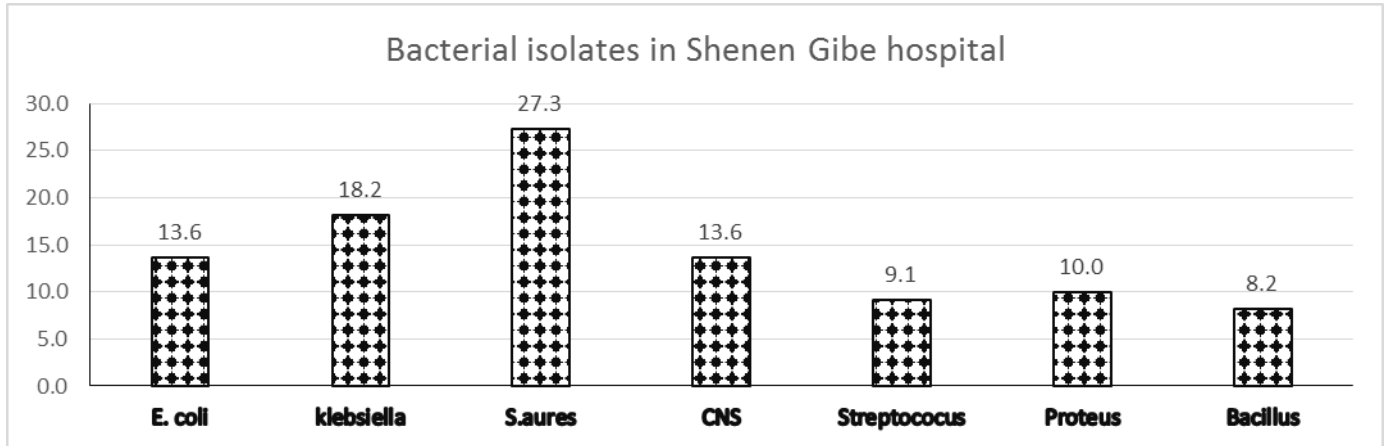
Figure 26. Bacterial isolates in shenen Gibe hospital in jimma town, 2016

5.20 Total microbial load in both studied hospitals

. Overall density of total bioaerosels Concentration for surveyed JUSH wards in were 3356.49 cfu/m^3 for morning and afternoon respectively (table 16). Based on the studied Shenen Gibe hospital wards overall density of total bioaerosels were 1288.93cfu/m^3. The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments suggested that total microbial load should not exceed 1000 CFU/m$^{3.}$ Based on the guideline all studied wards of JUSH were exceeded the limit. Whereas Shenen Gibe hospital two of the studied wards had exceeded the limit suggested by WHO.

Table 16. Overall Density of Total bioaerosels Concentration Based on Studied Hospitals Wards, jimma town, 2016 (cfu/m $^{3)}$

Hospital	Studied Wards For Each Hospital								Total average
JUSH	Emergency OPD	Intensive care unit	Operation Room	Laboratory	Medical male	Medical female	Pediatrics	Maternity	
Total concentration	5559.99	1816.62	1304.41	1174.10	3825.64	3828.04	5127.39	4215.77	3356.49
Shenen Gibe Hospital	Outpatient department	Operation Room	Delivery	Laboratory	Medical male	Medical female	Pediatrics	Gynecology	
Total concentration	4066.815	297.245	605.09	392.745	859.87	445.86	451.165	3326.695	1288.93

5.21 Drug susceptibility test against bacterial isolates of JUSH

The antimicrobial susceptibility test of the 110 isolates which are high prevalent in the studied wards were tested against five commonly used antibiotics in jimma university specialized hospital. The isolates were includes S.aures (n=68), klebsiella (n= 21), E.coli (n= 10) and Proteus spp (n=10). The result summarized in table. Of total 110 isolates with irrespective of their species all tested isolates were resistant to Ampicillin (10µg) and followed by Norfloxacine (10µg)) which were 34 isolates resistant to the drug.
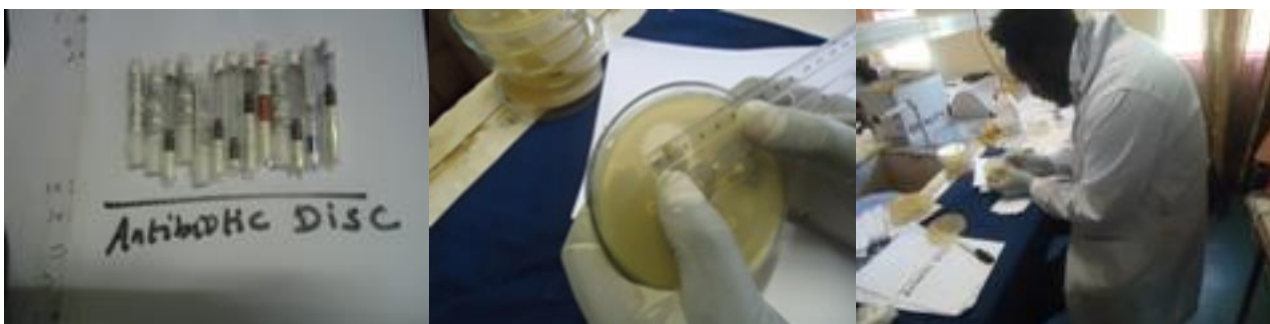
Figure 27. Antibiotic disks, measuring zones sizes of isolates of bacteria tested against five antibiotic in both hospitals

5.22 Drug susceptibility test against bacterial isolates of Shenen Gibe Hospital

The antimicrobial susceptibility of the 75 isolates which are high prevalent in the studied wards were tested against five commonly used antibiotics in Shenen Gibe hospital. The isolates were includes S.aures (n=30), klebsiella (n= 20), E.coli (n= 15) and Proteus spp (n=10). The result summarized in table. Of total 75 isolates with irrespective of their species all tested isolates were resistant to Ampicillin (10μg)) and followed by Norfloxacine(10μg)) which were 34 isolates resistant to the drug.

