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ABSTRACT

BACKGROUND: In both residential and hospital indoor environments, humans can be exposed to airborne microorganisms. The hospital's indoor air may contain a large number of disease-causing agents brought in by patients, staff, students, visitors, ventilation, or the outside. Hospitalized patients are at a higher risk of infection due to confined spaces, crowdedness, and poor infection prevention practices, which can accumulate and create favorable conditions for the growth and multiplication of microorganisms. Therefore, the aim of this study was to evaluate the indoor air bacterial load in Dilla University Hospital, Southern Ethiopia.

METHODS: An institutional-based cross-sectional study design was used to assess the bacterial load in the indoor air at Dilla University Hospital. To determine the bacterial load, a passive air sampling technique was used. The settle plate method was used to collect data, which involved exposing Petri-dishes filled with blood agar media to the indoor air of the sampled rooms for 60 minutes.

RESULT: A total of 72 indoor air samples were collected once a week for 2 weeks at 14-day intervals from 18 rooms in 8 wards, and samples were collected twice a day in the morning and afternoon. The mean bacterial concentrations ranged from 450 to 1585.83 CFU/m^3 after 60 minutes of culture media exposure. The mean bacterial concentrations in the obstetrics, surgical, pediatric, gynecology, and medical wards exceeded WHO guidelines. A high indoor air bacterial load was found in 58 (80.6%) of the samples in this study. Gram-positive bacteria in the air were the most common 51 (71%) of the bacterial population measured in all indoor environments. Fungal growth was found in 65 (90.3%) of the samples. Temperatures (26.5°C-28.3°C) and relative humidity (61.1%-67.8%) in the rooms were both above WHO guidelines, creating favorable conditions for bacterial growth and multiplication.

CONCLUSION: The majority of the wards at Dilla University Hospital had bacterial loads in the air that exceeded WHO guidelines. Overcrowding, high temperatures, inadequate ventilation, improper waste management, and a lack of traffic flow control mechanisms could all contribute to a high concentration of bacteria in the indoor air. To control the introduction of microorganisms by patients, students, caregivers, and visitors, it is critical to regularly monitor indoor air bacterial load and implement infection prevention and control measures.

KEYWORDS: Bacterial load, hospital wards, indoor air and settling plate

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Introduction

Humans require a near-constant supply of air. It is a fundamental human right for all people to have unrestricted access to clean air. The air quality in health-care facilities, offices, schools, day-care centers, and public buildings, where people spend a significant amount of their time, is critical in determining a healthy life and residents' well-being.[1,2]

Healthcare facilities can be sources of pathogenic bacteria that can lead to hospital-acquired infections (HAIs).[3] According to the Centers for Disease Control and Prevention, nearly 1.7 million hospitalized patients annually acquire HAIs while being treated for other health issues, and more than 98 000 patients (1 in 17) die as a result of these infections.[4] Bacteria were responsible for 90% of all HAIs. Bacterial indoor air quality reflects the health facility's sanitary conditions. Bacteria, mold, and viruses can grow on the pans of the ventilation system, as well as on the moist ceiling and floor.[5] Because most bacteria can live on dry surfaces for months and are resistant to disinfectants, their resistance has an impact on patient health.[6-8]

