Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

Monitoring of Air and Water Bacterial Load in the Operation Theatres and Intensive Care Units of Selected Hospitals in Dhaka city: A Cross-Sectional Study

Adeeba Khanduker[1]*, Md. Asaduzzaman Rajib[2], Lovely Barai[3], Nabila Khanduker[4], Mohammad Haroon Or Rashid[5]

[*1]Junior Consultant, Labaid Cancer Hospital and Super Speciality Center, 26 Green Road, Dhaka-1205, Bangladesh. Email: adeebakhanduker@gmail.com,
Orcid ID: 0000-0001-6442-7095
[2]Assistant Registrar, Department of Urology, National Institute of Kidney Disease and Urology, Dhaka, Bangladesh. Email: rajib3434@gmail.com,
Orcid ID: 0000-0001-8110-5629
[3]Professor, Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh.
Email: barai_lovely@yahoo.com,
Orcid ID: 0009-0003-8554-9229
[4]Associate Professor, Department of Surgery, Green Life Medical College & Hospital, Dhaka, Bangladesh.
Email: nabilaarmin@gmail.com,
Orcid ID: 0009-0005-8139-6020
[5]Junior Consultant, Department of Casualty,Dhaka Medical College Hospital, Dhaka, Bangladesh.
Email: haroonnbl5@gmail.com,
Orcid ID: 0009-0004-5242-7425

*Corresponding author

Received: 20 Februry 2023
Revised: 24 March 2023
Accepted: 06 April 2023
Published: 30 April 2023

Abstract

Background: Hospital milieu monitoring is an essential component for controlling healthcare associated infections (HCAIs) as it serves as the reservoir for pathogenic microbes. Aim of this study was to identify the bacterial load in Intensive care units (ICU) and Operation theaters (OT) air and water sources of selected tertiary care hospitals. **Material & Methods:** The study was organized in Microbiology department, BIRDEM General Hospital. A sum total 28 air samples & 6 water samples were collected from three selected hospitals and those were processed according to the set of protocols. **Results:** From air sampling, highest load of bacteria was found 480 CFU/dm²/hr in Hospital C ICU, 38.40 ± 9.99 CFU/dm²/hr in pre-OT samples & 218.2±43.35 CFU/dm²/hr in intra OT samples of Hospital C. From water sampling, unacceptable level of coliforms was found in all three hospitals. Among the non-pathogens, 24% - 37% Micrococcus spp. (normal flora) and 2% -18% Bacillus spp. (contaminants) were found in the OTs. Whereas pathogens found were Acinetobacter spp. (20.7%) followed by Pseudomonas spp. (19.4%), Klebsiella spp. (12.1%) ~~&amp~~; S. aureus (9.2%) in the ICUs. **Conclusion:** It could be deduced from the study that environmental sources such as air and water contaminations with multidrug resistant pathogens are an ultimate risk factor for all related to the healthcare settings, specially the indoor patients.

Keywords:- Hospital milieu, Healthcare associated infections.

INTRODUCTION

Prevalence of healthcare associated infections (HCAIs) had continued to increase due to contamination of hospital milieu by microbes, especially in the operating rooms and other specialized areas.[1,2,3] Patients are in significant threat mainly due to antibiotic resistant bacteria in Intensive care unit (ICU) and Operation Theatre (OT). ICUs and OTs are "hot zones" for the appearance and spread of bacterial resistance because here majority of invasive procedures takes place along with high antibiotic usage and due to the infection control measures which are mostly inadequate.[4] To detect the changing vogue of types and counts of bacterial load, environmental monitoring by the microbiological testing of water, air,

