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Investigating Microbial Contamination of Indoor Air, Environmental Surfaces, and Medical Equipment in a Southwestern Ethiopia Hospital

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ABSTRACT

INTRODUCTION: Healthcare-associated infections, primarily caused by microorganisms, are widespread in healthcare facilities. These infections pose a significant challenge, especially in low and middle-income countries, and have a detrimental impact on patient outcomes. It is crucial to assess the level of microbial load and associated factors to prevent the spread of these infections. The objective of this study was to assess the microbial load and identify the factors associated with it in various wards at Jimma Medical Center.

METHOD: A cross-sectional study conducted at Jimma Medical Center. Indoor air samples were collected using the settle plate method with a 1/1/1 scheme. Inanimate surfaces and medical equipment were sampled using Swabs from a 10 × 10 cm area. A total of 268 samples were collected from 10 rooms. Pertinent information regarding the associated factors was gathered using an observational checklist. A multiple linear regression model was used to identify any associations with the microbial load.

RESULT: Out of the total samples, 181 (67.5%) tested positive for culture, and 270 microbes were isolated. The average load of bacteria and fungi in the indoor air ranged from 124.4 to 1607 and 96 to 814.6 Colony-forming unit (CFU)/m³, respectively. The mean total aerobic colony counts of bacteria and fungi from all surfaces in the wards ranged from 5.25 to 43.3 CFU/cm². Crowdedness [$\beta = 2.748$ (95% Confidence Interval (CI): 1.057-4.44)], the presence of waste material [$\beta = 1.747$ (95% CI: 0.213-3.282)], and an unclean room [$\beta = 2.505$ (95% CI: 0.990-4.019)] were significantly associated with the microbial load.

CONCLUSION: The microbial load detected in indoor air, inanimate surfaces and medical equipment was posing potential health risks. Consequently, it is recommended to implement regular microbial surveillance of the hospital environment and enhance the infection prevention program to mitigate these concerns.

KEYWORDS: Microbial load, indoor air, inanimate surfaces, medical equipment, hospital infection control

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Introduction

Healthcare-associated infections (HCAIs) occur within 48 hours or more following Hospitalization or within 30 days after receiving medical care.¹ Due to microbial contamination of the hospital environment, particularly in operating rooms, intensive care units, and other specialized wards, the burden of HCAIs continued to rise, which resulted in a high rate of morbidity and mortality for patients.² In a hospital environment, the microorganisms that cause HCAIs include bacteria, viruses, and fungi. However, more than 90% of them are due to bacteria.³ The most common organisms causing HCAIs reported are *Staphylococcus aureus* (*S. aureus*), *Coagulase-negative staphylococci* (*CoNS*), *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*), *Klebsiella* species, and *proteus* species that may come from the patient, contaminated instruments, and the environment.^{4,5}

Approximately 1.4 million people worldwide suffer from a lack of clean and safe healthcare facilities.⁶ While it was previously believed that most HCAIs were transmitted from patient to patient, recent evidence suggests that medical staff and the clinical environment, such as surfaces and equipment, are also common sources of infection.⁷ The burden of HCAIs, both endemic and epidemic, poses significant risks to public health. In low and middle-income countries, 15 out of every 100 hospitalized patients will acquire at least one HCAI, compared to 7 patients in developed countries. In the United States (US), HCAIs affect 3.2% of all hospitalized patients, while in the European Union/European Economic Area, the rate is 6.5%.^{8,9} Prevalence rates of HCAIs range from 5.7% to 19.1% in low-income countries, with a pooled prevalence of 16.96% in Ethiopia,¹⁰ and 19.41% in Jimma.¹¹



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Table 1. Inanimate surfaces and medical equipment sample allocation.

TYPE OF WARD	INANIMATE SURFACE SAMPLED	MEDICAL EQUIPMENT SAMPLED	TOTAL
Pediatric ward	65	15	79
MICU	26	6	33
NICU	38	18	56
OR	46	14	60
Total	175	53	228

Abbreviations: MICU, medical intensive care unit; NICU, neonatal intensive care unit; OR, operating room.