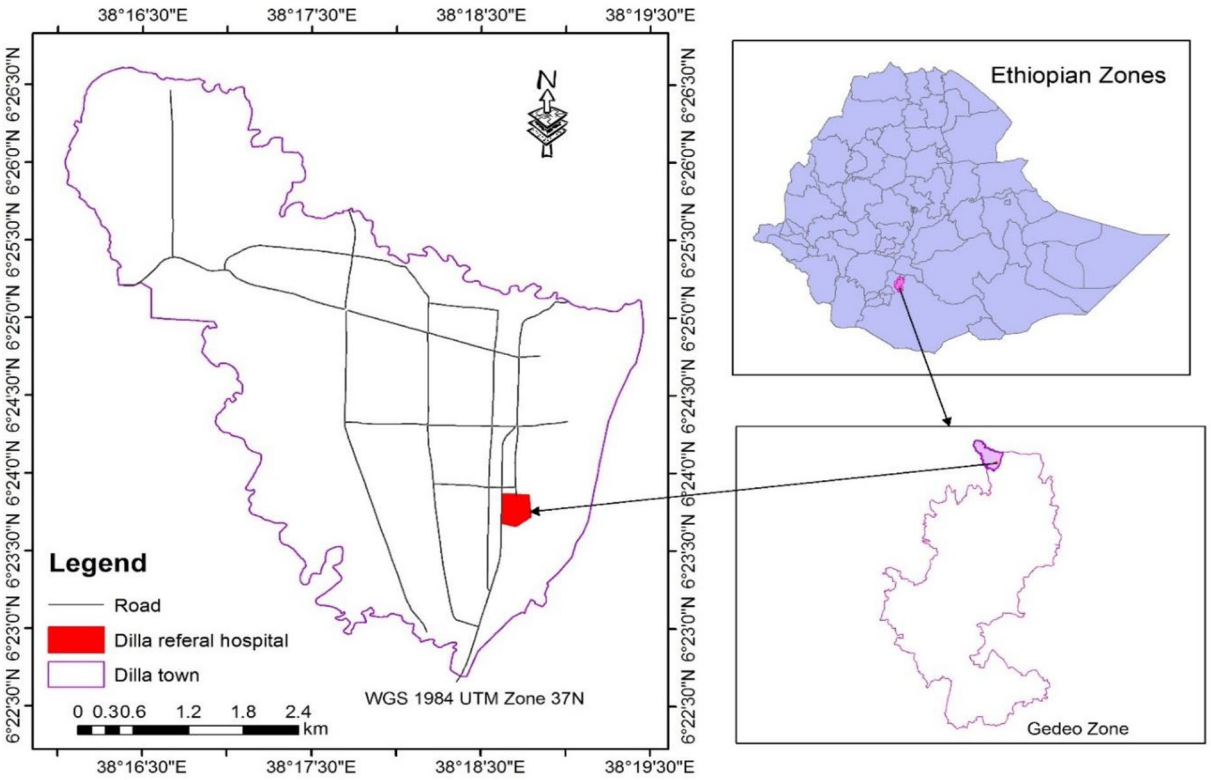

Figure 1. Location of the study area.

Bio-aerosol presence and multiplication are aided by a lack of fresh air caused by increased building insulation, poorly maintained or operated ventilation systems, and poorly regulated temperature and relative humidity levels. Poor indoor air quality in developing countries may be exacerbated by design flaws and insufficient ventilation.[9,10] Hospital indoor air quality is affected by the presence of unsanitary attached toilets, a poor waste management system, and a poor ventilation system.[11]

Most hospitals in developing countries lack an effective infection prevention and control program due to a lack of awareness, personnel, water, laboratory backup, and safe practices among health workers.[12] Poor indoor air quality has been linked to a variety of airborne infections, with the most common disease-causing bacterial agents.[11,13,14]

The microbial load in hospital indoor air is affected by the number of occupants, their activity, and the ventilation. Residents may be a source of microorganisms because bacteria are shed from the skin and respiratory tract. Gram-negative bacilli can be found in sinks, washbasins, drains, nebulizers, humidifiers, and cooling towers.[15-17]

There are significant correlations between airborne colony count and indoor air quality parameters; however, the relationship is not conclusive; indoor air quality parameters may influence microorganisms.[18] Several studies in Ethiopia revealed a high risk of infection in hospital wards as a result of a high bacterial load in the air. Bacterial air quality was found to be above standards[19] in almost all of the hospital wards studied in Hawassa, Gonder, Jimma, and Adama.[11,20-22] Therefore, determining the indoor air bacterial load helps to revise and design appropriate infection prevention methods to reduce the occurrence of health care acquired infections. There have been a few research on indoor air quality in Ethiopian hospitals, and those studies have revealed a high bacterial load in the majority of the hospitals. However, none of them provide sufficient information regarding the various environmental factors that contribute to indoor air bacteria load, such as temperature, humidity, inpatient room cleanliness, use of ventilation systems, and overcrowding. Therefore, the objective of this study was to evaluate the indoor air bacterial load Dilla University Hospital.