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

surfaces and equipment is useful.[5] Air bio-load which is present in the form of aerosols, may contain bacteria, yeasts, moulds, fungal spores and viruses. The possible sources of these bio-load may come from patients' own endogenous flora, or from health care persons and from sources of environment.[6] An estimate has been made that wound contamination will reduce by around 50%, if there is reduction of 13-fold in the airborne bacteria of operating rooms.[7] Another estimate shows the probable causes of HCAIs in the ICUs are due to 40-60% from patients' endogenous flora, 20-40% from the contaminated hands of healthcare workers (HCWs), 20-25% from resistant trends of antimicrobials and 20% from environmental contamination.[8] Bacteria including all the pathogens, opportunistic pathogens as well as the normal flora lives in diverse communities at ICUs. Bacteria of ICU environments are typically found to be associated with human. Confined space, limited and controlled access with strict cleaning procedures make the ICU environment less diverse than indoor environments. Air sampling and air quality testing is done worldwide in different hospitals to check the hygiene status of the environment. Two methods for air sampling are active (uses mechanical samplers) & passive (uses several agar plates). Passive air sampling known as settle plate method is widely used due to accessible and cost effective. Measurement of air colony count can be done by two methods. The first one is Koch's sedimentation method according to which recommended conventional operating theatres values: acceptable bio load of an empty theatre <35 CFU/m^3, during operation < 180 CFU/m^3, ultraclean super specialized theatre (e.g., for cardiac and joint replacement surgeries), the centre of an empty theatre should be less than <1 CFU/m^3.[9] Here colony count is expressed in CFU/m^3. Another method is 1/1/1 schedule (a sterile Petri dish of nine cm in diameter containing 5% Sheep's blood agar was kept open to the air for an hour. It should be a meter above from the floor and a meter away from the wall).[10] The total number of colony forming units (CFU) was calculated and results were expressed in CFU/dm^2/hr.[11] HCAIs can be caused by Gram-negative bacteria present in potable water. Most of the clinically important, opportunistic organisms include Pseudomonas spp., Burkholderia cepacia, Stenotrophomonas maltophilia, Ralstonia pickettii and Sphingomonas spp. are present in tap water. These organisms possess the risk of developing infection among the immunocompromised patients. Colonization by these organisms often precedes to the development of infection. Tap water acts as potential risk factor for exposure when it is used for direct patient care, as a water source for medical equipment and instruments, as a diluent for solutions and during the steps of instrument disinfection. The colonized patients may serve as a source of contamination, especially for moist environments of medical equipment (e.g., ventilators).[12] The aims of this study is to observe air quality in the form of total bacterial count by Settle plate method, detect water source contamination, identify the common bacterial pathogens and their antimicrobial susceptibility pattern in the ICUs and OTs of three selected hospitals.

MATERIAL AND METHODS

Study Setting, Design, and Period

A cross-sectional observational study was done from the time period of 15 March 2019 to 30

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

February 2020 at Microbiology Department, BIRDEM General Hospital, Dhaka. Three hospitals were selected by purposive sampling for this study and designated as hospital 'A', hospital 'B' and hospital 'C' in order to maintain privacy of the hospitals. Hospital 'A' is a private multidisciplinary hospital complex. It consists of 103 cabins, 747 ward beds, 21 beds in ICU and 8 Operation theatres in the OT complex. Hospital 'B' is a mono disciplinary private specialized hospital for cardiac treatment consisting of 78 beds including 8 ICU beds and 2 Operation theatres. Hospital 'C' is a tertiary level government general hospital. It consists of around 1700 beds, 36 ICU beds in 3 ICUs and 12 Operation theatres in the main OT complex. From Hospital 'A' among 8 OT, 5 were selected and 1 ICU. From Hospital 'B', 2 OT and 2 ICU were selected. And from Hospital 'C', randomly 5 OT were selected as every second or third from 12 OT in the main OT complex in order to keep the sample size same with Hospital 'A' and 'B' ICU. In total, samples were taken from 12 (5+2+5) OTs and 4 (1+2+1) ICUs of three hospitals.

Collection of air sample by settle plate method

Total 28 air samples were collected. Single samples were taken from the ICUs (as the Hospital 'B' ICU has 2 rooms, so 2 samples were taken from 2 rooms) in between 10 – 11a.m. From the total 12 Operating rooms, samples were collected twice from the same site before (7 – 8 a.m.) and during operative procedures (10 – 11 a.m.).During the air sampling period, sterile gloves, mouth masks and protective gown was used for preventing self-contamination of the media. Microbial air contamination index was based on the number counts of the microbial fallout onto petri dishes which were left open to the air for 1 hour, 1meter above the floor and minimum 1meter away from any wall or obstacle. Then the plates were covered with their lids and sealed in plastic bags, taken to the Microbiology laboratory and incubated at 37^{o}C for 24 hours in the incubator. In the following day, culture plates that showed discrete apparent colonies were counted using digital colony counter.[10]