A study conducted internationally revealed that the mortality rate among patients with HCAs was double (30%) compared to those without HCAs (15%).¹² In the United States, a national study estimated that HCAI-related deaths numbered 98,987.¹³ According to the European Centre for Disease Prevention and Control (CDC) reported an estimated 91 310 deaths in acute-care hospitals due to HCAs.¹⁴ Patients with HCAs in US hospitals had an additional length of hospital stay (LOS) of 26.3 days, while those without HCAs had a LOS of 5.69 days.¹⁵

The United States Centers for Disease Control and Prevention has reported that Hospitals in the USA alone incur annual direct medical costs of HCAs ranging from US\$ 35.7 to 45 billion. Meanwhile, the annual economic impact in Europe is as high as € 7 billion.¹⁶ There are several factors that affect microbial load in the hospital environment such as poor ventilation, the occupants and their activities, high dusting, overcrowding, welding operations, transmission through sneezing and coughing, the outdoor microbial load, and poor infection prevention practices.^{17,18} Despite the current level of medical knowledge, HCAs still cannot be completely eliminated; however the experience of countries that have been using surveillance and control programs for many years shows that with the help of such programs, the incidence of infection can be reduced.¹⁹

There is a dearth of data regarding the microbial quality of healthcare facilities in developing nations such as Ethiopia. However, the limited existing studies reveal bacterial loads that are unacceptably high.^{20,21} In order to combat this issue, a National Infection Prevention and Control (IPC) guideline have been developed for healthcare facilities in Ethiopia. However, healthcare providers often do not follow these guidelines, leading to poor microbiological quality in different hospital wards.²²

This study aims to investigate the degree of microbial load in indoor air, inanimate surfaces, and medical equipment samples in different wards at Jimma Medical Center (JMC), as well as any associated factors. Therefore, it will help to implement evidence-based decisions for improving infection prevention and control methods for health care facilities; which will intern reduce the occurrence of HCAs.

Materials and Methods

Study area

The study was conducted in Oromia region, Jimma zone, at Jimma Medical Centre (JMC). Jimma Medical Centre is 354km from the capital Addis Ababa in the southwest of Ethiopia. It is one of the teaching and referral Hospitals in the country, with a total of 800-bed capacity. JMC is a tertiary-level referral Hospital that provides inpatient, outpatient, and emergency and chronic clinic follow-up services for an estimated 15 million people annually in the Southwest part of the country.

Study design and period

A cross-sectional study was conducted from April 1 to June 22, 2022 to undertake the sample collection from indoor air and surfaces. Purposive sampling was used to select inpatient ward such as Pediatric ward, Intensive care unit (ICU), and Operation room (OR). These wards are selected by considering the high-risk nature of the patients admitted to these wards due to their weak immunity and open wounds from operation. Subsequently, a total of 10 rooms were randomly selected using the lottery method from our source population, consisting of 3 pediatric wards, 1 Medical Intensive Care Unit (MICU), 3 Neonatal Intensive Care Units (NICU), and 3 OR.

Sample size determination and sampling technique

Indoor air sample size was determined by taking into account the factors such as sampling site and time.²³ Two air samples were collected per day from each room with 2 repetitions which are $2 \times 10 \times 2 = 40$ indoor air samples. Samples of inanimate surfaces and medical equipment were taken from those which have frequent contact with patients and healthcare providers 175 inanimate surfaces and 53 medical equipment such as floor, walls, sink, IV stand, operation tables, cylinder, incubator, and trolley were included.²⁴⁻²⁶ Accordingly, a total of 268 samples were taken for this study. The sampling allocation for inanimate surfaces and medical equipment indicated in (Table 1).

Study variables

Dependent variable.
 Microbial load.
 Independent variable.
 Sampling time.
 Ventilation (open window or door).
 Status of the room.
 Presence of waste.
 Crowdedness (number of occupants/room area).