All S.aures isolates from JUSH and Shenen Gibe hospital were sensitive to vancomycine and gentamicin where as they were more resistant to Ampicillin and norfloxacin. Similar to this, the gram negative isolates were more resistant to for both Ampicillin and vancomycine. They were sensitive to gentamicin. Tables 28 and 29 showed the pattern of drug resistance in JUSH and Shenen Gibe hospital.

Figure 28.Drug susceptibility test against isolate of Shenen Gibe hospital after incubation overnight in jimma town, 2016

Table 17. Antimicrobial susceptibility of bacterial isolates of indoor air of Jimma University specialized hospital, jimma town, 2016

(N=110)		NOR (10µg)	VA (30µg)	CRO(30µg)	AM(10µg)	CN(10µg)
		N (%)	N (%)	N (%)	N (%)	N (%)
S. aureus (n=68)	S	33(47.9)	68(100)	30(44.9)	0	68(100)
	I	14(20.3)	0(0)	27(39.1)	10(14.70)	0(0)
	R	22(31.9)	0(0)	11(15.9)	58(85.29)	0(0)
Klebsiella Spp (n=21)	S	2(9.5)	19(90.47)	2(9.5)	0(0)	21(100)
	I	8(38.05)	2(9.52)	10(47.6)	0(0)	0(0)
	R	12(57.14)	0(0)	9(42.8)	21(100)	0(0)
Proteus spp (n=11)	S	6(54.54)	0(0)	3(27.27)	0(0)	11(100)
	I	3(27.27)	0(0)	7(63.63)	0(0)	0(0)
	R	2(18.18)	11(100)	1(9.09)	11(100)	0(0)
E. coli (n=10)	S	10(100)	0(0)	10(100)	0(0)	11(100)
	I	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	10(100)	0(0)	10(100)	0(0)
Total (N=110)	S	51(46.36)	87(79.09)	45(40.90)	0(0)	110(100)
	I	24(21.81)	2(1.81)	44(40.00)	10(9.09)	0(0)
	R	35(31.81)	21(19.09)	21(19.09)	100(90.90)	0(0)

Keys: S, susceptible; I, intermediate; R, resistance, NOR; Norfloxacin, VA, Vancomycin; CN, Gentamicin; AM, Ampicillin; CRO, Ceftriaxone

Table 18. Antimicrobial susceptibility of bacterial isolates of indoor air of Shenen Gibe hospital, jimma town, 2016

Antibiotic disks		NOR (10µg)	VA (30µg)	CRO(30µg)	AM(10µg)	CN(10µg)
Isolates (N=75)		N (%)	N (%)	N (%)	N (%)	N (%)
S. aureus (n=30)	S	15(50)	30(100)	14(46.6)	0	30(100)
	I	6(20)	0(0)	12(40)	5(16.66)	0(0)
	R	9(30)	0(0)	4(13.3)	25(83.33)	0(0)
Klebsiella Spp (n=20)	S	3(15)	19(95.5)	2(10)	0(0)	20(100)
	I	8(38.05)	1(5)	10(50)	0(0)	0(0)
	R	9(45)	0(0)	8(40)	20(100)	0(0)
Proteus spp (n=10)	S	5(55)	0(0)	2(20)	0(0)	10(100)
	I	3(33)	0(0)	6(60)	0(0)	0(0)
	R	2(22)	10(100)	2(20)	10(100)	0(0)
E. coli (n=15)	S	15(100)	0(0)	15(100)	0(0)	15(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	15(100)	0(0)	15(100)	0(0)
Total (N=75)	S	38(50.66)	49(65.33)	35(46.66)	0(0)	75(100)
	I	17(22.66)	1(1.33)	26(34.66)	5(6.66)	0(0)
	R	20(26.66)	25(33.33)	16(21.33)	70(93.33)	0(0)

Keys: S, susceptible; I, intermediate; R, resistance, NOR; Norfloxacin, VA, Vancomycin; CN, Gentamicin; AM, Ampicillin; CRO, Ceftriaxone

CHAPTER SIX: DISCUSSION

Currently many Occupational Hygienists possess highly developed skills in recognizing and assessing chemical hazards in the workplace, but generally are not as familiar with the biological contaminants in the workplace (48). The microbial qualities of indoor air of healthcare facilities have been linked with nosocomial infections. Nosocomial infections transmitted by the airborne route, especially fungal infection such as *aspergillosis*, are a major source of morbidity and mortality in immune compromised patients. Bio-aerosols, of which fungal spores are one of the major types of microorganisms, can be present in all hospital environments, and may be transmitted through indoor and outdoor air, visitors, patients, and air conditioners (38).

Both the fungal load (CFU/m^3) and the diversity of fungal genera isolated (JUSH and Shenen Gibe hospital) together generate a picture of the overall biological quality of the air. Abundance and diversity of fungal profiles should not be used independently when evaluating a workplace indoor air quality.

In this study sex bacterial species and seven fungal genera were identified from bioaerosels assessment in both surveyed hospitals. This study revealed that Jimma University Specialized hospital and Shenen Gibe zonal hospital had bacterial load in the range of between 6794.06 and 774.95 cfu/m^3 and 3630.57 and 127.39 cfu/m^3 respectively and it is in line with the study the study conducted in JUSH and Gonder University teaching Hospital (33,34). However, in current study JUSH was highly contaminated with bioaerosels than Shenen Gibe zonal hospital. The result of this study showed that JUSH had a higher degree of contamination with air borne bacteria and fungi indoor air rather than Shenen Gibe hospital. These high rates in JUSH might be attributed to the age of the building(JUSH was built in 1945) while the zonal Hospital was built 2012, poor and deficient hygienic conditions, poor housekeeping practice of studied wards and inappropriate disinfection procedure against air borne bacteria and fungi might raise the overall density of bioaerosels contaminates.

The study carried out in Nigeria within two hospitals (Faithdome and Eromosele) showed that the least microbial concentration in Operating Theater were recorded (25). In contrast, in this study both hospitals had the highest microbial concentration. In JUSH bacterial count in OR ranged 977 to 1221 cfu/m^3, while the fungal count ranged from 149 to 244 cfu/m^3. In shenen Gibe zonal hospital recorded bacterial count that ranged from 138 to 196 cfu/m^3 and a fungal count that ranged from 112 to 159 cfu/m^3. This variation microbial load among two hospital (JUSH and Shenen Gibe zonal hospital) might be the type of tasks

carried out, patient flow, visitors, and personnel. Since in shenen Gibe hospital only minor case was carried out the microbial concentration become low compare to Jimma University Specialized Hospital.