Materials and Methods

Study setting

The study was conducted at Dilla University Hospital in Dilla town, Gedeo zone, Southern Ethiopia. Dilla town is found at a distance of 365 km from the capital city of Ethiopia, Addis Ababa. Dilla University Hospital provides curative and rehabilitative services to over 2 million people in the SNNPR, Oromia and Sidama Regional State as catchment areas. The hospital has 8 wards with a capacity of 209 beds and is staffed by 546 health care workers (Figure 1).

Study design and period

An institutional-based cross-sectional study was conducted to determine indoor air bacterial load at Dilla University Hospital, Southern, Ethiopia from June to August 2021.

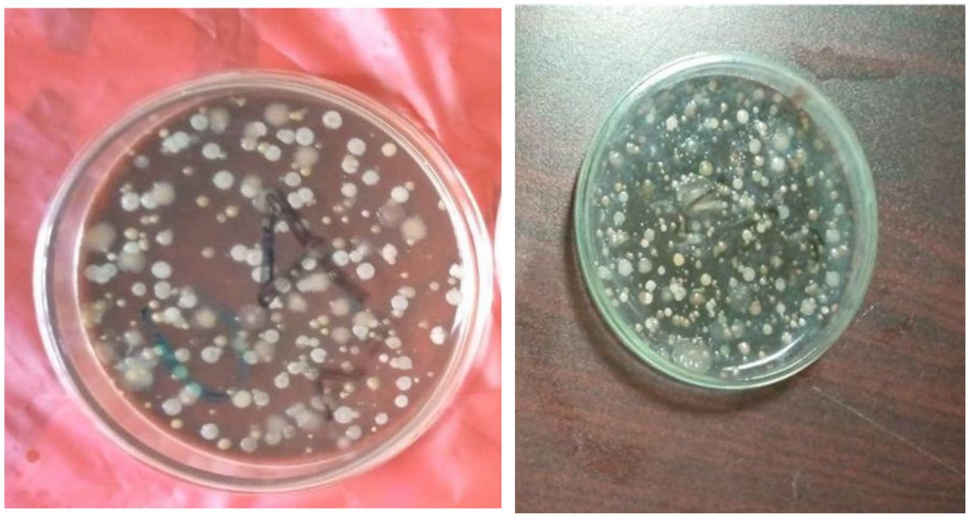

Figure 2. Cultured Petri dishes.

Data collection and validation

Blood agar for indoor air data collection. A laboratory technician prepared blood agar according to standard operating procedure (SOP) 1 day before the data collection day, dispensed it into petri dishes, and stored it in the refrigerator at 2°C to 8°C after labeling it with the name of the media, preparation date, and expire date. Each petri dish was coded, and the time when the blood agar was first exposed to indoor air was recorded. We cleaned the sample collection area to avoid contamination during sample collection, and we decontaminated the cold box and transported it with care to avoid contamination during transportation. We cleaned and decontaminated the incubator to avoid contamination during incubation.

Media preparation procedures

Culture media were prepared according to the manufacturer's instructions, and sterility was confirmed by incubating a representative of the batch at 35°C to 37°C for 48 to 72 hours and observing for growth. Those batches of media that showed any growth within 72 hours were discarded. All laboratory investigations and analyses were carried out in accordance with standard operating procedures (SOPs) developed by investigators.

Laboratory diagnosis

Gram stain. Gram staining was performed on sample taken from culture after overnight incubation using Crystal violet (as an initial stain), Gram's iodine (as a mordant or binding agent), acetone alcohol (20 volume of acetone: 19 volumes of methanol: 1 volume of water) (as a decolorizer), and Safranin (as a counter stain) to identify gram positive and gram-negative bacteria according to SOPs.