Microbial sampling

The collection of indoor air samples was conducted using the Settle Plate Method, following the 1/1/1 scheme, which involved sampling for a duration of 1 hour, at a height of 1 m above the floor, and approximately 1 m away from walls or significant obstructions.²³ A Petri dish containing 5% sheep blood agar and Sabourod dextrose agar was used for total bacterial and fungal load count. For gram-negative and gram-positive bacteria isolation, MacConkey agar and Mannitol salt agar were used. Sampling was done in the morning (10:00-11:00 am) and the afternoon (3:00-4:00 pm). Following the manufacturer's instructions, culture media were prepared, and sterility was confirmed by incubation at 37°C for 24 hours and monitoring for growth. After sampling, all samples were labeled and transported to the medical microbiology laboratory at Jimma University using an ice box. Each plate was incubated for 24 hours at 37°C under aerobic conditions for bacterial growth. While, the fungal culture plates were incubated at 28°C for 3 to 5 days. Colony-forming units (CFUs) were counted using a colony counter. After counting, the CFU/m³ was calculated using Omeliansky's equation.²⁷

$N = 5a \times 10^4 (bt)^{-1}$ Where N = microbial CFU/m³ of indoor air,
 where a = number of colonies per petri

dish b = dish surface area (cm²) t = exposure time (minutes)

To gather swab specimens, sterile cotton-tipped applicator sticks were soaked with sterile normal saline. A 10 × 10 cm² area was sampled, and for smaller equipment, the surface area was approximated, and the whole area was swabbed.²⁸ High-touch areas of different environmental surfaces (inanimate surfaces and medical equipment) were sampled in this study. The swabs were rotated with firm pressure over the target areas and then repeated at perpendicular angles for maximum recovery. One swab was used for each surface and the sample was taken on different days during the study period in the morning and afternoon sections. No prior notice was given to ward staff before sample collection. All samples were collected after the cleaning was completed. Samples were labeled properly and transported to the medical microbiology laboratory at Jimma University using an ice box within 30 minutes and processed

immediately. For a more accurate count, samples were vortexed to release the microbes from the swab. A total of 100 μL of the original swab suspension and 100 μL of the two dilutions were then inoculated on nutrient agar and Sabourod dextrose agar for total bacterial and fungal colony count. Mannitol salt agar, and MacConkey agar for gram-positive and gram-negative bacteria isolation using a sterile spreader. Samples were incubated at 37°C for 24 hours for bacteria and at 28°C for 3 to 5 days for fungi. The number of colonies was counted from the plate dilution with the most countable number. Multiplied by the appropriate dilution factor, and then divided by the area swabbed (100 cm²) to express the colony count as CFU/cm².²⁹

Confirmatory tests

For both air and swab samples, confirmatory tests were conducted for the selected microorganisms. *S. aureus* and *CoNS* were identified by catalase and coagulase test. Identification of Gram-negative bacteria was performed for pure colonies sub-cultured on nutrient agar for final identification of the isolates.³⁰ Identification was based on their characteristics or reaction on Kligler's Iron Agar, indole, H₂S production, citrate agar, lysine decarboxylase agar, and oxidase after 24-hour incubation. Indole test for *E. coli*, oxidase test for *Pseudomonas aeruginosa*, and citrate utilization test for *Klebsiella* species identification of bacteria were performed.³⁰ The identification of fungal colonies (*Aspergillus* spp) was conducted by visual and microscopic examinations. Visual emergence of fungal colonies arises on the third to fifth days from the incubation at 28°C. A compound microscope was used to determine the colonial feature and morphological structure of *Aspergillus* spp; morphology was determined by mounting the material in Lacto phenol and cotton blue.

