BACTERIOLOGICAL EVALUATION OF INDOOR AIR QUALITY IN SOME SELECTED UNITS AT THE UNIVERSITY OF CAPE COAST HOSPITAL IN GHANA

ABSTRACT

A hospital is an environment solely for diagnosing and treating patients. Contemporary research, however, reveals the possibility of users contracting diseases due to many factors, such as poor air quality.This research, therefore, delves into the critical indoor air quality assessment domain, focusing on selected units within the University of Cape Coast Hospital, Ghana. The study's primary objective was to conduct a comprehensive microbial assessment of indoor air quality in eight different units (emergency room, operating theater, out-patient department, consulting rooms, laboratory, male ward, female ward, and ear, nose, and throatunit) of the hospital, shedding light on potential airborne bacteria present. Indoor and outdoor air were sampled using Koch's sedimentation method. Colony forming units per cubic meter of air (cfu/m^3) were determined with the Omeliansky formula. The bacteriological load within the units revealed that the out-patient department had the highest bacterial concentration ($139.2\pm60.32\times10^2$cfu/m^3), immediately followed by Outdoor ($135.1\pm43.63\times10^2$cfu/m^3), whereas ear, nose, and throat unit recorded the least concentration ($0.4\pm0.57\times10^2$cfu/m^3). The remaining units range between $135.1\pm43.63\times10^2$cfu/m^3 and $0.4\pm0.57\times10^2$cfu/m^3. The morphological characteristics of the seven observed bacterial isolates (GSB 1-7) showed the presence of two cocci and five rods. Isolates 1 and 4 had a rhizoid form, isolates 2,3, and 5 had a circular form, while isolates 6 and 7 had filamentous forms. All isolates showed positive gram tests, and endospores were detected in isolates 1,6, and 7. Bacterial isolates were identified as *Bacillus mycoides, Micrococcus luteus, Staphylococcus epidermidis, Bacillus circulans, Staphylococcus saprophyticus, Bacillus subtilis*, and *Micrococcus* sp. These outcomes indicate bacterial contaminations in the indoor environment, likely to pose a significant risk to patients, workers,and visitor's safety. Therefore, rigorous monitoring and mitigation strategies are essential to ensure a safer environment in healthcare settings.

Keywords: Bacteria, microorganisms, contaminants, culture, indoor air quality

1. INTRODUCTION

Indoor air quality (IAQ) is defined by the US Environmental Protection Agency (EPA)as the air quality within and around buildings and structures, with a focus on the comfort and health of building occupants [1].Hospitals accommodatea wide range of microorganisms, including viruses, fungi, and bacteria. These pathogens can lead to nosocomial infections and pose a serious public health risk in relation to asthma, allergies, and respiratory infections [2,3]. Therefore, the microbial assessment of IAQ in hospitals is critical to maintaining a healthy indoor environment and forestalling the spread of airborne diseases [4].

Several studies have investigated the microbial composition of indoor air in hospitals. For instance, Poza et al. [5] assessed the bacterial diversity in different hospital wards and found that the microbial composition of indoor air differs depending on the type of ward. They also highlighted the importance of maintaining proper hospital ventilation and air filtration systems. Similarly, Sarca et al. [6] identified many microorganisms in hospital environments, including pathogenic bacteria, and suggested the need for effective infection control measures to prevent the spread of hospital-acquired infections.

The Coronavirus pandemic has additionally featured the significance of observing IAQ in hospitals. According to the Centers for Disease Control and Prevention (CDC), SARS-CoV-2 can remain viable in aerosols for up to three hours, emphasizing the importance of assessing the microbial quality of indoor air in healthcare settings [7]. Upon the receival of in-depth education to the general public on COVID-19, the utilization of personal protective equipment (PPE), personal hygiene, sterilization, disinfection, environmental cleaning, ventilation systems, and air purifiers was embraced in healthcare facilities to mitigate the spread of microorganisms. However, the effectiveness of these measures in controlling

the transmission of nosocomial infections in hospitals post-COVID remains uncertain, of which the University of Cape Coast is no exception.