Collection of water sample

Water sample was collected from the tap water source of the 3 ICU and 3 OT. In total 6 samples were collected. The tap was sterilized by using the flame of spirit lamp. A pre-sterilized glass bottle was then filled 200 ml from a gentle flow of water and cap of the bottle was replaced and brought to the laboratory. In the laboratory the filtration unit and suction device was assembled. Then by using sterile blunt-ended forceps, a sterile membrane filter (47 mm diameter, pore size 0.22 µm, Cellulose acetate membrane filter, Membrane solutions) was placed, grid-side uppermost, on the filter base and the unit reassembled. Suction was applied to draw 100 ml of water sample through the filter membrane. Then aseptically by sterile blunt-ended forceps the membrane was removed from the filtration unit and placed on the MacConkey agar media plate and incubated overnight. In the following day, the number of colonies were counted (both lactose fermenter and non-fermenter) and expressed per 100 ml of water.[13]

Interpretation

Coliform bacteria 1-10/100 ml is acceptable and heterotrophic organism (e.g. *Pseudomonas*) <50000 CFU/100 ml is acceptable.[14] The

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

colonies were evaluated for the growth of potential pathogenic bacteria. This was done initially by observing the colony characteristics, pattern of haemolysis and by microscopy of Gram-stained smears. Final identification was done by practising the standard bacteriological techniques. Antimicrobial susceptibility of all isolates was observed by performing Kirby Bauer modified disc diffusion technique using Muller Hinton agar plates. The zones of inhibition were interpreted according to CLSI guideline.[15]

RESULTS

A total of 34 samples were included in the study from the three tertiary care hospitals, out of them 28 were air samples and 6 were water samples. Among the 28 air samples, (10+4+10) samples were taken from 3 Hospital's OT and (1+2+1) samples were taken from 3 hospital's ICU. The 6 water samples were collected from each of the 3 ICUs and 3 OTs.

Table 1: Standard range of bacterial load according to microbial air contamination index of Fisher.

Site	Standard (CFU/dm²/hr)		
	Optimal	Acceptable	Unacceptable
Operating room (active)	0-60	61-90	>90
Operating room (passive)	0-4	5-8	≥9
Intensive care unit	0-250	251-450	>450

Table 2: Bacterial load of air in ICUs & OTs of different hospitals.

Passive air samples	Mean(±SD) of Bacterial load in CFU/dm²/hr (Hygiene level)			ANOVA test p-value
	Hospital 'A'	Hospital 'B'	Hospital 'C'	
ICU	380.00 ± 0.00 (A)	270.00 ± 28.28 (A)	480.00 ± 0.00 (UA)	B vs C = 0.013
Pre OT	33.60 ± 12.18 (UA)	14.00 ± 5.66 (UA)	38.40 ± 9.99 (UA)	B vs C = 0.010
Intra OT	186.00±22.53 (UA)	77.50±17.68 (A)	218.2±43.35 (UA)	A vs B = 0.004 B vs C = 0.001

According to microbial air contamination index of Fisher: 251-450 CFU/ dm²/hr in ICU is acceptable, 60-90 CFU/ dm²/hr in Pre-OT is acceptable, 60-90 CFU/ dm²/hr in intra OT is acceptable, UA = Unacceptable, A = Acceptable.

[Table 2] presents the bacterial load of air in the ICUs and OTs. The finding showed that ICU of Hospital 'C' had the highest bacterial load of 480 CFU/dm² and the Operating room of Hospital 'B' had the lowest bacterial load of 77.5 CFU/dm² during active period. The pre-OT bacterial load was found in unacceptable range for all the OTs.

Table 3: Bacterial load & quality of water in ICUs& OTs of different hospitals.

