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Indoor Air Bacterial Load, Isolate and Antimicrobial Susceptibility Patterns at Hawassa University Comprehensive Specialized Hospital Wards

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Abstract: *Air is the greatest dissemination agent of pathogenic microbes which cause significant problems in the hospital environment.*

Objectives of the Study: *To determine bacterial indoor air concentrations, to identify the bacterial isolates and determine antibiotics resistance patterns present in indoor air in a hospital wards at Hawassa University comprehensive specialized Hospital.*

Methodology: *In this cross sectional study, 6 wards in Hawassa comprehensive specialized hospital were studied. Sample collection was carried out twice a day, mornings and afternoon with an interval of 14 days. Sedimentation technique using open petri dishes containing sheep blood agar was used. An openculture medium was placed 1 meter above the floor for 1 hour at selected sections of the wards and transported aseptically to the Microbiology laboratory. Types and number of colonies were determined in the laboratory. Then isolates were identified by appropriate bacteriological techniques. The SPSS version 16.0 software was used for data management. P- Value of < 0.05 was used as Statistical association.*

Result: *The finding of this study indicated that the highest indoor bacterial count was observed at medical common ward which is 7958.98 CFU/ m^3 and a least count was recorded at neonatal intensive care unit of 930 CFU/ m^3 withthe average count of 4420 $\pm$ 1642 CFU/ m^3. Out of 96 indoor air sampled 41 (43%) were below satisfactory limits. Among the 6 sampled wards, neonatal intensive care unit was the most unsatisfactory area for patients with 11/16 (69%) air sampled were undesirable. The predominant isolates were S. aureus(36%), Coagulase negative staphylococci (28.8.9%), followed by Pseudomonas spp. (13.5%). The study revealed that, similar to others findings most of isolates were resistant for the tested antibiotics.*

Conclusions: *Regardless of number of students and visitors, the neonatal intensive care units need to get appropriate emphasis to prevent newborns from nosocomial infection risk as their immunity debilitated. Bacteriology of the isolates remained troublesome because of the resistance pattern of the isolates to commonly prescribed antibiotics. Therefore, hospitals should have enhanced practice of good sanitation protocols and infection control measures.*

Keywords: *Bacterial isolate; Indoor Air quality; drug resistance*

1. INTRODUCTION

Air is the greatest dissemination agent of pathogenic microbes which cause significant problems in the hospital environment particularly in operating room (OR), intensive care unit (ICU) and neonatal ward/room. Insufficient ventilation, high movement of personnel and improper management of hospital monitoring are main sources of indoor air contamination in the hospital [4].

Measures that often taken to prevent nosocomial infections are effective uses of antiseptics, disinfectants, adequate cleaning, sterilization and isolation of patients with highly infectious diseases [8].However, less attention is paid for indoor air as been a probable contributing factor to hospital acquired infections. Airborne bacteria in the hospital environment have been a major source of post-

operative infection and a serious problem in the intensive care unit(ICU). Many of these isolates (bacteria) are shown to be resistant to common antiseptics used in hospitals [9].

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. Climatic conditions such as excessive humidity and moisture of walls and ceilings may facilitate microorganism's colonization. Beyond this physical parameters such as temperature and humidity are known to influence the ability of microorganisms to survive and be airborne. In Ethiopian setting where hot and humid climatic conditions prevail, it is necessary to monitor airborne microbial concentrations and determine if there are variations in the microbial concentrations and their types with changing antibiotic sensitivity pattern. This study is undertaken to determine bacterial indoor air concentrations, to characterize the microorganisms and determine antibiotic resistance patterns present in indoor air in a hospital ward at Hawassa comprehensive specialized Hospital [9].

This study therefore was aimed at investigating the bacterial isolate, load and antibiotics resistance patterns of indoor air in different wards and units of Hawassa University Comprehensive Specialized Hospital (HU- CSH). It will also provide important information on the quality of indoor air for hospital mangers.

2. METHODS

2.1. Study Area and Period

This study was carried out at Hawassa University, Comprehensive Specialized Hospital, located about 270 km from the Addis Ababa, capital city Ethiopia. This hospital serves as a referral center for both public and private hospitals in South Nations Nationalities People Regional State (SNNPR) the neighboring oromia region. It is also a training institution for undergraduates, post graduate, resident post-graduate doctors besides it serves as a research center.Hospital based cross-sectional study was conducted between July to September 2017 in Hawassa University comprehensive specialized Hospital.