Data collection procedures

Microbiological analysis method. Air samples were collected from the hospital's surgery, emergency, orthopedic, obstetric, medical, pediatrics, gynecology, and Neonatal Intensive Care Units (NICU) wards, which all provided patient care services. Bacteria and fungi were collected using a passive air sampling technique: the settle plate method with (90 mm) or 9 cm diameter petri dishes on blood agar enriched with 5% sheep blood (Becton, Dickinson and Company). Plates were left open according to the 1/1/1 method (1 m from the walls, 1 m from the floor, and for 1 hour), then covered with their lids. Samples were collected twice a day, at 9:00 a.m. and 2:30 p.m., and were immediately placed in a cold box before being transported to the Dilla University Hospital microbiology laboratory for further analysis. Each plate was incubated for 24 to 48 hours at 37°C under aerobic conditions, and colony-forming units (CFUs) were counted using a bacteria colony counter. After counting the colony forming units (CFU), the CFU/m^3 was calculated using Omeliansky's equation.[23,24]

$N = 5a \times 10^4 (bt)^{-1}$, Where N = microbial CFU/m^2 of indoor air; a = number of colonies per petri dish; b = dish surface (cm^2); t = exposure time (minutes)[25,26] (Figure 2). We used Sabouroad dextrose agar medium for fungal culture and incubated it for 3 days at 25°C. Gentamicin, chloramphenicol, and cycloheximide were added to Sabouraud dextrose agar to inhibit the overgrowth of both gram positive and gram-negative bacteria, and cycloheximide was added to inhibit the growth of saprophytic fungi.[27] All antibiotics were added after the media had been autoclaved and cooled to 45°C to 50°C.

Sample quality management

Before the data collection period, microbiologists provided training to data collectors. To ensure the sample's quality, every necessary procedure was followed from collection to analysis, including sterilization of sampling equipment, the use of personal protective clothing, gloves, and a cold box to transport the sample, proper handling of sterilized materials, and the safe incubation of samples.

To avoid contamination, monitoring was performed during transportation and analysis at the laboratory. Before the real data collection for the air sample, a per-test was performed. The per-test for air sampling was designed to determine the media exposure period to the indoor hospital room. Before using the culture media, any physical changes such as cracks, excessive moisture, color, hemolysis, dehydration, contamination, and expiration dates were examined. The temperature of the incubator and refrigerator was monitored on a daily basis.

Observational checklist and measurement

An observational checklist and measurement were used to assess cleanliness, frequency of cleaning, proper storage of patient food, presence of odor and flies, number of windows, and waste management systems. Two environmental health professionals were brought in to evaluate the associated factors for indoor air quality. The room's temperature and humidity were measured using Digital LCD Thermometer Hygrometer Humidity Meter Room Indoor Temperature clock. In addition, one microbiologist participated in the collection and analysis of the samples.

Operational definition

Indoor air: It defines as air within a building occupied for at least 1 hour by people of varying states of health. Indoor air quality can be defined as the totality of attributes of indoor air that affect a person's health and wellbeing.[28]

Cleanliness: Conditions with trash free walk ways, mopped floors, stainless floor and wall, organized patients' drawers and table that are free from left items indicate cleanliness.