Regarding ICU, this unit has to deal with critical cases and there must be sufficient strategies to reduce the microbial rate, however, this study showed that high rate in JUSH. This might be correlated to the fact that the ward was crowded and poor ventilation system. Similar study conducted in Venzuela public General hospital (OR and ICU) showed very low concentration of bioaerosels (8).

Another factor which might be contributed in the rising of bioaerosels in JUSH is the number of beds. JUSH has 426 beds and Shenen Gibe hospital has only 50 beds. This high beds number beds in JUSH means high patients, personnel, and visitors. There were multiple patients per room and high number of people in the rooms and in the corridor. These results indicate influences the rate of air borne bacteria and fungi.

Quantitative study of different hospital wards showed that the pediatrics and emergency OPD had the highest total bacterial counted followed by maternity and medical male and female ward respectively. The high microbial count recorded for JUSH as compared to Shenen Gibe zonal hospital could be due to the size and patients flow.

The bacterial loads of previous study conducted in JUSH (surgical, medical and maternity) were in line with current study(49){FormattingCitation}. The bacterial concentration counts in the studied wards of surgical, medical and maternity were 3975 and 394 and 4161 cfu/m^3 respectively. These wards were characterized by crowded with visitors in addition to the hospitals personnel and patients.

Currently there is no uniform standard available on the level of indoor microbial load in health care facilities (50). The work conducted by WHO expert group on assessment oh health risks of biological agent indoor environment, suggested that total microbial load should not exceed 1000 cfu/m^3 (51). Some scholars suggested that the bacterial limit in health care facilities should not be greater than 750 (52). Bioaerosels concentrations ranging from 4500 to 10,000 cfu/m^3 also have been suggested as upper limit for ubiquitous bacterial aerosols (33). Other authors consider that 300 cfu/m^3 and 750 cfu/m^3 should be the limit for fungi and bacteria respectively (5).

The quantitave interpretation of the results describing the air quality in the wards of JUSH and Shenen Gibe zonal hospital were evaluated based on the sanitary standard, for the non-premises formulated by the European commissions in 1993 (53) and the work conducted by WHO expert group on assessment on

health risks of biological agent indoor environment (51,54). According to this classification all wards of Jimma University specialized hospital that were in included in the study were contaminated. The environment of these hospitals were also contaminated Based on the guideline published by WHO in 1988 (11). JUSH was highly contaminated than Shenen Gibe hospital. These might be because of the number of the capacity of hospital to serve the patients, occupants in the ward, patients, visitors and personnel during sampling time.

In JUSH, poor housekeeping, and unhygienic attached toilets and poor solid waste management were assessed during the study. These situations do have certain implication with to indoor air quality. The microbial load concentration in the wards may be due to the presence unhygienic attached toilets and poor waste management. This verification is supported research conducted in Gonder University Teaching Hospital (2).

The microbial isolates characterized and identified included seven bacterial and six fungal isolates in both hospitals, they include, *Staphylococcus aureus, CNS, Klebsiella, Escherichia coli, Bacillus* sp., *Proteus sp* and *Streptococcus* sp. for the bacterial isolates, while the fungal isolates includes *Aspergillus* sp. *Alternaria spp, Fusarium spp Penicillium spp Mucor spp* and *Trichophyton spp* (29). Among the fungal isolate, *Alternaria spp* and *Aspergillus* were the dominate species in both studied hospital which account (33.01%, 30.21% and 29.6%, 27.13%) respectively. Most study conducted in this area supported this finding (9,55,56).

 Staphylococcus aureus, was reported to be the most prevalent bacterial isolate followed by coagulase negative Staphylococci in JUSH and *Staphylococcus aureus* and *Klebsiella spp were in Shenen Gibe hospital* among studied hospitals wards. The prevalence of these species were supported by studies conducted in Ethiopia health care facilities (23,39). *S. aureus* common in all studied wards of both hospitals. The identified bacterial and fungal species of indoor air of both hospital might be a source of nosocomial infections in the hospitals. *S. aureus* can be cause of various diseases such as a post-operative infections, urinary tract infection, skin infections and respiratory and food poising.

Klebsiella spp and *Escherichia coli* are associated with urinary tract infections among catheterized patients. *Bacillus spp* are spore forming bacteria that can survive for long periods in the environment causing serious medical problems. *Aspergillus* has also been associated with incidence of nosocomial infection in immune compromised patients. Apart from these infections, allergic reactions have been reported following inhalation of fungal spores, making it important to pay attention to their presence in hospital air (50).

According to data and information obtained in this study, regular monitoring is essential to assess the effectiveness of the control of air and to detect irregular introduction of airborne particles via patients, visitors and/or medical staff. Moreover, microbiological survey data should be used to clearly define specific guidelines for air quality especially in controlled environments in hospitals. The compliance with good hygiene practices can however also reduce the number of nosocomial infections and their growing resistance to antibiotics remaining always a global concern (37).

Different studies elaborated that the concentration of airborne microbes varies among geographical location and is also influenced by environmental and climate factors. Kind of hospitals along the type of wards and time of sampling is significant factors that influence the rate of bioaerosels (53). Similarly in this study three factors were investigated to determine how these factors affected microbial count, namely the kind of hospital, the type of wards and the time of sampling. The statistical analysis showed that the concentrations of bacterial and fungal that were measured in both hospitals were significantly different from each other (p-value=0.000). However, the concentrations of bacterial and fungal that were measured at different sampling time (morning and afternoon) were not significantly different (p-value =0.980 and 0.180) respectively in both hospitals. Moreover, the concentrations of bacterial and fungal that were measured at different wards in both hospitals were also significantly different (p-value =0.000).