2.2. Sites and Sample Collection

Samples were collected from the following wards/units: Pediatrics ward (PW), Neonatalintensive care unit (NICU), Surgical ward (MSW), Medical ward (MM),Gynecology ward (GW) and Orthopedic ward. A total of 96 indoor air samples were collected using settle plate or Passive Air Sampling method following 1/1/1 schedule (a nine cm in diameter sterile Petri dish with 5% Sheep's blood agar was left open to the air for an hour, a meter above the floor and a meter from the wall) in all units twice a day, mornings (9.00 am – 10.00 am) and evenings (4.00 pm – 5.00 pm) for an interval of 14 days. (18). During air sampling sterile gloves, mouth masks and protective gown was worn to prevent self-contamination of the 5% Sheep's blood agar plate (Oxoid, UK).

2.3. Sample Processing and Laboratory Analysis

Culture plates was transported to the microbiology laboratory and incubated aerobically for 24 hours at $37c^{o}$. The culture plates that show distinct colonies was counted using plate colony counter. Then colonies were examined for the growth of potential pathogenic bacteria initially by colony characteristics, hemolysis and microscopic examination of Gram stained smears. Then, these suspected colonies were sub cultured on Manitol salt agar (MSA) and MacConkey used accordingly. And finally, further identification was done by carrying out catalase, coagulase, mannitol fermentation, and bacitracin susceptibility tests for gram positive and citrate utilization, urea, motility and others for gram negative bacteria by following standard bacteriological techniques (24, 25).

The antimicrobial susceptibility testing was done on Mueller-Hinton agar (Oxoid, UK) for every potential pathogenic bacteria isolates with 13 antibiotics each by Kirby-Bauer disk diffusion method matching the test organism to 0.5 McFarland turbidity standards. Then, the susceptibility result was interpreted according to the principles established by Clinical and Laboratory Standards Institute, 2016 (CLSI) by measuring the zone diameter of inhibition [25].

Reference strains *S. aureus*(ATCC 25923); *E. coli* (ATCC 25922) and *P. aeruginosa*(ATCC 27853) was used as a quality control for culture and susceptibility testing throughout the study.

2.4. Data Processing and Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0. A chi-square test was used to screen the possible association between the times of collection among different wards. P-values of <0.05 was considered as statistically significant.

2.5. Ethical Consideration

This proposal was presented to the School of Medical Laboratory Science and then endorsed by the school commission. Then official permission was obtained from the Hospital chief clinical director.

3. RESULT

In this study a total of 96 indoor samples were collected from 12 rooms in six different wards once per week for 4 times with 14 day interval. The two neonatal intensive care units were sampled 4 times repeated twice a day morning and afternoon. While the rest of the wards, as they have more than 4 rooms, we have selected 4 rooms from each by lottery method and sampled 4 times repeated twice a day morning and afternoon. Almost all common rooms hold 6 bed each and the private rooms have either one or two beds. The finding of this study indicated that out of the total wards included in this study the highest indoor bacterial count was observed at medical common ward which is 7958.98 CFU/ m^3 and a least count was recorded at neonatal intensive care unit of 930 CFU/ m^3 the average count of 4420 ± 1642 CFU/ m^3 was revealed.

Table1. *Mean bacterial counts of air samples in different rooms of HU-CSH, 2017*

Rooms	Observed Mean ± SDV		Standard[1] (CFU/dm^2)		
	CFU/dm^2 No. (mean value)	CFU/m^3 No.(mean value)	Optimal	Acceptable	Unacceptable
NICU 1	8 (132.6) ±50.2	8 (1737.5) ±657.1	0-60	61-90	>91
NICU 2	8 (177.6) ±125.2	8 (2327) ±1640.5			
GW C	8 (446.75) ± 36.8	8 (5853.9) ±480.8	0-250	251-450	>451
GW P	8 (402) ± 81.5	8 (4611.5) ± 1336.9			
SW C	8 (391.8) ± 98.9	8 (5132.2) ±1296			
SW P	8 (338.9) ±77	8 (4439.5) ± 1008.7			
MW C	8 (419.5) ±95.2	8 (5169.3) ±1476.2			
MW P	8 (327.1) ±58.7	8 (4285.5) ±769.4			
OPW 1	8 (363.6) ± 74.5	8 (4436.5) ±1011.4			
OPW 2	8 (405.8) ±104.9	8 (4990.6) ± 1543.4			
PEDW 1	8 (349) ±64.6	8 (4244.8) ±802			
PEDW 2	8 (444) ±119	8 (5818.5) ±1562			
Total	96 (349.9) ±125.3	96 (4420.6) ±1642.3			