Observational assessment

To evaluate the contributing factors, including sampling time, ventilation system, crowdedness, room condition, and the presence of waste material, a checklist was employed. Data were collected for each sampling time across all the selected rooms.^{21,31}

Data quality management

Before the actual sample collection, training and discussion with sample collectors and laboratory professionals were done by the principal investigator. Standard operating procedures were used for the specific purpose of all laboratory procedures. To keep the quality of the samples starting from the preparation of media, sample collection to analysis, aseptic techniques including sterilization of sampling equipment, culture media preparation following manufacturer's instructions, and media sterility was confirmed by incubating at 37°C for 24 hours and observing for growth, use of controls during sample collection

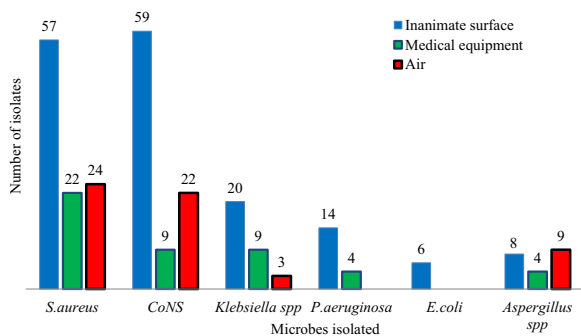


Figure 1. Types of microbes isolated from indoor air, inanimate surface and medical equipment at JMC, 2022.

Abbreviations: CoNS, Coagulase-negative staphylococci; *E. coli*, *Escherichia coli*; *P. aruginosa*, *Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*.

from randomly selected rooms, personal protective clothing, gloves, facemask, sample label, and ice box to transport the sample was used.

Data analyses

Data were coded by assigning a unique identification number. The Data were entered into Epi-data version 3.1, then cleaned and exported to Statistical Package for Social Sciences (SPSS) version 26 for further analysis. The generated data were compiled and presented using descriptive statistics. Using a multiple linear regression model, the relationship between the dependent and independent variables was evaluated. To fit the model, all variables were transformed to $\log(x+1)$. Homoscedasticity (Constant variance), randomness of residuals, errors are uncorrelated with the individual predictors, normality of the error distribution, were examined by plotting of the residuals against predicted value. Multicollinearity among independent variables was tested and Variance Inflation Factor (VIF) was less than 10. Unstandardized coefficients (β) were used to illustrate the impact of a unit increase in the independent variables on the microbial load; *P* value less than .05 was considered a significant association and 95% confidence intervals (CIs) were calculated.

Results

Microbial isolates at selected wards in JMC

A total of 268 samples (175 swabs from inanimate surfaces 53 from medical equipment, and 40 indoor air samples) were collected from 4 different wards and processed during the study period. Of all processed samples, 181 (67.5%) yielded growth of a total of 270 microbial isolates. Of which 249 bacteria and 21 were fungal isolates. Gram-positive bacteria isolates predominate at 193 (77.5%) followed by gram-negative bacteria at 56 (22.5%). The majority of the isolated microbes at 212 (78.5%) were recovered from inanimate surfaces and medical equipment samples, and the rest 58 (21.5%) were from indoor air (Figure 1).

When we look at the distribution of isolates from different wards, the highest microbial isolate were recovered from the

Table 2. The microbial load of indoor air in the clinical bedrooms at JMC, 2022.

TYPE OF WARD	TIME	BACTERIA (CFU/M ³)	FUNGI (CFU/M ³)
Pediatric ward	Morning	1607	814.6
	Afternoon	1348.8	570.5
MICU	Morning	340.7	321
	Afternoon	235.9	307.6
NICU	Morning	467.3	476
	Afternoon	310.3	287.4
OR	Morning	146.3	206
	Afternoon	124.4	96

Abbreviations: MICU, medical intensive care unit; NICU, neonatal intensive care unit; OR, operating room.

pediatric ward at 111 (41.1%), followed by NICU with 69 (25.6%), OR at 55 (20.3%), and MICU at 35 (13%). *S. aureus* was the dominant bacteria isolate from all wards 103 (38%).