6.1 Antibiotic resistance test

All species isolated in JUSH 110(100) and Shenen Gibe hospital 70(93.33%) were resistant to Ampicillin, which is locally the antibiotic of choice for the treatment of infections caused by these bacteria. Similarly study conducted in Hawassa University Referral hospital and previous in JUSH revealed (23,39). Similar pattern of resistance to ampicillin has been reported earlier. In contrast all isolated species bacteria from JUSH 110(100%) and Shenen Gibe hospital 75(100%) of were susceptible for Gentamicin. Under this study in both hospitals displayed the same antibiotic susceptibility pattern.

Ampicillin is one of the most commonly prescribed antibiotic forms in healthcare facilities in Ethiopia. Irrational prescription of the antibiotic and its misuse by patients might have contributed for the resistance of most of the isolates to the antibiotic.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

According to the results obtained in the current study, the degrees of bacterial and fungal loads were far beyond the acceptable limit in both hospitals. But the status of pollution level in jimma university specialized hospital was very high compared to Shenen Gibe hospitals. Jimma University specialized were highly contaminated. Therefore, the number of personnel, patient per room, housekeeping practice and number of visitors should be consider.

Emergency Outpatient department had the highest microbial load in both hospitals. Relatively the lowest microbial load recorded in Operation room and laboratory.

Diversified bioaerosels were identified from both studied hospitals. These can be causative agent of hospital acquired infections in highly sensitive environment of hospitals such as Operating Theatre, Intensive Care unit and pediatrics.

The bacterial profile of indoor air sample in Jimma University Specialized hospital and Shenen Gibe hospital were similar and the difference in prevalence.

S. aureus and *coagulase negative Staphylococci* were the most frequently isolated species among potential pathogenic bacteria with isolation rate of 68(41.46%), 37(22.6%) in Jimma University Specialized hospital. *S. aureus* 30(27.3 %) and *Klebsiella spp* 20 (18.1 %%) were the most prevalent species in Shenen Gibe hospital.

Among the identified fungal isolates from both studied wards *Alternaria spp* and *Aspergillus spp* were high prevalent in both hospitals.

High prevalence of isolated species of bacteria which were tested against five antibiotics showed the resistance pattern to commonly prescribed antibiotic.

Ampicillin was resistant drug under this study by all tested species. While Gentamicin drugs were susceptible drug against all tested species in both studied hospitals.

Every studied wards of Shenen Gibe hospitas there were Aspergillus spp compared to Jimma University specialized hospital.

Both hospitals were no practicing regular cleaning, sweeping and disinfection of the surfaces.

In this finding type of hospital, types of wards were identified as a factor which affects the microbial load of both hospitals studied wards.

Time of sampling had not effect on the microbial load among studied wards.

7.1 Recommendation

Based on the findings the following suggestions and critical interventions are forwarded to improve the microbial indoor air quality of both studied hospitals.

To JUSH and SHENEN GIBE HOSPITAL

Regular cleaning, sweeping and disinfection should be practiced for improving of indoor air quality.

Better and well-constructed ventilation systems should be provided in both as this would go a long way in reducing the microbial load in hospitals.

Regular surveillance of microbiological quality of indoor air of hospitals should be done.

Information, education and communication programs should be established on the microbial indoor air quality for patients, visitors and professionals.

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Annex I: Materials, procedure and Reagents Required

1. Urea hydrolysis test

This procedure provides instructions for the differentiation of bacteria on the basis of urea hydrolysis.

Procedure

Take nutrient broth
Label the tube
Sterilize wire loop using the Bunsen burner
Using wire loop take a heavy inoculum of growth from an 18-24 hour pure culture
Suspend in Nutrient Broth
Vortex the suspension
Incubate at 37ºC incubator
When the suspension become turbid take drop of suspension aseptically and add drops into urea slant
Incubate the inoculated media at 37ºC incubator for overnight
Observe change of color on the media

2. Motility test

This procedure provides instructions for performing the detection of motility of gram-negative enteric bacilli.

Procedure

Step	Action
1	Take nutrient broth
2	Label the tube
3	Take pure colony on MacConkey Agar Plate near Bunsen burner
4	Suspend in Nutrient Broth
5	Vortex the suspension
6	Incubate at 37ºC incubator
7	When the suspension become turbid take drop of suspension aseptically and stab the medium not drop the broth
8	Incubate the inoculated media at 37ºC incubator for overnight

| 9 | Observe change of color (diffusion of bacteria) on the media |

3. Lysine Iron Agar

This procedure provides instructions for the differentiation of microorganisms on the basis of lysine decarboxylase and hydrogen sulfide production.

Step	Action
1	Take nutrient broth
2	Label the tube
3	Take pure colony on MacConkey Agar Plate near Bunsen burner
4	Suspend in Nutrient Broth
5	Vortex the suspension
6	Incubate at 37ºC incubator
7	When the suspension become turbid take drop of suspension aseptically and add drops into LIA Slant
8	Stab the butt of LIA by the Pasteur pipette
9	Incubate the inoculated media at 37ºC incubator for overnight
10	Observe change of color on the media

4. KIA =kligler iron agar

This procedure provides instructions for the differentiation of microorganisms on the basis of dextrose and lactose fermentation and hydrogen sulfide production

Step	Action
1	Take nutrient broth
2	Label the tube
3	Take pure colony on MacConkey Agar Plate near Bunsen burner
4	Suspend in Nutrient Broth
5	Vortex the suspension
6	Incubate at 37ºC incubator

7	When the suspension become turbid take drop of suspension aseptically and add drops into KIA Slant
8	Stab the but of KIA by the Pasteur pipette
9	Incubate the inoculated media at 37ºC incubator for overnight
10	Observe change of color on the media

5. Simmon Citrate utilization test

This procedure provides instructions to differentiate Citrate utilizing and Non utilizing bacteria.

Step	Action
1	Take nutrient Broth tube
2	Label the tube
3	Take pure colony using wire loop from MacConkey Agar Plate near Bunsen burner
4	Suspend in Nutrient Broth
5	Vortex the suspension
6	Incubate at 37ºC incubator
7	When the suspension become turbid take drop of suspension aseptically and add drops into Citrate Slant
8	Incubate the inoculated media at 37ºC incubator for overnight
9	Observe change of color on the media

Gram staining

This procedure provides instructions to perform gram's stain.