Key: *NICU= neonatal intensive care unit; GW=Gynecology ward; SW-Surgical Ward; MW= Medical ward; OPW= Orthopedics ward; PED= Pediatrics ward; C= common; P= private room*

Compared to the standard set by Fisher's index of microbial air contamination (Pasquarella et al., 2000), air microbial count of neonatal intensive care unit and wards must not exceed 90 and 450 CFU/dm^2 respectively (Table 1). In this study about 41 (43%) air samples collected from different wards were below satisfactory limits **(Table 2)**. Though the acceptability of wards with the standard has no statistically significant association (p= 0.163), among the 6 sampled wards, neonatal care unit was the most unsatisfactory area for patients with 11/16 (69%) air sampled were undesirable and gynecology ward followed by 8 out of 16 air sampled **(Fig 1)**.

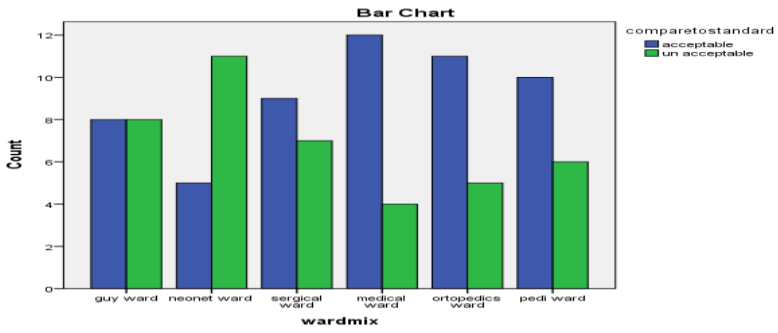

Figure1. *Frequency of acceptable samples from indoor-air of different rooms at HU-CSH, 2017*

From 96 indoor air samples, a total of 111 bacterial isolates were attained. Among *Staphylococcus aureus* was the predominate isolates with36% (40/111) followed by coagulase negative *Staphylococcus* (CoNS) 28.8% (32/111). The gram negatives were very small in proportion than gram positives, only 19.8% (22/111), *Pseudomonas spp*. (13.5%) and *Klebsiella Spp*(6.3%)**(Fig 2)**.

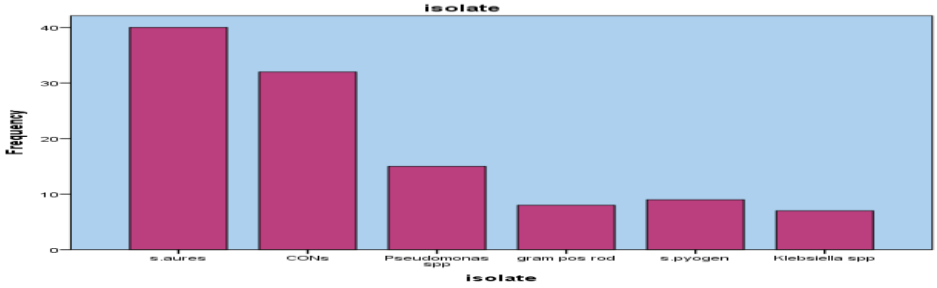

Figure2. *Frequency of bacterial isolates from indoor-air of different rooms at HU-CSH, 2017*

Table2. *Level of acceptability of indoor air of different site against the standard in HU-CSH, 2017*

Rooms	Level of acceptability of indoor air No (%)		p-value
	Acceptable	Unacceptable	
Neonate intensive care unit	5 (31)	11 (69)	
Gynecology wards	8(50)	8(50)	
Surgical wards	9(56)	7(44)	P= 0.163
Medical wards	12(75)	4(35)	
Orthopedics wards	11(69)	5(31)	
Pediatric wards	10 (62.5)	6(37.5)	
Total	55(57.3)	41(42.7)	

Regarding time of sample collection the afternoon samples were more intolerable 22/48 (46%) than the morning samples 19/48 (39.5%) p-value of 0.53. At the mean time *S. aureus* is very common in the afternoon while *CoNS* was dominant in the morning **(Fig 3)**.