Water samples	Bacterial load in CFU/100 ml (Hygiene level)					
	Hospital 'A'		Hospital 'B'		Hospital 'C'	
	Coliforms	Heterotrophs	Coliforms	Heterotrophs	Coliforms	Heterotrophs
ICU	16 (UA)	164 (A)	12 (UA)	148 (A)	18 (UA)	172 (A)
OT	15 (UA)	131 (A)	12 (UA)	108 (A)	12 (UA)	112 (A)

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

UA = Unacceptable, A = Acceptable, Coliform bacteria 1-10/100 ml is acceptable &heterotrophic bacteria <50000/100 ml is acceptable.[13,14]

[Table 3] shows the waterload of bacteria in the ICUs and OTs. Highest coliform bacteria were found 18 per 100 ml in Hospital 'C' ICU whereas lowest was 12 per 100 ml in Hospital 'B'. In all the three hospitals the coliform bacterial count was not acceptable as they were above 10 per 100 ml. In contrast, the heterotrophic bacterial count was within normal range in all the ICUs and OTs.

Table 4: Frequency of isolated organisms from air and water sources of ICU and OT environments in three hospitals.

Isolated organisms	Hospital 'A'			Hospital 'B'			Hospital 'C'		
	Pre OT (33)	Intra OT (186)	ICU (380)	Pre OT (14)	Intra OT (77)	ICU (270)	Pre OT (38)	Intra OT (218)	ICU (480)
S. aureus	5 (15.2)	25(13.4)	35(9.2)	4(28.6)	7(9.2)	33(12.2)	8(21.1)	20(9.2)	50(10.4)
CONS	8 (24.2)	30(16.1)	25(6.6)	4(28.6)	10(12.9)	53(19.6)	12(31.5)	43(19.7)	33(6.9)
Bacillus spp.	5 (15.2)	29(15.6)	30(7.9)	2(14.3)	10(12.9)	50(18.6)	1(2.5)	40(18.3)	46(9.6)
Micrococcus spp.	8 (24.2)	50(26.9)	42(11.1)	4(28.5)	28(36.4)	62(22.9)	2(5.3)	60(27.5)	64(13.3)
E. coli	0	2 (1.1)	15(3.9)	0	0	2(0.7)	2(5.3)	6(2.7)	27(5.6)
Klebsiella spp.	0	0	46(12.1)	0	0	0	0	2(0.9)	32(6.7)
Pseudomonas spp.	3 (9.1)	28(15.1)	74(19.4)	0	12(15.6)	38(14.1)	5(13.2)	25(11.5)	78(16.2)
Flavobacterium spp.	4 (12.1)	22(11.8)	34(8.9)	0	10(12.9)	32(11.9)	8(21.1)	22(10.2)	66(13.7)
Acinetobacter spp.	0	0	79(20.7)	0	0	0	0	0	84(17.5)

[Table 4] shows the frequency of isolated organisms in the air of high-risk areas of three hospitals. In the pre-OT state, CoNS was found highest (31.6%) in Hospital 'C'. Micrococcus spp. was found highest both in Intra OT state of Hospital "B" (36.4%) as well as among the ICU of Hospital "A". Among the Gram-negative organisms, Pseudomonas spp. (14.1%) was found highest in the ICUs.

Table 5: Antimicrobial agent resistance patterns of isolated Enterobacteriaceae, Acinetobacter spp., Pseudomonas spp. and Staphylococcus spp. from air and water environment of ICUs & OTs of three hospitals.

Antibiotics tested	Enterobacteriaceae			Pseudomonas spp.			Acinetobacter spp.			S. aureus		
	Hos 'A' n = 63	Hos 'B' n = 02	Hos 'C' n = 69	Hos 'A' n = 105	Hos 'B' n = 50	Hos 'C' n = 108	Hos 'A' n = 79	Hos 'B' n = 0	Hos 'C' n = 84	Hos 'A' n = 65	Hos 'B' n = 44	Hos 'C' n = 78
Ceftriaxone	34(53.9)	1(50.0)	50(72.4)	-	-	-	60(75.9)	-	64(76.19)	-	-	-
Ceftazidime	34(53.9)	1(50.0)	50(72.4)	63(60.0)	25(50.0)	61(56.5)	60(75.9)	-	64(76.19)	-	-	-
Cefotaxime	34(53.9)	1(50.0)	50(72.4)	-	-	-	60(75.9)	-	64(76.19)	-	-	-
Cefixime	34(53.9)	1(50.0)	50(72.4)	-	-	-	60(75.9)	-	64(76.19)	-	-	-
Amoxiclav	34(53.9)	1(50.0)	50(72.4)	-	-	-	60(75.9)	-	64(76.19)	22(33.8)	8(18.2)	16(20.5)
Piperacillin-Tazobactam (PIT)	13(20.6)	0	20(28.9)	63(60.0)	18(36.0)	52(48.1)	60(75.9)	-	60(71.42)	-	-	-
Aztreonam	34(53.9)	1(50.0)	50(72.4)	48(45.7)	12(24.0)	57(52.7)	60(75.9)	-	64(76.19)	-	-	-
Imipenem	5(7.9)	0	13(18.8)	48(45.7)	25(50.0)	61(56.5)	60(75.9)	-	60(71.42)	-	-	-