Principle

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains purple while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space.

Step	Action
1.	Label a glass microscope slide with the laboratory accession number.
2.	Take discreet colony from culture and Prepare a thin smear of the grindings the size of a quarter.
3	**Heat fixation** a) Pass air-dried smears through a flame two or three times. **Do not overheat**. b) Allow slide to cool before staining.
4	Flood the prepared slide with crystal violet for one minute.
5	Rinse the slide gently with tap water.
6	Flood the slide with Gram's iodine for one minute.
7	Rinse the slide gently with tap water.
8	Working with one slide at a time, flood the slide with decolorizer for 5 seconds and rinse with tap water. Repeat decolorization step for thick smears.
9	Flood the slide with safranin for one minute.
10	Rinse the slide gently with tap water.
11	Drain the slide in an upright position. Blot the back of the slide and place on a slide warmer or heating block to completely dry.
12	Observe under microscope using oil immersion.

Result Interpretation

Gram-positive bacteria and yeast will stain blue to purple. Gram-negative bacteria will stain pink to red.

Lacto phenol cotton blue staining for fungus examinations

Staining of fungus from culture

1. Take a grease free slide
2. Add a drop of lactophenol cotton blue o slide
3. Sterilize the inculation loop or needle and cool it transfer mycellial groth on to the lactophenol stain and press it gently so that it easily mix it the stain
4. Take a clean cover slip with help of a forceps place the cover slip mycelia groth plus LCB
5. With the help of blotting paper, wipe the excess stain
6. Observe the preparation under low and high power objectives of the microscopic

Kirby-Bauer disk diffusions susceptibility test protocol

1. Allow a Muller Hinton agar to come to room temperature.

2. Plates may be placed in a 35°c incubator until dry usually 10 to 30 minutes.

3. Appropriate label each MH agar plate for Each organisms to be tested.

Preparation

1. Use the sterile inoculating loop or needle, touch four or five isolated colonies of the organisms to be tested.

2. Suspend the organisms in 2 ml of sterile saline.

3. Vortex the saline tube to create a smooth suspension.

4. Adjust the turbidity of this suspensions to a 0.5 Mcfarland standard by adding more organisms if the suspensions is too light or diluting with sterile saline if the suspensions is too heavy.

5. Use this suspensions within 15 minutes of preparation

Inoculation of the Muller Hinton plates

1. Plate Dip a sterile swab into the inoculum tube.

2. Rotate the swab against the side of the tube or above the fluid level using firm pressure, to remove excess fluid. The swab should not be dripping wet.

3. Inoculating the dried surface of MH agar by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distributions of the inoculum.

4. Rime the plate with the swab to pick up any excess liquid

5. Discard the swab into an appropriate container.

6. Leaving the lid slightly agar, allow the plate to sit at room temperature at least 3 to 5 minutes, but not more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

7. Place the appropriate antimicrobial impregnated disk on the surface of the agar

8. Do not move a disk once it has contacted the agar surface

9. Using the forceps carefully remove one disk from cartridge

10. Invert the plate and place them in 35°c air incubator for 16 to 18 hours.

Measuring zone sizes

1. Following incubation, measures the zone size to the nearest milimetre using a ruler or caliper
2. When measuring zone diameter, always round up the next millimeter
3. Recorded the zone size on the recording sheet
4. Growth up to the edge of the disks can be reported as zone of 0mm.
5. Using the published CLSI guidelines, determine the susceptibility or resistance of the organisms to each drug tested.
6. The result of the Kirby-Bauer disk diffusions susceptibility test are reported as only susceptible, intermediate or resistant.

Materials and media
Gloves
Mouth mask
Marker
Oil immersion
Petri dishes
Gown
Slide 22mmx22mm
Slide cover
Autoclaves
Oven
Digital balance
Spoon
Cold box
Alcohol 70%, 97%
Cotton
Thermometer
Plaster
Measuring cylinder
Refrigeter
Safety cabinet

Distilled water
Tap water
Ruler
Aluminum foil
Applicator sticks
Light microscope
Lactophenol cotton blue
Crystal violet
Saffrine
Iodine
Pipets
Tartaric acid
Antimicrobials disks
Forceps
Racks
Test tubes
Inoculating needle
White nail polish
Normal saline 5%
Fitter paper
Rubbing plastic
Syringe
Coagulase
Hydrogen per oxide
Sterile swabs
Urea Agar
Pasteur pipette
Rubber tit
Wire loop
Incubator 37ºC

Bunsen burner
Pasteur pipette
0.5 Mcfarland standared
Blood agar base
Sterile 5% sheep blood
Nutrient agar
Macconkey agar
Potato dextrose agar
Manitol salt agar
Muller Hinton agar
Urea Agar
Semi solid agar
Klingler iron aga
triple sugar iron agar
Lysine iron agar
Xylene
Beakers
Round bottom flask
Matches
Focus screen
Urea supplement
Weigh trays
Hot plate

Annex II: Microbial Indoor Air Quality Assessment Checklist

Jimma University

College of Medical Science and Public health

Department of Environmental Health Science and Technology

Microbial indoor air quality assessment checklist in public health care facilities in Jimma town, 2016