Airborne microbial load in the wards

According to the results observed from the analyzed air samples, 37 of them (92.5%) were found to have bacteria and fungi present. The concentration of these microorganisms in terms of CFU/m³ indicated in Table 2. The highest mean concentration of bacteria found in the air was 1607 CFU/m³ while the highest concentration of fungi was 814.6 CFU/m³. Moreover, the average bacterial load was found to be higher in the morning (between 10:00 and 11:00 am) than in the afternoon (between 3:00 and 4:00 pm) in the examined rooms.

A substantial literature survey indicated that there are no uniform international standards available for assessing the extent of microbial load from indoor air. Therefore, we have used the European standards for non-industrial premises to compare our results and it indicates that the pediatric ward had a high level of microbial load. While MICU, NICU and OR wards showed an intermediate level (Table 3).

Microbial load from environmental surfaces

The mean aerobic colony count (ACC) from surfaces in the Hospital was higher than the acceptable limits at <5 CFU/cm².²⁴ The mean total aerobic colony counts from all inanimate surfaces and medical equipment in the investigated wards were 43.3, 28.8, 32.18 CFU/cm² for the pediatric ward, MICU, NICU, and OR, respectively. The highest mean bacterial colony count was reported in pediatric wards at 43.3 CFU/cm², and the least was in OR at 18 CFU/cm² as shown in Table 4.

Based on the distribution of specimens, among the 228 inanimate surfaces and medical equipment samples 212 microbial isolates were recovered. The dominant bacteria isolates

Table 3. Microbial contamination level of studied rooms at JMC according to the European Commission standards for non-industrial premises, 2022.

MICROBE	RANGE OF VALUES (CFU/M ³)	POLLUTION DEGREE	TYPE OF WARD AND TIME			
			PEDIATRIC WARD	MICU	NICU	OR
Bacteria	<50	Very low				
	50-100	Low				
	100-500	Intermediate		✓	✓	✓
	500-2000	High	✓			
	>2000	Very high				
Fungi	<25	Very low				
	25-100	Low				
	100-500	Intermediate		✓	✓	✓
	500-2000	High	✓			
	>2000	Very high				

Abbreviations: MICU, medical intensive care unit; NICU, neonatal intensive care unit; OR, operating room.

Table 4. The microbial load of inanimate surfaces and medical equipment at JMC, 2022.

TYPE OF WARD	BACTERIAL COLONIES IN CFU/CM ²	FUNGAL COLONIES IN CFU/CM ²
Pediatric ward	43.3	32.3
MICU	28.8	22.5
NICU	32	20.2
OR	18	5.25

Abbreviations: MICU, medical intensive care unit; NICU, neonatal intensive care unit; OR, operating room.

were *S. aureus* 79 (37.3%) and *CoNS* 68 (32%). The highest microbial growth was documented from floors accounting for 37 (17.5%), and the least was on OR lamp 2 (0.9%). Details of microbial distribution on the inanimate surfaces and medical equipment are shown in Table 5.

Factors associated with microbial load

In this study, independent variables which were: - sampling time, open windows/doors (the hospital was using natural ventilation system), crowdedness of the room, presence of waste material in the room, and unclean room, were analyzed using a multiple linear regression model to find any probable association with the microbial load. The final multiple linear regression model explained about 20.8% of the variation in microbial load. Three variables were identified as positive predictors of microbial load (Table 6), which were crowdedness [$\beta = 2.748$ (95% CI: 1.057-4.44)], presence of waste material [$\beta = 1.747$

(95% CI: 0.213-3.282)], and unclean room [$\beta = 2.505$ (95% CI: 0.990-4.019)].

During our study period the number of patients in each ward per room was in pediatric ward 8 patients, NICU 2 to 5 patients, MICU from 4 to 6 patients. The hospital used a natural ventilation system at the time. The findings also indicated variations in the level of microbial load across different types of wards. Among air, inanimate surfaces, and medical equipment samples, the pediatric ward exhibited the highest microbial load (Figures 2 and 3).