Poor IAQ in hospital units can adversely affect human health and spread infectious agents, contributing to the risk of nosocomial infections [8]. Lack of updated awareness of the microbial quality of air at the hospital (especially post-COVID) can pose serious health risks to patients, staff, and visitors. Such knowledge postulates the necessity to conduct a microbial air quality assessment within eight units at the University of Cape Coast Hospital in the Cape Coast Metropolis, Ghana.

This present study, therefore, aims to conduct a bacteriological assessment of the indoor air in eight (8) units (the emergency room, operating theaters, OPD, consulting rooms, laboratory room, male ward, female ward, and ENT with the outdoor air as a control) of the University of Cape Coast Hospital in Ghana by estimating the microbial loads and identifying bacteria associated with each unit of the Hospital.

2. MATERIAL AND METHODS

Materials and equipment were acquired from the Research Laboratory of the Department of Laboratory Technology at the University of Cape Coast.

2.1 Study Area and Design

This study was conducted (with the consent of the hospital authorities) in eight (8) different units (the emergency room, operating theaters, OPD, consulting rooms, laboratory room, male ward, female ward, and ENT) with the Outdoor air as a control at the University of Cape Coast Hospital (5.1167° North and 1.2669° West) within the Cape Coast Metropolis of the Central Region of Ghana.

2.2 Sampling Technique

The sedimentation method (settling plate technique) was utilized during purposive sampling as adopted by Chadeganipour et al. [9]. The air, both indoor and outdoor, was sampled from each unit between 11:00 am and 2:00 pm with an exposure time of 20 minutes with two plates positioned on the floor and at the breathing area (1.5 meters above floor level) for indoor areas and 10 metres from the main hospital entrance for outdoor.

2.3 Bacteriological Analysis

Petri plates containing 20 ml of nutrient media with the sampled air were incubated for 48 hours at 37 ^{0}C to ensure a controlled bacterial colony growth. Bacterial colonies were enumerated with a colony counter and expressed as colony-forming units per cubic meter of air (cfu/m^{3}) using the Omeliansky formula[10],

$$N = 5a \times 10^{4} ((bt)^{-1}) \quad \ldots\ldots (1)$$

where,

N = colony forming units per cubic meter of air,

a = number of colonies per petri dish,

b = dish square centimeter,

t = time of exposure (minutes).

The morphology (shape, size, color, opacity, elevation, and margin) of all the colonies on each petri dish were viewed and recorded.

2.3.1 Colony Isolation

Pure cultures of the individual colonies were isolated using the streaking method as employed by Asem et al. [11]. A 50 ml of nutrient agar was prepared, heated for an even mixture, transferred into seven glass test tubes, and sterilized in an autoclave for 15 minutes at 121 ^{0}C. After sterilization, the test tubes were slanted and allowed to dry at 40 ^{0}C before the seven different colonies from the Petri dishes were aseptically transferred into a separate test tube, labeled, and incubated for 24 hours at 37 ^{0}C.

2.3.1 Bacterial Identification

Aseptically, smears of each isolate were prepared and heat-fixed on slides labeled I_1-I_7 for their Gram reactions. The endospore staining technique was further used to determine the spore-forming status of each isolate. Discrete colonies were subcultured for biochemical tests (catalase, citrate, motility, and indole tests) in the identification of the isolates using the Center for Food Security and Public Health-Iowa State University, Bergey's Manual of Determinative Bacteriology, and UK SMI-Identification of *Bacillus* species [12].

2.4 Statistical Analysis

Descriptive analysis of the data was conducted using Microsoft Excel version 16.0 and presented in tables.

3. RESULTS AND DISCUSSION

The bacterial concentrations of indoor and outdoor units at the University of Cape Coast Hospital as means with standard deviation and total percentage count are presented in Table 1. From the table, OPD recorded the highest mean with a corresponding percent load of 27.03%, followed by LAB 21.52%, with the rest having a percentage less than 10. However, the outdoor had a mean value close to that of OPD with a percentage load of 26.21%.