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

Amikacin	9(14.3)	0	20(28.9)	36(34.3)	12(24.0)	52(48.1)	60(75.9)	-	60(71.42)	03(4.6)	03(6.8)	8(10.3)
Gentamicin	9(14.3)	0	20(28.9)	36(34.3)	12(24.0)	57(52.7)	60(75.9)	-	60(71.42)	03(4.6)	03(6.8)	8(10.3)
Ciprofloxacin	13(20.6)	1(50.0)	13(18.8)	63(60.0)	18(36.0)	52(48.1)	60(75.9)	-	60(71.42)	11(16.9)	8(18.2)	16(20.5)
Cotrimoxazole	9(14.3)	1(50.0)	18(26.1)	85(80.9)	38(76.0)	87(80.5)	60(75.9)	-	64(76.19)	11(16.9)	8(18.2)	16(20.5)
Cefoxitin	-	-	-	-	-	-	-	-	-	11(16.9)	0	24(30.8)
Oxacillin	-	-	-	-	-	-	-	-	-	11(16.9)	0	24(30.8)
Vancomycin	-	-	-	-	-	-	-	-	-	0	0	0
Erythromycin	-	-	-	-	-	-	-	-	-	22(33.8)	8(18.2)	16(20.5)
Clindamycin	-	-	-	-	-	-	-	-	-	22(33.8)	8(18.2)	16(20.5)

[Table 5] depicted the antimicrobial drug resistance patterns of isolated Enterobacteriaceae, Acinetobacter spp., Pseudomonas spp. and Staphylococcus spp. from environment of ICUs and OTs of the selected hospitals. Enterobacteriaceae isolated from ICU & OT environment of three different hospitals showed high resistance pattern to 3rd generation Cephalosporin (50 – 75%), Aztreonam (50 – 75%) & Amoxiclav (50–75%). Isolated Acinetobacter spp. depicted approximately 75% resistance (except Hospital 'B') to 3rd generation Cephalosporin, Beta-lactamase inhibitor combinations, Aztreonam, Imipenem, Aminoglycosides, Ciprofloxacin & Cotrimoxazole. However, they were found 100% sensitive to Tigecycline & Colistin. Pseudomonas spp. isolated from this environment were found 50 – 60% resistant to Ceftazidime, 35 – 50% to Piperacillin-Tazobactam (PIT), 45 – 57% to Aztreonam, 45 – 60% to Imipenem, 30 – 50% to Aminoglycosides and 38 - 50% to Ciprofloxacin.

DISCUSSION

Micro flora or microbial contamination of the hospital milieu especially the "hot zones" like OT and ICU, possess a high infection risk for patients. It is an important source of HCAI as they can lead to colonization and even infection of the patients. These increases the vulnerability, morbidity, longer hospital stays, cross-infection and huge economic loss of the patients and the surfacing of multi-drug resistant (MDR) pathogens. For preventing these consequences and reducing the bacterial load, maintaining environmental hygiene by controlling the bio-burden is a must to do now. With this perspective, our study was conducted to monitor environmental hygiene in the three selected tertiary care hospitals. This was done by counting and identifying the air and water bacterial of those environments.

Bacterial load in air of ICU was counted according to the microbial air contamination index of Fisher [Table 2]. According to this index acceptable range in ICU is 251–450 CFU/dm²/hr. The range of Hospital 'A' and 'B' ICU was within the acceptable range. A study done in Czech Republic is in accordance with the findings of these two results.[16] The range of hospital 'C' was slightly higher than Fisher's range that coincides with the studies of Hawassa, Ethiopia and India.[17,18] The finding shows that the hygienic state of the Hospital 'C' ICU under study is in unacceptable range. Human trafficking is the possible explanation of this increased bacterial load in air of ICU, as Hospital 'C' is a government general hospital where visitor's restriction is not well maintained.