Discussion

The microbiological quality of hospitals can be considered a reflection of the hygienic conditions existing in the environment. Different studies have reported that air, inanimate surfaces, and medical equipment of the healthcare service units are contaminated by different pathogens which might serve as a source of infection. This study was carried out to gain insight into the distribution of microbial load at JMC and identify the associated factors.

From our findings, the overall microbial contamination rate of this study was 181(67.5%). Lower result was reported from Bahir Dar, Ethiopia 39.6%.³² However, higher microbial contamination rates have been reported (84.4%) from Nepal,³³ (88.4%) from Tigray, Ethiopia,²⁵ and 74.7% from Hawassa, Ethiopia.²⁶ The observed variation in microbial load could potentially be attributed to differences in the hospital community's population and the sampling time frame. The pediatric ward had the highest microbiological load across samples of air, inanimate surfaces, and medical equipment. This might be because of the presence of the high number of patients, which

Table 5. Types of microbes isolate on inanimate surfaces and medical equipment in studied rooms at JMC, 2022.

TYPE AND NUMBER OF SCREENED INANIMATE SURFACES AND MEDICAL EQUIPMENT	IDENTIFIED SPECIES						
	<i>S. AUREUS</i>	<i>CONS</i>	<i>KLEBSIELA SPP</i>	<i>P. AERUGINOSA</i>	<i>E. COLI</i>	<i>ASPERGILLUS SPP</i>	TOTAL
Floor (n=36)	13	10	6	4	2	2	37
Wall (n=36)	4	13	1	-	-	-	18
Bed rails (n=17)	8	6	3	1	1	1	20
Door handles (n=13)	5	6	2	1	-	-	14
Locker (n=12)	7	3	-	-	2	1	13
Light switch (n=13)	5	6	2	1	-	1	15
Chair (n=14)	6	2	3	2	-	-	13
Table (n=6)	1	1	1	2	-	-	5
Sink (n=13)	5	5	1	2	1	1	15
Trolley (n=6)	-	4	-	-	-	-	4
OR table (n=3)	2	1	-	1	-	-	4
OR lamp (n=3)	1	-	-	-	-	1	2
Iv stand (n=17)	5	1	6	1	-	3	16
Cylinder(n=17)	8	2	2	1	-	-	13
Patient monitor (n=6)	2	1	1	2	-	-	6
Suction machine (n=3)	2	1	-	-	-	-	3
Radiant warmer (n=5)	2	2	-	-	-	-	4
Incubator (n=3)	-	2	1	-	-	1	4
Phototherapy machine (n=2)	1	-	-	-	-	-	1
Anesthesia machine (n=3)	2	2	-	-	-	1	5
Total=228	79 (37.3)	68 (32)	29 (13.7)	18 (8.5)	6 (2.8)	12 (5.7)	212

Table 6. Multiple linear regressions of independent variables associated with microbial load in JMC (n=268).

VARIABLES	UNSTANDARDIZED COEFFICIENTS		STANDARDIZED COEFFICIENT BETA	T	P-VALUE	95% CONFIDENCE INTERVAL FOR BETA	
	BETA	STD. ERROR				LOWER BOUND	UPPER BOUND
Constant	-1.091	0.484		-2.255	0.025	-2.044	-.139
Sampling time	0.835	0.665	0.072	1.256	0.210	-.474	2.144
Open windows/doors	0.355	0.436	0.052	0.814	0.416	-.504	1.214
Crowdedness	2.748	0.859	0.235	3.199	0.002**	1.057	4.440
Presence of waste material	1.747	0.779	0.150	2.242	0.026**	0.213	3.282
Unclean room	2.505	0.769	0.216	3.257	0.001**	0.990	4.019