Table 1. Airborne bacterial concentrations in mean and percentages from indoor andoutdoor units within the University of Cape Coast Hospital.

Environment	Sampling Sites	Mean ± SD [CFU/m^3 (10^2)]
Indoor	ENT	0.4±0.57
	EM	24.2±10.25
	TR	2.9±2.47
	MW	11.8±1.20
	FW	19.3±3.89
	OPD	139.2±60.32
	CR	50.5±45.18
	LAB	110.8±7.21
Outdoor (Control)	HE	135.1±43.63
Average bacterial load		54.9±19.41

Legend: ENT= Ear, Nose and Throat; EM= Emergency Unit; TR= Theatre Room;MW=Male Ward; FW= Female Ward; OPD= Out-Patient Department; CR=Consulting Room; LAB = Laboratory; *HE= Hospital Exterior*.

Seven (7) bacterial genera were isolated and described according to their morphological characteristics. From Table 2, five rods and two cocci were identified. By observation, GSB 1 and 4, GSB 2,3 and 5, and GSB 6 and 7 were seen to be rhizoids, circular and filamentous, respectively.

Table 2. Morphological characteristics of bacterial isolates from the outdoor andindoor air at the University of Cape Coast Hospital.

Isolates	Morphological Characteristics							
	Form	**Color**	**Opacity**	**Surface**	**Elevation**	**Margin**	**Size**	**Shape**
GSB 1	Rhizoid	White	Opaque	Smooth	Raised	Entire	Small	Rods
GSB 2	Circular	Yellow	Opaque	Smooth	Raised	Entire	Small	Rods
GSB 3	Circular	Yellow	Translucent	Smooth	Raised	Entire	Small	Rods
GSB 4	Rhizoid	Brown	Opaque	Smooth	Flat	Lobate	Medium	Rods
GSB 5	Circular	White	Opaque	Smooth	Raised	Entire	Medium	Coccus
GSB 6	Filamentous	White	Opaque	Rough	Flat	Entire	Large	Rods
GSB 7	Filamentous	Yellow	Opaque	Rough	Raised	Entire	Medium	Coccus

Legend: GSB 1-7 is used as a coded name to represent the bacteria isolates yet to be identified.

The Gram's reactions, endospore staining results, and four different biochemical tests carried out on the seven (7) isolates have been described in Table 3. The Gram's reaction proved all isolates positive, while only three of the seven isolates had endospores present. Four different biochemical tests were employed for identification.

Table 3.Gram reaction, endospore staining, and biochemical testcharacteristics of isolates within indoor air sampled from the University of Cape Coast Hospital.

Isolates code	Gram Reactions	Endospore Staining	Biochemical tests				Organisms
			Motility	Catalase	Indole	Citrate	
GSB 1	+	+	-	+	-	-	*Bacillus mycoides*
GSB 2	+	-	-	+	-	-	*Micrococcus luteus*
GSB 3	+	-	-	+	-	-	*Staphylococcus epidermides*
GSB 4	+	+	+	+	-	+	*Bacillus circulans*
GSB 5	+	-	-	+	-	-	*Staphylococcus saprophyticus*
GSB 6	+	+	+	+	-	+	*Bacillus subtilis*
GSB 7	+	-	-	+	-	+	*Micrococcus sp.*

Lengend: += positive, -= negative

Table 4 illustrates the frequency and percentage of occurrence of the individual isolated bacteria genera within the selected units. With a total bacteria count of 1655(100%), *M. luteus* recorded the highest count of 456(27.6%), followed closely by *S. saprophyticus* 446(27.0%), with *S. epidermides*297(17.9%), *B. mycoides* 265(16.0%), *Micrococcus* sp. 104(6.3%), *B. subtilis* 86(5.2%), *B. circulans*1(0.1%) accordingly.An account of the indoor air reveals OPD as the unit with the highest bacteria concentration, while ENT recorded the lowest.

Table 4. Frequency and percentage of occurrence of the various bacteria isolateswithin the selected units of the Hospital.