1. Name of Healthcare facility_____

2. Name of ward/Unit_____

3. Sample code : petri dish number _____, _____,_____,_____

4. Type of microbes need to be sampled _____

5. Date of Sampling_____ Morning_____ Afternoon_____

6. Number of petri dish for fungal sampling in the morning _____

7. Number of petri dish for bacteria sampling in afternoon _____

8. Petri dish diameter _____ cm

9. Relative humidity_____%

10. Room temperature_____ ºC

11. Type of Ventilation   1. Natural   2. Mechanical   3. Both mechanical and natural 4.other___

12. If there is mechanical ventilation, is it functional currently 1. Yes   2. No 3. Not known

13. Is there any restriction rule for movement of individual in the ward? 1. Yes   2. No

14. Cleanliness of wards /Unit 1. Clean   2. Not clean

15. Housekeeping frequency per day 1. Once per day 2. Twice per day 3. Three per day

16. Method of housekeeping 1. Dry method (without water) 2. Wet method (with water) 3. Others_____

17. Number of Beds _____

18. Number of Patient_____

19. Number of personnel_____

20. Number of relative/ visitors_____

Name of sample collector _____ sign_____ date

Part two Laboratory result format of fungal identifications and bacteria

Date ----------------------------------

1. Number of petri dish positive for fungal growth_____

2. Enumerated fungal colony on each petri dish

P1_____

P2_____

P3_____

P4_____

3.   Species/genera of fungal isolated_____

4.   Number of petri dishes for bacterial growth_____

5.   Enumerated bacteria colony on each petri dis

P1_____

P2_____

P3_____

P4_____

Bacterial isolates _____

6.   Gram positive rod_____

7.   Gram positive coci (cluster)_____

8.   Gram positive coci (chain)_____

9.   Gram negative rod_____

Annex III: Tukey honest significance difference test result

Showed Tukey honest significance difference test result of fungal load in JUSH, 2016

(I) WARD	(J) WARD	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intensive care unit	Emergency OPD	-476.38125*	38.40128	.000	-601.8695	-350.8930
	operating Room	54.40375	38.40128	.918	-71.0845	179.8920
	Medical laboratory	-11.94375	38.40128	1.000	-137.4320	113.5445
	Medical Ward male	-238.85500*	38.40128	.000	-364.3432	-113.3668
	Medical Ward female	-240.18125*	38.40128	.000	-365.6695	-114.6930
	Surgical Ward male	-335.72375*	38.40128	.000	-461.2120	-210.2355
	Surgical ward female	-140.66000*	38.40128	.016	-266.1482	-15.1718
	Pediatrics	-19.90500	38.40128	1.000	-145.3932	105.5832
	Maternity	-175.16000*	38.40128	.001	-300.6482	-49.6718
operating Room	Emergency OPD	-530.78500*	38.40128	.000	-656.2732	-405.2968
	Intensive care unit	-54.40375	38.40128	.918	-179.8920	71.0845
	Medical laboratory	-66.34750	38.40128	.776	-191.8357	59.1407
	Medical Ward male	-293.25875*	38.40128	.000	-418.7470	-167.7705
	Medical Ward female	-294.58500*	38.40128	.000	-420.0732	-169.0968
	Surgical Ward male	-390.12750*	38.40128	.000	-515.6157	-264.6393
	Surgical ward female	-195.06375*	38.40128	.000	-320.5520	-69.5755
	Pediatrics	-74.30875	38.40128	.646	-199.7970	51.1795
	Maternity	-229.56375*	38.40128	.000	-355.0520	-104.0755
Medical laboratory	Emergency OPD	-464.43750*	38.40128	.000	-589.9257	-338.9493
	Intensive care unit	11.94375	38.40128	1.000	-113.5445	137.4320
	operating Room	66.34750	38.40128	.776	-59.1407	191.8357
	Medical Ward male	-226.91125*	38.40128	.000	-352.3995	-101.4230
	Medical Ward female	-228.23750*	38.40128	.000	-353.7257	-102.7493
	Surgical Ward male	-323.78000*	38.40128	.000	-449.2682	-198.2918
	Surgical ward female	-128.71625*	38.40128	.040	-254.2045	-3.2280
	Pediatrics	-7.96125	38.40128	1.000	-133.4495	117.5270
	Maternity	-163.21625*	38.40128	.002	-288.7045	-37.7280
Medical Ward male	Emergency OPD	-237.52625*	38.40128	.000	-363.0145	-112.0380
	Intensive care unit	238.85500*	38.40128	.000	113.3668	364.3432
	operating Room	293.25875*	38.40128	.000	167.7705	418.7470

	Medical laboratory	226.91125*	38.40128	.000	101.4230	352.3995
	Medical Ward female	-1.32625	38.40128	1.000	-126.8145	124.1620
	Surgical Ward male	-96.86875	38.40128	.275	-222.3570	28.6195
	Surgical ward female	98.19500	38.40128	.258	-27.2932	223.6832
	Pediatrics	218.95000*	38.40128	.000	93.4618	344.4382
	Maternity	63.69500	38.40128	.814	-61.7932	189.1832
Medical Ward female	Emergency OPD	-236.20000*	38.40128	.000	-361.6882	-110.7118
	Intensive care unit	240.18125*	38.40128	.000	114.6930	365.6695
	operating Room	294.58500*	38.40128	.000	169.0968	420.0732
	Medical laboratory	228.23750*	38.40128	.000	102.7493	353.7257
	Medical Ward male	1.32625	38.40128	1.000	-124.1620	126.8145
	Surgical Ward male	-95.54250	38.40128	.293	-221.0307	29.9457
	Surgical ward female	99.52125	38.40128	.241	-25.9670	225.0095
	Pediatrics	220.27625*	38.40128	.000	94.7880	345.7645
	Maternity	65.02125	38.40128	.795	-60.4670	190.5095
Surgical Ward male	Emergency OPD	-140.65750*	38.40128	.016	-266.1457	-15.1693
	Intensive care unit	335.72375*	38.40128	.000	210.2355	461.2120
	operating Room	390.12750*	38.40128	.000	264.6393	515.6157
	Medical laboratory	323.78000*	38.40128	.000	198.2918	449.2682
	Medical Ward male	96.86875	38.40128	.275	-28.6195	222.3570
	Medical Ward female	95.54250	38.40128	.293	-29.9457	221.0307
	Surgical ward female	195.06375*	38.40128	.000	69.5755	320.5520
	Pediatrics	315.81875*	38.40128	.000	190.3305	441.3070
	Maternity	160.56375*	38.40128	.003	35.0755	286.0520
Surgical ward female	Emergency OPD	-335.72125*	38.40128	.000	-461.2095	-210.2330
	Intensive care unit	140.66000*	38.40128	.016	15.1718	266.1482
	operating Room	195.06375*	38.40128	.000	69.5755	320.5520
	Medical laboratory	128.71625*	38.40128	.040	3.2280	254.2045
	Medical Ward male	-98.19500	38.40128	.258	-223.6832	27.2932
	Medical Ward female	-99.52125	38.40128	.241	-225.0095	25.9670
	Surgical Ward male	-195.06375*	38.40128	.000	-320.5520	-69.5755
	Pediatrics	120.75500	38.40128	.069	-4.7332	246.2432
	Maternity	-34.50000	38.40128	.996	-159.9882	90.9882
Pediatrics	Emergency OPD	-456.47625*	38.40128	.000	-581.9645	-330.9880
	Intensive care unit	19.90500	38.40128	1.000	-105.5832	145.3932
	operating Room	74.30875	38.40128	.646	-51.1795	199.7970
	Medical laboratory	7.96125	38.40128	1.000	-117.5270	133.4495
	Medical Ward male	-218.95000*	38.40128	.000	-344.4382	-93.4618
	Medical Ward female	-220.27625*	38.40128	.000	-345.7645	-94.7880