Bacterial load in air of OT was also done according to the microbial air contamination index of Fisher [Table 2]. According to this index pre-OT acceptable range is 5 – 8

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

CFU/dm²/hr, intra OT acceptable range is 60 – 90 CFU/dm²/hr. The mean colony count of air obtained from operating rooms were 33.60 CFU/dm²/hr (unacceptable), 14 CFU/ dm²/hr (unacceptable) and 38.40 CFU/dm²/hr (unacceptable)during pre–operation time in Hospital 'A', 'B' and 'C' OT respectively (Table-3.1). During intra- operation time the mean colony count of air in Hospital 'A' was 186 CFU/dm²/hr (unacceptable), in Hospital 'B' was 77.50 CFU/dm²/hr (acceptable) and in Hospital 'C' was 218.2 CFU/ dm²/hr (unacceptable)(Table-3.1). The hygienic level of air during intra OT time observed hygienic in Hospital 'B' which is a monodisciplinary specialized cardiac hospital. The observed poor quality of air during intra OT time in Hospital 'A' & 'C' may be explained by multidisciplinary private & government hospitals where implementation of infection control OT protocol was not up to the standard. This finding is comparable to the study done in Jimma, Ethiopia, where ORs were not found in acceptable range according to the Fisher's index.[19] The findings of Hospital 'B' was compatible with study done in Northern Ethiopia,[20] which reported mean colony count of17.2 CFU/dm²/hr during pre-OT and 91.8 CFU/ dm²/hr, during intra OT time. Qudiesat et al. in Jordan also reported similar findings.[21]

Water quality testing was done by counting coliform and heterotrophic bacteria in the water]Table 3]. Coliform bacteria 1-10/100 ml is acceptable and heterotrophic organism (e.g.,Pseudomonas, Flavobacterium, Acinetobacter) <50000 CFU/100 ml is acceptable.[14] According to the findings of this study, the range of coliform bacteria was not acceptable in all three hospitals indicating poor quality of supply water system. A source of HCAIs could be the wet environmental sites such as hospital water systems and taps, water baths, sink drains and hydrotherapy pools where many Gram-negative opportunistic pathogens can survive and proliferate. These water friendly organisms include Pseudomonas, Serratia, Acinetobacter and Flavobacterium species which are non-fermenting, inherently antibiotic resistant and are even capable for acquiring multipleresistance factors.[22]

Non-pathogenic organisms were isolated predominantly in the OTs of three hospitals (15 – 38 %) [Table 4]. Among them [Table 5] 24% - 37% were Micrococcus spp. (normal flora) and 2% - 18% were Bacillus species (contaminants). This finding was in accordance with the study of Kiranmai in Telangana, India,[23] where Bacillus spp.45% (contaminants) and Micrococcus spp. 33%(normal flora) were reported. In our study, the highest pathogens isolated were Staphylococcus aureus (15% - 29%) followed by Pseudomonas spp. (9% - 16%).No MRSA was found from the OT samples in this study. Pseudomonas spp. was the only isolate which was detected on both air and water sources of OTs.

In the scenario of organisms' isolation from ICU samples, the least contaminated ICU was found from Hospital 'B' which is a private monodisciplinary cardiac specialized hospital. Among the isolated pathogens in ICUs, Acinetobacter spp. was found to be predominant (20.7%) followed by Pseudomonas spp. (19.4%), Klebsiella spp. (12.1%) & S.aureus(9.2%). In case of non-pathogens, Micrococcus spp. was found to be predominant (22.9%) followed by Bacillus spp.