Isolates	**Hospital units (Indoors)**								**Outdoor** (Control)	**Total**
	ENT	EM	TR	MW	FW	OPD	CR	LAB		
B. mycoides	0(-)	9(0.5)	0(-)	0(-)	29(1.8)	22(1.3)	66(4.0)	55(3.3)	84(5.1)	265(16.0)
B. circulans	0(-)	0(-)	0(-)	1(0.1)	0(-)	0(-)	0(-)	0(-)	0(-)	1(0.1)
B. subtilis	0(-)	0(-)	1(0.1)	7(0.4)	0(-)	0(-)	2(0.1)	54(3.3)	22(1.3)	86(5.2)
Micrococcus sp.	0(-)	0(-)	0(-)	0(-)	0(-)	5(0.3)	91(5.5)	0(-)	8(0.5)	104(6.3)
M. luteus	2(0.1)	21(1.3)	6(0.4)	7(0.4)	11(0.7)	159(9.6)	17(1.0)	206(12.5)	27(1.6)	456(27.6)
S. saprophyticus	0(-)	40(2.4)	4(0.2)	20(1.2)	8(0.5)	112(6.8)	26(1.6)	77(4.7)	159(9.6)	446(27.0)
S. epidermides	0(-)	13(0.8)	0(-)	0(-)	0(-)	169(10.2)	0(-)	0(-)	115(7.0)	297(17.9)
Total (%)	2(0.1)	83(5.0)	11(0.7)	35(2.1)	48(3.0)	467(28.2)	202(12.2)	392(23.7)	415(25.1)	**1655(100)**

**Values in parenthesis represent the percentage occurrence of isolates within the selected Hospital unit and the parenthesis with a minus sign (-)= Null.*

The results of this study revealed the presence of airborne bacteria in the indoor and outdoor air of the Hospital and the dominance of bacteria aerosols. From the indoor units, OPD recorded the highest airborne bacterial contamination, followed by the Laboratory,Consulting room, Emergency room, Female ward, Male ward, Theatre room, and the lowest being ENT, with corresponding CFU/m^3 (10^2) respectively being 139.2±60.32, 110.8±7.21, 50.5±45.18, 24.2±10.25, 19.3±3.89, 11.8±1.20, 2.9±2.47, 0.4±0.57. The outdoor recorded 135.1±43.63 CFU/m^3 (10^2), which is also higher. With 54.9±19.4 CFU/m^3(10^2) as the average bacterial load, the study confirms contamination levels in the Hospital according to recommended guidelines [13-15]. This indicates the presence of some factors contributing to increased microbial contamination within the indoor environment. A similar observation was present in a study by Gizaw et al. [16]. The study revealed marked variations in microbial loads at the selected Hospital units, with higher bacterial counts for indoor environments than outdoor ones. The variations in microbial populations have been attributed to a combination of factors. The elevated bacterial count within the OPD suggests a higher density of patients, limited ventilation, and medical procedures that could lead to the dispersion of microorganisms into the air. Comparing the units with high bacterial counts to those units with low bacterial counts, similar reasons aforestated could be ascribed, in addition to poor cleaning methods and the microbial diversity of the patients that visit the facility[16]. The units that use proper and persistent decontamination regimes are less likely to be contaminated, recording low bacterial counts.These findings are in line with a review conducted by Larry et al [17]. Their work showed that OPD is more contaminated than the other units and shares the same reasons stated above.

From the cultural and morphology characteristics of bacteria investigated with results presented in Table 2, four of the seven isolates were rods, and two were coccus. Isolates GSB 1 and 4, GSB 2, 3, and 5, and GSB 6 and 7 showed rhizoids, circular and filamentous, respectively, which reflects the heterogeneity of microorganisms in the hospital environment [18].

The observation of the Gram's reaction in Table 3 shows that all seven isolates are Gram positives. According to a study by Dalton et al. [19], Gram-positive bacteria often contribute to healthcare-associated infections (HAIs), posing a potential risk to patients and healthcare workers. On the other hand, the predominance of Gram-positive bacteria in indoor air does not necessarily indicate a negative IAQ. It, however, underscores the need for continuous monitoring and interventions to reduce the potential risks associated with such microorganisms.