	Surgical Ward male	-315.81875[*]	38.40128	.000	-441.3070	-190.3305
	Surgical ward female	-120.75500	38.40128	.069	-246.2432	4.7332
	Maternity	-155.25500[*]	38.40128	.005	-280.7432	-29.7668
	Emergency OPD	-301.22125[*]	38.40128	.000	-426.7095	-175.7330
	Intensive care unit	175.16000[*]	38.40128	.001	49.6718	300.6482
	operating Room	229.56375[*]	38.40128	.000	104.0755	355.0520
	Medical laboratory	163.21625[*]	38.40128	.002	37.7280	288.7045
Maternity	Medical Ward male	-63.69500	38.40128	.814	-189.1832	61.7932
	Medical Ward female	-65.02125	38.40128	.795	-190.5095	60.4670
	Surgical Ward male	-160.56375[*]	38.40128	.003	-286.0520	-35.0755
	Surgical ward female	34.50000	38.40128	.996	-90.9882	159.9882
	Pediatrics	155.25500[*]	38.40128	.005	29.7668	280.7432
The mean difference is significant at the 0.05 level						

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wed Tukey honest significance difference test result of fungal load in Shenen Gibe hospital, 2016

(I) WARDS	(J) WARDS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	OPD	-550.68875[*]	26.97378	.000	-635.6098	-465.7677
	GYNACOLOGY	-136.67625[*]	26.97378	.000	-221.5973	-51.7552
	DELIVERY	-161.88750[*]	26.97378	.000	-246.8086	-76.9664
OR	MEDICAL LABORATORY	-58.38500	26.97378	.388	-143.3061	26.5361
	MEDICAL MALE	-283.96750[*]	26.97378	.000	-368.8886	-199.0464
	MEDICAL FEMALE	-90.23125[*]	26.97378	.030	-175.1523	-5.3102
	PEDIATRICS	-87.57750[*]	26.97378	.039	-172.4986	-2.6564
	OR	136.67625[*]	26.97378	.000	51.7552	221.5973
	OPD	-414.01250[*]	26.97378	.000	-498.9336	-329.0914
	DELIVERY	-25.21125	26.97378	.981	-110.1323	59.7098
GYNACOLOGY	MEDICAL LABORATORY	78.29125	26.97378	.091	-6.6298	163.2123
	MEDICAL MALE	-147.29125[*]	26.97378	.000	-232.2123	-62.3702
	MEDICAL FEMALE	46.44500	26.97378	.673	-38.4761	131.3661
	PEDIATRICS	49.09875	26.97378	.609	-35.8223	134.0198
	OR	161.88750[*]	26.97378	.000	76.9664	246.8086
	OPD	-388.80125[*]	26.97378	.000	-473.7223	-303.8802
	GYNACOLOGY	25.21125	26.97378	.981	-59.7098	110.1323
DELIVERY	MEDICAL LABORATORY	103.50250[*]	26.97378	.007	18.5814	188.4236
	MEDICAL MALE	-122.08000[*]	26.97378	.001	-207.0011	-37.1589
	MEDICAL FEMALE	71.65625	26.97378	.158	-13.2648	156.5773
	PEDIATRICS	74.31000	26.97378	.128	-10.6111	159.2311
MEDICAL	OR	58.38500	26.97378	.388	-26.5361	143.3061

LABORATORY	OPD	-492.30375*	26.97378	.000	-577.2248	-407.3827
	GYNACOLOGY	-78.29125	26.97378	.091	-163.2123	6.6298
	DELIVERY	-103.50250*	26.97378	.007	-188.4236	-18.5814
	MEDICAL MALE	-225.58250*	26.97378	.000	-310.5036	-140.6614
	MEDICAL FEMALE	-31.84625	26.97378	.934	-116.7673	53.0748
	PEDIATRICS	-29.19250	26.97378	.958	-114.1136	55.7286
MEDICAL MALE	OR	283.96750*	26.97378	.000	199.0464	368.8886
	OPD	-266.72125*	26.97378	.000	-351.6423	-181.8002
	GYNACOLOGY	147.29125*	26.97378	.000	62.3702	232.2123
	DELIVERY	122.08000*	26.97378	.001	37.1589	207.0011
	MEDICAL LABORATORY	225.58250*	26.97378	.000	140.6614	310.5036
	MEDICAL FEMALE	193.73625*	26.97378	.000	108.8152	278.6573
	PEDIATRICS	196.39000*	26.97378	.000	111.4689	281.3111
MEDICAL FEMALE	OR	90.23125*	26.97378	.030	5.3102	175.1523
	OPD	-460.45750*	26.97378	.000	-545.3786	-375.5364
	GYNACOLOGY	-46.44500	26.97378	.673	-131.3661	38.4761
	DELIVERY	-71.65625	26.97378	.158	-156.5773	13.2648
	MEDICAL LABORATORY	31.84625	26.97378	.934	-53.0748	116.7673
	MEDICAL MALE	-193.73625*	26.97378	.000	-278.6573	-108.8152
	PEDIATRICS	2.65375	26.97378	1.000	-82.2673	87.5748
PEDATERICS	OR	87.57750*	26.97378	.039	2.6564	172.4986
	OPD	-463.11125*	26.97378	.000	-548.0323	-378.1902
	GYNACOLOGY	-49.09875	26.97378	.609	-134.0198	35.8223
	DELIVERY	-74.31000	26.97378	.128	-159.2311	10.6111
	MEDICAL LABORATORY	29.19250	26.97378	.958	-55.7286	114.1136
	MEDICAL MALE	-196.39000*	26.97378	.000	-281.3111	-111.4689
	MEDICAL FEMALE	-2.65375	26.97378	1.000	-87.5748	82.2673
*. The mean difference is significant at the 0.05 level.						