WHO expert group bacterial load standard: Less than 1000 CFU/m^3 is acceptable.[29]

European Commission for non-industrial premises sanitary standard for bacterial load. A bacterial load of less than 50 CFU/m^3 is considered "Very low," 50 to 100 CFU/m^3 is considered "Low," 100 to 500 CFU/m^3 is considered "intermediate," 500 to 2000 CFU/m^3 is considered "High," and more than 2000 CFU/m^3 is considered "Very high."[30]

Results

A total of 72 indoor air samples were taken from 18 rooms in 8 wards once a week for 2 weeks at a 14-day interval, and sampled twice a day in the morning and afternoon for this study. According to the findings of this study, the highest indoor bacterial count was found in the pediatric ward at the stable room, which was 2200 CFU/m^3 after 60 minutes of culture media exposure, and the lowest count was found in the Neonatal Intensive Care Unit (NICU) at pre-term critical, which was 300 CFU/m^3 after 60 minutes of culture media exposure time (Table 1)

The mean bacterial concentrations in the obstetrics, surgical, pediatric, gynecological, and medical wards exceeded WHO guidelines. However, mean bacterial concentrations in the Neonatal Intensive Care Unit (NICU), Orthopedics Ward, and Emergency Department were all within the WHO guidelines range. The temperatures and relative humidity (RH) levels in the rooms were 26.5°C to 28.3°C and 61.1% to 67.8%, respectively, which were above WHO guidelines (Table 2).

Indoor air bacterial load of hospital wards

According to the European Commission for non-industrial premises sanitary standard for bacterial load, 58 (80.6%) of the samples in this study had high indoor air bacterial load (500-2000 CFU/m^3), while 8 (11.1%) had very high indoor air bacterial load (>2000 CFU/m^3) (Figure 3). Gram staining was used to identify gram positive and gram-negative bacteria from a culture based on their gram reaction using the 4 basic gram staining reagents: crystal violet, gram's iodine, acetone–alcohol, and safranin, with gram-positive bacteria stained blue to purple (Figure 4) and gram-negative bacteria-stained pink to red (Figure 5). Based on gram reaction, 71% was gram positive while 29% was gram negative. Fungal growth was found in 65 (90.3%) of the samples.

Observational result

Health care waste management. Dilla University Hospital had poor waste collection, sorting, transportation, and disposal systems. Waste generated in inpatient wards was not collected on a daily basis, resulting in waste overflow in some wards. Contaminated waste containers were not properly cleaned or disinfected after waste disposal.

Handwashing and latrine facilities and practices. Almost all inpatient wards lack hand washing facilities, and those that do exist are dirty and insufficient. People or air currents from these dirty hand washing facilities and toilets may carry microorganisms to the wards. This may contribute to poor indoor air quality in the hospital, which may result in a high microbial load in the air.

Traffic flow control and ventilation. Microbial contamination can be significant in heavily trafficked areas and areas where soiled surgical instruments and other equipment are first processed. Controlling the flow of visitors, patients, and staff is critical to preventing disease transmission in healthcare facilities. According to the findings of this study, wards are overcrowded, natural ventilation in the rooms is below the WHO guidelines (the majority of ward windows are closed), and there is no artificial (mechanical) ventilation. Close contact was observed between patients, caregivers, and attendants. In addition, most wards lack traffic flow control methods such as signs

Table 1. Bacterial counts in air samples collected from different rooms at Dilla University Hospital, 2021.

DEPARTMENT/WARDS	SAMPLE COLLECTION UNIT	BACTERIAL GROWTH BY CFU/M³			
		WEEK 1		WEEK 3	
		MORNING	AFTERNOON	MORNING	AFTERNOON
Obstetrics	Delivery room	1120	1115	1180	990
	Waiting room	2120	2100	1950	1670
	Postnatal room	1190	2195	1650	1750
Surgical	Female room	1050	1000	1060	970
	Male room	1125	980	1325	990
	Pediatric surgery room	1220	1015	1120	1045
Pediatrics	Stable room	2110	1500	2200	970
	Phase one	1540	1050	990	785
Emergency	Yellow room	1200	710	1250	990
	Pediatrics room	890	680	620	890
Medical	Male critical room	2120	925	2000	1550
	Female critical room	1480	1040	950	850
NICU	Preterm critical	510	300	500	380
	Term critical	400	480	540	490
Gynecology	High risk	930	640	800	870
	Maternity room	2140	690	2130	755
Orthopedics ward	Orthopedics inside	1070	765	1150	885
	Orthopedics outside	1000	880	950	700

Abbreviations: CFU, Colony Forming Unit; NICU, Neonatal Intensive Care Unit.