**Significant at .05 level.

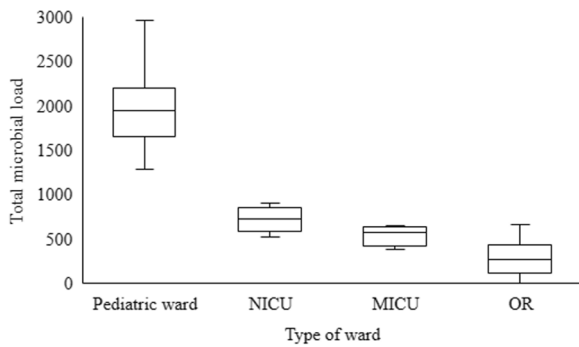


Figure 2. Indoor air microbial load distribution in different wards in JMC.

lead to higher number of visitors, and medical staffs in the ward during sampling time. This might contribute to an increment of the pathogenic organisms that could cause HCAs for patients and health workers.

In the present study, 103 (38.1%) *S. aureus*, and 90 (33.3%) *CoNS* were the predominant isolates. This finding was found to be consistent with previous studies in Ethiopia.^{34,35} This may be due to *S. aureus* constituting part of the normal human flora, inhabiting the skin, and mucous membranes and regularly being shed onto the Hospital environment by patients and medical personnel, whereupon they persist. These isolates were also indicators of inadequate clinical sanitation.

Of the total (40) Hospital indoor air samples processed during the study period, 37 (92.5%) had microbial growth. This implies that many pathogenic microbes remain suspended in the air. The finding was comparable with studies done in Hawasa and Sodo Ethiopia where the recovery rate was 96.9% and 90.2%, respectively.^{36,37} The microbial isolated from the indoor air sample were 24 (41.4%) *S. aureus*, 22 (38%) *CoNS*, 9 (15.5%) *Aspergillus* spp, and 3 (5.2%) *Klebsiella* spp. All these microbes are known infectious pathogens, especially among immune-compromised patients admitted to the Hospital. The result was in line with studies done in Bahir Dar, Ethiopia³² and Hawasa.³⁸ The reason for high *S. aureus* could be due to the inability of gram-negative bacteria to survive in an aerosolized state for a long period because of the inability to resist conditions like drying.

The mean indoor air bacterial load found in the present study was 124.4 to 1607 CFU/m³ and this was nearly coherent with the results reported from Dilla, Ethiopia 450 to 1585.8 CFU/m³.³⁹ On the contrary higher values of microbial load were reported from Harar 148.4 to 2883.2 CFU/m³,³¹ Jimma 3106 to 9733 CFU/m³,²⁰ and Arba Minch 1914 ± 1081.4 CFU/m³²¹ all in Ethiopia. The disparity could be attributed to the difference in IPC practice, length of plate exposure time, period of sample collection, and hospital settings.

The study found that the average indoor air fungal load ranged from 96 to 814.6 CFU/m³. These results were higher than previous studies in India (0-262 CFU/m³).⁴⁰ In contrast, the results were lower than a previous study at Arba Minch,

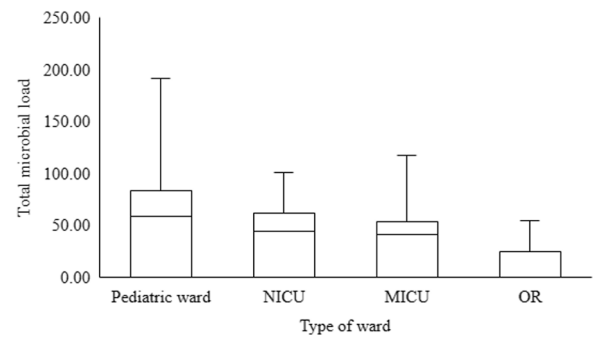


Figure 3. The microbial load of inanimate surfaces and medical equipment in different wards of JMC.