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

(18.6%) & Coagulase negative *Staphylococcus* (19.6%) [Table 5]. This finding suits the findings of Huang et al. in Taiwan.[24] The finding of Acinetobacter spp. and Pseudomonas spp. in air and water source in total from the whole ICU environment was noticeable. Shamsizadeh et al. found data close to our study.[25]

The resistance pattern of antimicrobial agents was found very high among the isolated enterobacteriaceae of this study from all three hospitals [Table 6]. In Hospital 'A' 53.9% of enterobacteriaceae were found ESBL positive, where in Hospital 'B' & 'C' 50% &72.4% isolates were found ESBL positive respectively. Antimicrobial resistance of enterobacteriaceae were also found high for Ciprofloxacin, Imipenem & Aminoglycosides. The resistance rate of enterobacteriaceae was found close with Deepa in Karnataka, India.[18] Acinetobacter spp. was found >70% resistant to all drugs [Table 6] which was also comparable to the study of Deepa.[18] In case of Pseudomonas spp. Ceftazidime was found 50 -60% resistant, Piperacillin-Tazobactum35 – 60%, Imipenem 45 – 60%, Aminoglycosides & Ciprofloxacin 20 – 60% resistant. All the Gram-negative isolate showed high sensitivity to Colistin & Tigecycline [Table 6].

The pathogens which circulate in the "hot zones" of the hospitals are considered as the most common source of contamination and infection due to their tremendous capability to survive a long life in the hospital milieu even in adverse environmental conditions.[26,27] Detection of high bacterial burden comprising 'indicator' organisms Acinetobacter spp., Pseudomonas spp., E. coli and Klebsiella spp. in the present study documents the increase chance of HAI in Hospital 'A' & Hospital 'C'. The findings also reflect inadequate practice of infection control measures in these hospitals. Periodic cleaning & washing of hospital milieu at regular interval to decrease the bacterial load especially in the ICUs and OTs should be done. Continuous focus on microbiological surveillance, consciousness of the staffs including clinicians to maintain good infection control practice will surely enhance infection prevention and control.

CONCLUSIONS

High bacterial load of indoor air and water is implied as a potential risk factor both for surgical site infections as well as the ICU patients as there is a linear relationship between infection rate and bacterial load in air. The resistance pattern of pathogens towards the antimicrobial agents were found very high. Highest resistance was found among the Acinetobacter isolates which is alarming as they were found throughout the environment (both in air and water sources). As the ICUs and OTs are reflecting the entire hospital's environment, the hygiene status was detected better in Hospital 'B' which is monodisciplinary private specialized cardiac hospital compared to Hospital 'A' and Hospital 'C'. This might be due to differences in the practice and implementation of infection control protocol in those respective hospitals. As the patients getting exposed to OT and ICU environments are highly susceptible to microbial infection, adequate attention should be given in the maintenance of hospital environmental hygiene.