Table 2. The mean bacterial concentrations, temperature, and humidity of inpatient units at Dilla University Hospital, Southern Ethiopia, 2021.

WARDS	BACTERIA (CFU/M³)	TEMPERATURE (°C)	RH (%)	WHO GUIDELINES
Obstetrics	1585.83	27	61.1	Bacteria (<1000 CFU/m³) Temperature (22°C-26.1°C) RH (30%-60%)
Surgical	1075	27.3	62.3	
Pediatrics	1393.12	27.2	66.5	
Emergency	903.75	26.5	61.2	
Medical	1364.37	27.9	67.8	
NICU	450	26.6	64.2	
Gynecology	1119.37	28.2	62.9	
Orthopedics ward	925	28.3	67	

Abbreviations: CFU, Colony Forming Unit; NICU, Neonatal Intensive Care Unit; RH, Relative Humidity.

(eg, authorized personnel only), reminders (eg, red line on the floor), and physical barriers to control the flow of visitors, patients, and staff (eg, closed doors).

Environmental cleaning. Clean techniques are essential for all healthcare providers, patients, and attendants on a daily basis because they help in the prevention of infections. According to

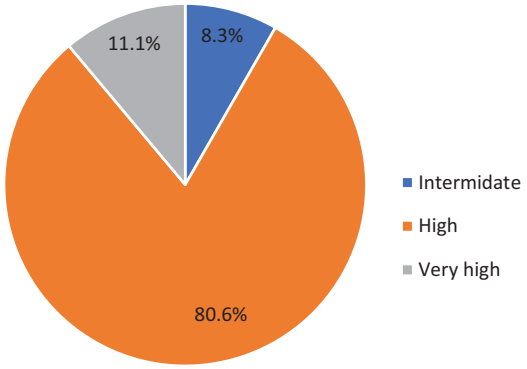

Figure 3. Bacterial contamination of indoor air of Dilla University Hospital, Southern Ethiopia, 2021.

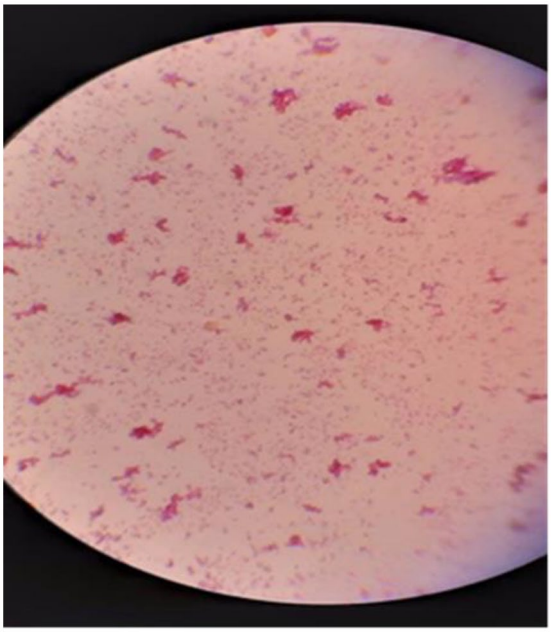

Figure 4. Gram-positive rod bacteria.