Table3. *Time variation of indoor air samples against the standard HU-CSH 2017*

	Number of sample N=48(%)			p-value
	Morning	Afternoon	Total %	
Acceptable	29 (60.4)	26 (54.2)	55	P=0.53
Unacceptable	19 (39.6)	22 (45.8)	41	
Total	48	48	96	

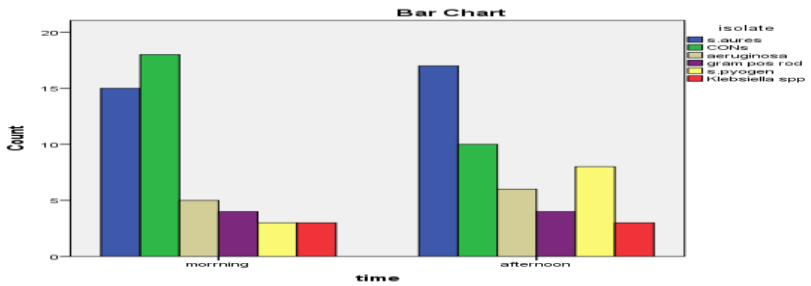

Figure3. *Frequency of bacterial isolates by time of samples at HU, CMHS, 2017*

In this study most of isolates were resistant for the tested antibiotics. The antibiotic resistance occurrences of the bacterial isolates are shown in table 3. We have tested the bacterial isolates for 10different antibiotics. High level resistance> 70% was seen for Penicillin by Gram-positive isolates;75% of *S. aureus* and 72% of the *CoNS* being resistant. Again around 60% of all the rest isolates were resistant to erythromycin. According to this studies finding, the most effective antibiotics for *S. aureus, S. pyogen and CoNS were* erythromycin and trimethoprim-sulphamethoxazole. most antibiotics being resistant against the two gram-negative isolates, *pseudomonasspp* and *K. pneumonia* however, we can say both of them have an average of above 60% resistant for most antibiotics tested and Almost all of the bacterial isolates in this study show multidrug resistant**(Table 4)**.

Table4. *Antimicrobial resistance pattern of bacterial isolates in HU-CSH, 2017*

Antibiotics tested	Bacterial isolates				
	S.aureus (n=40)	*CoNS (n=32)*	*Pseudomonas Spp (n=15)*	*S.pyogens (n=9)*	*K. pneumonia (n=7)*
Gentamicin	21(52.5)	21(65.6)	6(90)	6(66.7)	3(42.9)
Ampicillin	11(27.5)	13(40.6)	6(82)	9(0)	1(74.3)
Chloramphenicol	13(32.5)	15(46.9)	6(78)	5(55.6)	4(57.1)
Clindamycin	13(32.5)	19(59.4)	ND	6(55.7)	ND
Ciprofloxacin	10(25)	16(50)	3(89)	3(33.3)	2(88.6)
Erythromycin	24(60)	19(59.4)	9(60)	8(88.9)	4(57.1)
SXT	7(17.5)	9(28.1)	2(76.7)	ND	1(64.3)
Ceftriaxone	12(30)	13(40.6)	5(80.4)	4(44.4)	2(78.6)
Tetracycline	26(65)	25(78.1)	15(100)	8(88.9)	6(85.7)
Penicillin	29(75.5)	23(71.9)	ND	9(0)	ND

Key: *ND= not done, SXT-Trimethoprim-sulphamethoxazole*

4. DISCUSSION

Different kind of microbial inhabitants are found in hospital indoor environments and they could serve as primary sources of indoor air contamination, perhaps posing severe health risk to patients and health workers. This is because they are confined aerosols which enables them build up to infectious level (26). Specific activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological matter (4).

The study of indoor bacterial load in hospital environments is important to understand the dissemination of airborne microbes particularly the pathogenic ones (8). It is believed that the hospital environment where patients are treated has an important influence on the prospect of such patients recovering or acquiring infection that may complicate their conditions (13). Hence, it is essential to assess intermittently the quality of the indoor air in the hospital environments. For this reason the number and type of airborne microorganisms can be used to determine the degree of cleanliness (8).

The result of this study indicates that the mean colony counts achieved from all neonatal rooms were beyond the acceptable limit while the rest wards mean value of colony count obtained were almost in the acceptable range of bacteriological standard set by Pasquarella (1). Even though mean CFU count laid within the permissibility range, around 41 samples of the collected 96 samples from different rooms shows the intolerable rang. The reason for unacceptable range of neonate unit might be due to the fact that the rooms are not well ventilated and not well isolated from other staff rooms and believed as a normal ward than neonatal intensive careunit. Moreover the rooms are very small and accumulate many neonates at once. The location of the neonates' room is very important in order to reduce the microbial exchange with the other units through the air. At the mean time neonate's room had no good ventilation system and was located near the other units of the hospital.