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

REFERENCES

1. Bhalla A, Aron DC, Donskey CJ. Staphylococcus aureus intestinal colonization is associated with increased frequency of S. aureus on skin of hospitalized patients. BMC Infect Dis. 2007;7:105. doi: 10.1186/1471-2334-7-105.
2. Bonten MJ, Hayden MK, Nathan C, van Voorhis J, Matushek M, Slaughter S, et al. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. Lancet. 1996;348(9042):1615-9. doi: 10.1016/S0140-6736(96)02331-8.
3. Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications. Infect Control Hosp Epidemiol. 1997;18(9):622-7.
4. Hanberger H, Arman D, Gill H, Jindrák V, Kalenic S, Kurcz A, et al. Surveillance of microbial resistance in European Intensive Care Units: a first report from the Care-ICU programme for improved infection control. Intensive Care Med. 2009;35(1):91-100. doi: 10.1007/s00134-008-1237-y.
5. Sandle T. Environmental monitoring risk assessment. J GXP Compliance. 2006;10(2):54-74.
6. Sehulster L, Chinn RY; CDC; HICPAC. Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep. 2003;52(RR-10):1-42.
7. Whyte W, Hambraeus A, Laurell G, Hoborn J. The relative importance of the routes and sources of wound contamination during general surgery. II. Airborne. J Hosp Infect. 1992;22(1):41-54. doi: 10.1016/0195-6701(92)90129-a.
8. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis. 2013;26(4):338-44. doi: 10.1097/QCO.0b013e3283630f04.
9. Landrin A, Bissery A, Kac G. Monitoring air sampling in operating theatres: can particle counting replace microbiological sampling? J Hosp Infect. 2005;61(1):27-9. doi: 10.1016/j.jhin.2005.03.002.
10. Pasquarella C, Pitzurra O, Savino A. The index of microbial air contamination. J Hosp Infect. 2000;46(4):241-56. doi: 10.1053/jhin.2000.0820.
11. Andersson AE, Bergh I, Karlsson J, Eriksson BI, Nilsson K. Traffic flow in the operating room: an explorative and descriptive study on air quality during orthopedic trauma implant surgery. Am J Inf Control. 2012;40(8):750-5.
12. CDC issues new environmental guidelines. OR Manager. 2003;19(8):20-2.
13. Jensen PA, Lambert LA, Iademarco MF, Ridzon R; CDC. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR Recomm Rep. 2005;54(RR-17):1-141.
14. Amanidaz N, Zafarzadeh A, Mahvi AH. The Interaction between Heterotrophic Bacteria and Coliform, Fecal Coliform, Fecal Streptococci Bacteria in the Water Supply Networks. Iran J Public Health. 2015;44(12):1685-92.
15. Weinstein MP, Lewis JS 2nd. The Clinical and Laboratory Standards Institute Subcommittee on Antimicrobial Susceptibility Testing: Background, Organization, Functions, and Processes. J Clin Microbiol. 2020;58(3):e01864-19. doi: 10.1128/JCM.01864-19.
16. Vackova MH, Smetana J, Chlibek R, Bostikova V, Splino M. Microbial air load at the transplant intensive care unit. Mil Med Sci Lett. 2011;80:52-7.
17. Hailemariam M, Worku M, Azerefegne E. Intensive care units and operating rooms bacterial load and antibiotic susceptibility pattern. J Surg. 2016;4(2):60-4.
18. Deepa S, Abishek MU, Venkatesha D. The air as harbinger of infections in critical care units. Med Sci. 2014;8:8-13.
19. Genet C, Kibru G, Tsegaye W. Indoor air bacterial load and antibiotic susceptibility pattern of isolates in operating rooms and surgical wards at jimma university specialized hospital, southwest ethiopia. Ethiop J Health Sci. 2011;21(1):9-17. doi: 10.4314/ejhs.v21i1.69039.
20. Tesfaye T, Berhe Y, Gebreselassie K. Microbial contamination of operating Theatre at Ayder Referral Hospital, Northern Ethiopia. Int J Pharma Sci Res. 2015;6(10):1264-1267.
21. Qudiesat K, Abu-Elteen K, Elkarmi A, Hamad M, Abussaud M. Assessment of airborne pathogens in healthcare settings. Afr J Microbiol Res. 2009;3(2):66-76.
22. Berrouane YF, McNutt LA, Buschelman BJ, Rhomberg PR, Sanford MD, Hollis RJ, et al.

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-3 | May- June 2023
DOI: 10.53339/aimdr.2023.9.3.15
Page no- 113-122| Section- Research Article (Miscellaneous)

Outbreak of severe Pseudomonas aeruginosa infections caused by a contaminated drain in a whirlpool bathtub. Clin Infect Dis. 2000;31(6):1331-7. doi: 10.1086/317501.

23. Kiranmai S, Madhavi K. Microbiological surveillance of operation theatres, intensive care units and labor room of a teaching hospital in Telangana, India. Int J Res Med Sci. 2016;4(12):5256-60.
24. Kiranmai S, Madhavi K. Microbiological surveillance of operation theatres, intensive care units and labor room of a teaching hospital in Telangana, India. Int J Res Med Sci. 2016;4(12):5256-60.
25. Shamsizadeh Z, Nikaeen M, Nasr Esfahani B, Mirhoseini SH, Hatamzadeh M, Hassanzadeh A. Detection of antibiotic resistant Acinetobacter baumannii in various hospital environments: potential sources for transmission of Acinetobacter infections. Environ Health Prev Med. 2017;22(1):44. doi: 10.1186/s12199-017-0653-4.
26. Talon D. The role of the hospital environment in the epidemiology of multi-resistant bacteria. J Hosp Infect. 1999;43(1):13-7. doi: 10.1053/jhin.1999.0613.
27. Verdeil X, Bertrand MA, Roche R, Lareng MB, Pous J. Clinical and microbiological epidemiology of nosocomial infections in surgical wards: results of a prospective study of 3422 patients in a Toulouse hospital. Med Mal Infect. 1990;20(5):222-8.

Source of Support: Nil, Conflict of Interest: None declare