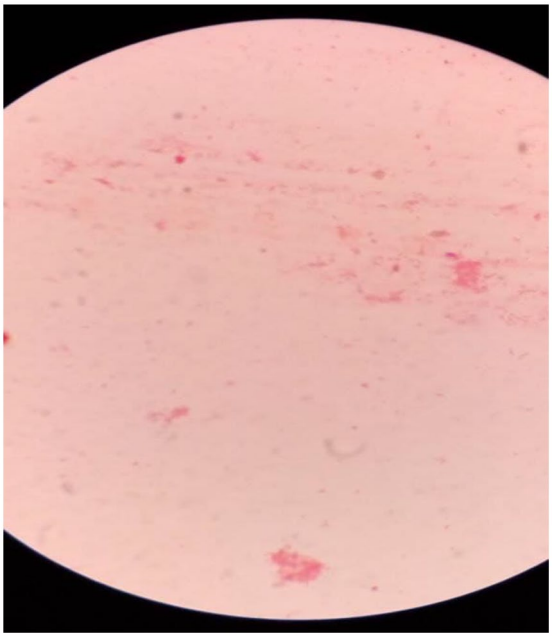

Figure 5. Gram-negative cocci bacteria.

the study's findings, the majority of wards do not adhere to environmental cleaning guidelines, preferring dry sweeping and dusting over wet mopping, which may increase the generation of bio-aerosols in the indoor air. The patient beds and surroundings, as well as the floors, walls, ceilings, and doors, including door handles, were not cleaned and disinfected on a regular basis.

Discussion

The bacterial indoor air quality reflects the sanitary conditions of the health care facility. Because most bacteria can survive for months on dry surfaces and are resistant to disinfectants, their resistance has an impact on patient health. Using a passive air sampling technique called the settle plate method, the bacterial load in the Dilla University Hospital's indoor air ranged from 300 to 2200 CFU/m^3. This study's findings were higher than those of studies conducted at the University of Gondar Teaching Hospital in Northwest Ethiopia[11] and in different locations of a Portuguese Hospital[31] but, lower than those of studies conducted in Jimma University Specialized Hospital[20] and in hospitals in Dutse, Jigawa State.[32] The disparity could be attributed to differences in Infection Prevention and Control (IPC) practices, temperature and humidity variations, traffic flow control mechanisms, and hospital settings.

The obstetrics ward had the highest mean indoor air bacterial concentration, while the Neonatal Intensive Care Unit (NICU) ward had the lowest. This study's findings were supported by a case study at Jimma University Specialized Hospital.[20] The high bacterial load in the obstetrics ward could be attributed to poor room cleanliness, overcrowding of rooms by Medicine and health science students, patients, and visitors, insufficient ventilation, attachment of unsanitary latrines and shower facilities, and improper health care waste management.

The average indoor air bacterial load ranged from 450 to 1585.83 CFU/m^3. The findings of the study were lower than a study conducted at Jimma University Specialized Hospital, which ranged from 2123 to 9733 CFU/m^3,[20] and higher than a study conducted in a tertiary care hospital in India, which ranged from 65.52 to 1179 CFU/m^3,[9] and the University of Gondar Teaching Hospital in Northwest Ethiopia, which ranged from 480 to 1468 CFU/m^3.[11] The high concentration of bacteria in the indoor air in this study could be attributed to poor health care waste management, overcrowding of wards by students, patients, and visitors, and poor sanitation of attached latrine facilities. Furthermore, this study revealed that all wards lacked artificial ventilation and relied solely on natural ventilation. Pollutants from the waste disposal area and the unsanitary outdoor environment may enter the room through the windows and doors.

According to the results of the study, 37 (51.4%) of the indoor air samples exceeded the WHO expert group's bacterial load standard (<1000 CFU/m^3). The findings of this study were supported by research conducted at the University of Gondar Teaching Hospital in Northwest Ethiopia[11] and the Hawassa University Comprehensive Specialized Hospital Wards.[22]

According to the European Commission's non-industrial premises sanitary standard for bacterial load, 58 (80.6%) of the samples in this study had high indoor air bacterial load (500-2000 CFU/m^3) and 8 (11.1%) had very high indoor air bacterial load (>2000 CFU/m^3). The majority of wards were overcrowded, natural ventilation in the rooms is below WHO guidelines, and there is no artificial (mechanical) ventilation, all of which could contribute to room contamination. Patients, caregivers, and visitors were found to be in close contact with one another. Furthermore, latrines and splashes of blood or body fluids were not cleaned on a regular basis, and cleaning was done with only water and no disinfectants. This study's findings were supported by research from Nigeria,[33] the University of Gondar Teaching Hospital in Northwest Ethiopia,[11] and the Hawassa University Comprehensive Specialized Hospital Wards.[22]