Ethiopia (1533.7 ± 858.8 CFU/m³)²¹ and Jimma, Ethiopia (2123-4168 CFU/m³).²⁰ The highest indoor air microbial load was recorded in the morning, this was in line with studies from Bahir Dar and Hawasa.^{32,41} This may be attributed to the higher human traffic both visitors and health science students who conduct round sessions with the patients doctors during the morning. This could initiate aerosolization of dust particles resulting in binding of the particles to the suspended microbes in the air and fallout in numbers due to gravitation. The results were lower than a previous study at the hospital, which found a high microbial load among the wards.²⁰ This may be due to the absence of active IPC team at the time, as reported by another study in the hospital.⁴² Currently, the hospital has moved to a new and larger building, but the findings still suggest a need for closer monitoring.

The mean bacterial and fungal load of inanimate surfaces and medical equipment from the pediatric ward, MICU, NICU, and OR were (43.3 and 32.3 CFU/cm²), (28.8 and 22.5 CFU/cm²), (32 and 20.2 CFU/cm²), and (18 and 5.25 CFU/cm²), respectively. This result exceeded Dancer's acceptable standard limits, which stipulate that the mean aerobic count from surfaces should be less than 5 CFU/cm².²⁴ The findings of bacterial load in NICU & OR were comparable to the result from Bahir Dar Hospital, which determined 27 and 14 CFU/cm², respectively.³² Of the total 228 inanimate surfaces and medical equipment samples, 144 (63.2%) contamination rates were detected in this study. This was in line with Black Lion Hospital, Ethiopia (60.3%).⁴³ On the other hand, a study conducted in Uganda reported a contamination rate of 44.2%,⁴⁴ in Bahir Dar and Hawassa Ethiopia reported 26.3%,³² and 50.4%,²⁶ respectively, which was lower than this study. Higher results have also been reported from 71.7 % from Arba Minch,⁴⁵ and 88.5% from Tigray, Ethiopia.²⁵ The discrepancies in contamination rates observed might be due to differences in hand hygiene practice, the frequency of decontamination, and the nature of the medical equipment and inanimate surfaces.

Among analyzed factors multiple linear regression model showed that higher crowdedness was found more prone to be contaminated with high microbial load. This finding was

supported by a study in Arba Minch which indicated crowding index contributed to a high microbial load by 12.5 times.²¹ As per this study presence of waste in the room had a significant association with the microbial load. This result was contrary to a study in Arba Minch where the presence of waste did not have a significant association with the microbial load. In this study, unclean rooms were associated with a higher microbial load. The finding was comparable to the study in Harar and Arba Minch which indicated that an unclean environment affected the microbial load by 12.9 and 5.8 times respectively.^{21,31}

This study is difficult to generalize to a larger area because of the single study area used. However, the study could imply the need for further large-scale studies targeting microbial load and associated factors in tertiary care Hospitals to strengthen this finding by the use of a larger sample size and multiple locations in Ethiopia.

Limitation

This study was conducted in a single hospital setting and did not identify the antibiotic resistance patterns of identified microbes.

Conclusion

In summary, our study findings indicated that almost all wards had an intermediate level of indoor air microbial load range according to European Commission standards. However, the microbial load detected on inanimate surfaces and medical equipment surpassed the prescribed limit, posing potential health risks. *S. aureus* was identified as the predominant isolate. The presence of waste, crowdedness, and unclean room conditions showed a significant association with the microbial load. Consequently, it is recommended to implement regular microbial surveillance of the hospital environment and enhance the infection prevention program to mitigate these concerns.

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Author Contributions

MMB was in charge of conceptualization, study design, sample collection, analysis, and interpretation, as well as the development and revision of the manuscript. AA and SM assisted with the conception and design of the study, interpretation of the findings, and preparation and editing of the paper. GK and BY were involved in data management, analysis and interpretation. The final manuscript was examined and approved by all authors.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author.

Ethical Consideration

The study initially obtained ethical clearance from the Institutional Review Board (IRB) of Jimma University under IRB number 440/22. Additionally, approval to conduct the study was obtained from the Hospital administration.

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