Regarding the other wards we can say they are relatively safe as the mean CFU count shows (table 1) though, around 30/80 (37.5%) were unacceptable. The recorded unacceptable values might be due to the human trafficking particularly patient assistants and students in these rooms. In addition Intensive disinfection procedures might not performed along the day to reduce the microbial rates as much as possible. The result of this study was comparable with the previous results (21, 22) and findings with the Northern Ethiopia (19) also similar with findings in Jordan (31). However our result shows safest environment compared to the finding compared to study done in Jimma Ethiopia (17) which reported that both ORs were not in the acceptable range.

When we compare the mean CFU count of common wards with private rooms the common rooms of gynecology, medical and surgical have the highest count also have the highest unsatisfactory level. The higher bacterial load compared to the standard could be sign of inadequate cleaning practices in the rooms. The other main reason might be due to higher number of patients with visitors per room. So that we can say that the higher load of indoor bacterial count in reality put the occupants at an increased threat for infection. Air born pathogen in hospital can be affected by weather condition, season, the outdoor microbial load and number of patients and visitors, number of medical teams and students, indoor ventilation system and design, cleaning procedure and detergents used (27). So to our

observation the higher number of CFU count might be the overcrowding number of students and visitors in addition to poor follow up on amount of detergent mix used during cleaning. For this hospital might check for deficit in line with set up. The more the bed number in room means a high number of patients, personnel, and visitors occupying the rooms consequently influences the bacterial load contamination rate in that specific rooms.

The study also indicates that the time variation in sample collection have slight difference. Samples collected in the afternoon were more unacceptable 22/48 (45.8%) than the morning samples 19/48 (39.5 %) but not have significant association, p-value of 0.53. Higher mean CFUs of bacteria also detected in indoor-air samples collected at afternoon; yet again, there was no significant difference in mean bacterial CFUs with periods of sample collection, $p > 0.05$. At the mean time *s. aureus* is very common in the afternoon while CoNS predominate in the morning. This is similar with finding in Jimma (17), in Adama (23). But, disagree with finding in Northern Ethiopia (19). On the other hand, Mengistu et al reported that the colony counts were found to be higher in the morning than in the afternoon. High bacterial indoor-air load during afternoon might be due to higher human traffic both visitors and personnel in the rooms during these times, which 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

In this study, similar to earlier study and others finding, the most frequently isolated bacteria were *Staphylococcus aureus, CoNS* and *pseudomonas spp*. As already recognized these isolates are the main cause of infections of the skin, deeper tissue and organs (22). Again these microorganisms are predictable to be primary agents of nosocomial infections in hospitals (29). These bacteria are commonly found on physicians and nursing staff's clothing [26, 28], cell phones, stethoscopes, blood-pressure cuffs and computer key board [29, 30]. The existence of multiple traits might indicate their domination in hospital environment. Similar to the previous studies,(21, 22) *S. aureus*was the most frequently detected isolate in this study followed by*CoNS*. Similar species of bacteria were reported from studies done in Jimma (17, 32), northern Ethiopia (19, 20). Bacteria isolates reported in different wards were almost similar but Surgical wards and gynecology contained higher load of bacteria compared to the other rooms, however the difference was not statistically significant ($P>0.05$).

As already reported in a different place most of isolates were resistant for the tested antibiotics. Penicillin was almost resisted by Gram-positive isolates; 75% of S. aureus and 72% of the *CoNS* being resistant. A similarly high prevalence of resistance of *CoNS* and *S. aureus* to penicillin has also been reported in earlier study (21, 22), Jimma Ethiopia (17), Adama (23) and others report elsewhere (19, 20). The two gram negative isolates, pseudomonas and k. pneumonia, almost resistant for most antibiotics tested which is reported in different place (22). In general nearly all of the bacterial isolates in this study show multidrug resistant.

5. CONCLUSION

In conclusion, the results in this study visibly recommend that; regardless of number of students and visitors, the neonatal care unit need to get appropriate emphasis to safe newborns from nosocomial infection risk as their immune debilitated. Bacteriology of the isolates remained troublesome because of the resistance pattern of the isolates to commonly prescribed antibiotics. Therefore, hospitals should have enhanced practice of good sanitation protocols and infection control measures. Regularly, monitoring of hospital indoor bacterial load is particularly recommended. Thorough application of infection prevention and patient's safety rules of known effective methods that we can combat nosocomial infections.

DATA AVAILABILITY STATEMENT

All the data supporting the result were shown in the paper, and can be applicable from corresponding author

AUTHORS' CONTRIBUTIONS

All authors participated in proposal writing, data collection, analysis, interpretation and critical review of the manuscript. All authors read and approved the final manuscript for publication.

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