Showed Tukey honest significance difference test result of bacterial load in Shenen Gibe hospital, 2016

(I) WARDS	(J) WARDS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
OPD	MINOR OPERATING ROOM	3217.88875*	69.38459	.000	2999.4464	3436.3311
	GYNACOLOGY	325.10875*	69.38459	.000	106.6664	543.5511
	DELIVERY	3077.22625*	69.38459	.000	2858.7839	3295.6686
	MEDICAL LABORATORY	3180.73500*	69.38459	.000	2962.2927	3399.1773
	MEDICAL MALE	2955.14125*	69.38459	.000	2736.6989	3173.5836
	MEDICAL FEMALE	3148.88500*	69.38459	.000	2930.4427	3367.3273
	PEDATERICS	3151.53625*	69.38459	.000	2933.0939	3369.9786
MINOR OPERATING ROOM	EMERGENCY OPD	-3217.88875*	69.38459	.000	-3436.3311	-2999.4464
	GYNACOLOGY	-2892.78000*	69.38459	.000	-3111.2223	-2674.3377
	DELIVERY	-140.66250	69.38459	.474	-359.1048	77.7798
	MEDICAL LABORATORY	-37.15375	69.38459	.999	-255.5961	181.2886
	MEDICAL MALE	-262.74750*	69.38459	.008	-481.1898	-44.3052
	MEDICAL FEMALE	-69.00375	69.38459	.973	-287.4461	149.4386
	PEDATERICS	-66.35250	69.38459	.979	-284.7948	152.0898
GYNACOLOGY	EMERGENCY OPD	-325.10875*	69.38459	.000	-543.5511	-106.6664
	MINOR OPERATING ROOM	2892.78000*	69.38459	.000	2674.3377	3111.2223
	DELIVERY	2752.11750*	69.38459	.000	2533.6752	2970.5598
	MEDICAL LABORATORY	2855.62625*	69.38459	.000	2637.1839	3074.0686
	MEDICAL MALE	2630.03250*	69.38459	.000	2411.5902	2848.4748
	MEDICAL FEMALE	2823.77625*	69.38459	.000	2605.3339	3042.2186
	PEDATERICS	2826.42750*	69.38459	.000	2607.9852	3044.8698
DELIVERY	EMERGENCY OPD	-3077.22625*	69.38459	.000	-3295.6686	-2858.7839
	MINOR OPERATING ROOM	140.66250	69.38459	.474	-77.7798	359.1048
	GYNACOLOGY	-2752.11750*	69.38459	.000	-2970.5598	-2533.6752
	MEDICAL LABORATORY	103.50875	69.38459	.808	-114.9336	321.9511
	MEDICAL MALE	-122.08500	69.38459	.649	-340.5273	96.3573
	MEDICAL FEMALE	71.65875	69.38459	.967	-146.7836	290.1011
	PEDATERICS	74.31000	69.38459	.960	-144.1323	292.7523
MEDICAL LABORATORY	EMERGENCY OPD	-3180.73500*	69.38459	.000	-3399.1773	-2962.2927
	MINOR OPERATING ROOM	37.15375	69.38459	.999	-181.2886	255.5961
	GYNACOLOGY	-2855.62625*	69.38459	.000	-3074.0686	-2637.1839
	DELIVERY	-103.50875	69.38459	.808	-321.9511	114.9336

	MEDICAL MALE	-225.59375*	69.38459	.038	-444.0361	-7.1514
	MEDICAL FEMALE	-31.85000	69.38459	1.000	-250.2923	186.5923
	PEDATERICS	-29.19875	69.38459	1.000	-247.6411	189.2436
MEDICAL MALE	EMERGENCY OPD	-2955.14125*	69.38459	.000	-3173.5836	-2736.6989
	MINOR OPERATING ROOM	262.74750*	69.38459	.008	44.3052	481.1898
	GYNACOLOGY	-2630.03250*	69.38459	.000	-2848.4748	-2411.5902
	DELIVERY	122.08500	69.38459	.649	-96.3573	340.5273
	MEDICAL LABORATORY	225.59375*	69.38459	.038	7.1514	444.0361
	MEDICAL FEMALE	193.74375	69.38459	.118	-24.6986	412.1861
	PEDATERICS	196.39500	69.38459	.108	-22.0473	414.8373
MEDICAL FEMALE	EMERGENCY OPD	-3148.88500*	69.38459	.000	-3367.3273	-2930.4427
	MINOR OPERATING ROOM	69.00375	69.38459	.973	-149.4386	287.4461
	GYNACOLOGY	-2823.77625*	69.38459	.000	-3042.2186	-2605.3339
	DELIVERY	-71.65875	69.38459	.967	-290.1011	146.7836
	MEDICAL LABORATORY	31.85000	69.38459	1.000	-186.5923	250.2923
	MEDICAL MALE	-193.74375	69.38459	.118	-412.1861	24.6986
	PEDATERICS	2.65125	69.38459	1.000	-215.7911	221.0936
PEDATERICS	EMERGENCY OPD	-3151.53625*	69.38459	.000	-3369.9786	-2933.0939
	MINOR OPERATING ROOM	66.35250	69.38459	.979	-152.0898	284.7948
	GYNACOLOGY	-2826.42750*	69.38459	.000	-3044.8698	-2607.9852
	DELIVERY	-74.31000	69.38459	.960	-292.7523	144.1323
	MEDICAL LABORATORY	29.19875	69.38459	1.000	-189.2436	247.6411
	MEDICAL MALE	-196.39500	69.38459	.108	-414.8373	22.0473
	MEDICAL FEMALE	-2.65125	69.38459	1.000	-221.0936	215.7911

*. The mean difference is significant at the 0.05 level.