After gram staining, both gram positive and gram-negative bacteria were identified in this study, with the majority of detected bacteria being gram positive 51 (71%). This study's findings were higher than those of a previous study conducted in Hawassa, Ethiopia, which reported 43.1%.[34] However, it was lower than studies conducted at Ayder Referral Hospital in Northern Ethiopia, which reported 87.3%[35] and Felege Hiwot Referral Hospital (FHRH) in Northwest Ethiopia, which reported 81.6%.[14] Possible explanations for the high distribution of gram-positive bacteria include the hospital's dry conditions and transmission through the skin, nasal cavity, and boils of healthcare workers, patients, visitors, and students.

Gram-negative cocci and Gram-positive rod bacteria were the most commonly identified bacteria in this study. The findings of this study were supported by a study conducted in hospitals in Dutse, Jigawa State[32] and study conducted in different sites of in a Portuguese Hospital.[31] In this study, fungal growth was found in 65 (90.3%) of the samples. The findings of this study were supported by the studies conducted in Nigeria,[33] at the University of Gondar Teaching Hospital in Northwest Ethiopia[11] and at Hawassa University Comprehensive Specialized Hospital Wards.[22]

In addition, the temperature and humidity levels in the wards were measured and analyzed. Temperatures of 20°C to 22°C and Relative Humidity levels of 30% to 60% were not conducive to the growth and multiplication of microorganisms.[33,36] However, in this study, the room temperature ranged from 26.5 to 28.3°C, and the relative humidity ranged from 60.5% to 69.5%, which was suitable for microorganism growth and multiplication. This study's findings were supported by those of another conducted at the University of Gondar Teaching Hospital in Northwest Ethiopia, where temperatures ranged from 26.5°C to 29.5°C and humidity ranged from 64.5% to 85%.[11] Temperature and relative humidity were found to be favorable for bacterial growth and multiplication in ward indoor air in this study. This may explain why the bacterial load in the Dilla University Hospital's indoor air has increased.

Conclusion

The majority of Dilla University Hospital's wards had indoor air bacterial loads that exceeded WHO guidelines and European Commission non-industrial premises sanitary standards. In the obstetrics, surgical, pediatric, gynecological, and medical wards, mean bacterial concentrations exceeded WHO guidelines. In this study, gram-positive rod bacteria and gram-negative cocci were found. Fungal growth was found in 90% of the samples.

According to the observational results, the main environmental factors that contributed to this high range of bacterial load were low cleaning frequency, the presence of flies, humidity, poor ventilation, improper waste management, soiled walls, high room temperature, and a large number of visitors and medicine and health sciences students. Therefore, regularly monitoring and evaluating indoor air bacterial load, as well as implementing infection prevention and control measures to control the introduction of microorganisms into the hospital by patients, visitors, and students, in order to reduce the risk of infection for inpatients, particularly the immune compromised, elderly, and children. Furthermore, each room should be inspected on a regular basis to determine if there is any condition or situation that may promote microbial growth, such as a leak or a sanitation problem. Reduce the number of visitors and health science students in order to avoid overcrowding in the wards' rooms and corridors. Enable for the proper use of windows in managing the temperature of the room and dilution of the microbial load.

Author Contributions

ZA: conceived and designed the study. ZA, KD, AA, GHW, ASA, GGK, HEH, AKW, and MT collected, analyzed, and interpreted the data and wrote the manuscript. The final manuscript was critically reviewed and approved by all of the authors.

Availability of Data

Data will be made available by request.

Ethical Consideration

Both verbal and written consent were considered and supportive letters from Dilla University and different stakeholders has been secured and also Approval from IRB of the University was secured before the implementation of the research.

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