The presence of highly resistant spores allows bacteria to endure extreme heat, desiccation, and chemical exposure. In this study, three bacterial isolates (GSB 1, GSB 4, and GSB 6) exhibited the presence of endospores, with the remaining four isolates (GSB 2, GSB 3, GSB 5, and GSB 7) being non-spore-forming. The occurrence of spore-forming bacteria in the hospital environment is the possibility of its proximity to the outdoor sources, the movement of patients and hospital staff, the equipment, and the Hospital's ventilation system. The presence of these spores highlights the importance of adequate air filtration, cleaning, and disinfection protocols to mitigate potential health risks associated with microorganisms in healthcare facilities [20,21]

The study employed four biochemical tests, and the findings in Table 3 showed that all seven isolates were negative for the indole test and positive for the catalase test. In contrast, GSB 4 and GSB 6 recorded positive for the motility test, with all the remaining isolates being negative. For the citrate test, GSB 4, GSB 6, and GSB 7 showed positive, while the other isolates recorded negative.

The morphological examination unveiled unique features aiding in identifying the seven (7) isolates. With a total bacteria count of 1655(100%) revealed in Table 4, *M. luteus* recorded the highest count of 456(27.6%), followed closely by *S. saprophyticus* 446(27.0%), with *S. epidermides*297(17.9%), *B. mycoides* 265(16.0%), *Micrococcus* sp. 104(6.3%), *B. subtilis* 86(5.2%), *B. circulans*1(0.1%) accordingly. Similar to the selected units, OPD had the highest count of 467(28.2%), followed by LAB 392(23.7%), with Consulting Room 202(12.2%), Emergency Room 83(5.0%), Female Ward 48(3.0%), Male Ward 35(2.1%), Theatre room 11(0.7%), and the ENT 2(0.1%). Considering the bacterial genera in terms of the number of isolates it recorded, Outdoor (control) became imminent. OPD and Consulting room, each followed by five isolates each; Emergency room and Laboratory were next with four (4) isolates. With three isolates each, the Male and Female wards, two for the Theatre room, and one for ENT. The total load from the indoor study represents about three times that of the outdoor, suggesting poor indoor ambient conditions and the endogenous contamination of the Hospital's air, as emphasized by Yafetto and Adator[22].

All the discussed variations can point to several factors, including varying levels of patient traffic and activities, poor ventilation and airflow systems, patient medical conditions, specialized medical procedures, average hospital hygiene and cleaning practices, favorable proliferation conditions for microorganisms coming from within and without of the selected indoor units, and some previous contamination events within the units.

The seven identified isolates in the indoor air of the University of Cape Coast Hospital may pose various dangers, primarily in the context of healthcare-associated infections (HAIs). These bacteria spread diseases among vulnerable patients with compromised immune systems, leading to extended hospital stays, increased mortality rates, and heightened healthcare costs [23]. Moreover, the potential for antibiotic resistance development within *Staphylococcus* species is a concerning threat. Additionally, the presence of these bacteria in various hospital units raises cross-contamination risks, increasing the complexity of infection control efforts.

4. CONCLUSION

This study has revealed a diverse range of putative bacterial contaminants (*Bacillus mycoides*, *Micrococcus luteus,Staphylococcus epidermidis*, *Bacillus circulans*, *Staphylococcus saprophyticus*, *Bacillus subtilis*, and *Micrococcus* spp.) within the indoor environment of the Hospital. The average bacterialconcentration in this research is $54.9x10^2$ CFU/m^3,confirming contamination levels within the hospital units. These risk findings underscore the critical importance of robust infection prevention and control measures within healthcare settings. To mitigate these risks, hospitals must prioritize rigorous infection control measures like stringent hand hygiene, ensure regular and effective standard cleaning and surface disinfection, maintain proper ventilation systems in each unit, and monitor contamination levels. Ongoing efforts are essential to safeguard patient and healthcare worker safety and enhance the overall quality of healthcare.

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