

Original Article

Environmental surface sampling for qualitative & quantitative detection of microbial burden in high risk areas of three hospitals in Dhaka city

Adeeba Khanduker¹, K.M. Shahidul Islam², Lovely Barai², Sk. Shehab Hasan³, Mufradul Islam Rafi², Md. Robiul Islam Rubel²

¹Department of Microbiology, Labaid Cancer Hospital & Super Speciality Center, Dhaka, Bangladesh, ²Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh, ³Department of Microbiology & Clinical Pathology, National Heart Foundation Hospital & Research Institute, Dhaka, Bangladesh.

Abstract

Background and objectives: Acquiring infection from intensive care units & operation theatres is considered as a significant public health problem around the world. Microbial burden may vary across different ICUs and OTs, albeit these sectors remain underexplored especially in resource poor country. Purpose of the study was qualitative and quantitative assessment of microbial burden in ICUs and OTs' environmental surfaces of three tertiary care hospitals in Dhaka city.

Materials and methods: The study was conducted in the department of Microbiology, BIRDEM General Hospital. A total 232 environmental surface swab samples & 86 hand & nasal swab samples of ICU staffs were collected from the selected three hospitals. The samples were processed according to standard operating procedures.

Results: Highest bacterial load from inanimate surface was found on sinks (155 CFU/cm²), ICU bedrails (7.56 ± 0.76 CFU/cm²) and OT sandals (66 CFU/cm²). Pathogenic isolates were found more in ICUs & non-pathogenic isolates were found more in OTs. Among the non-pathogens, *Micrococcus* spp. (37.8%) and *Bacillus* spp. (19.8%) were found from OTs. Among the pathogens, *Acinetobacter* spp. was predominant (21.2%) followed by *Pseudomonas* spp. (19.2%) in the ICUs. Overall cleanliness & hygiene status of Hospital 'A' & 'C' were found unacceptable whereas Hospital 'B' was found marginally bearable/tolerable.

Conclusion: It could be inferred from the study that health care workers and environmental surface contaminations with MDR pathogens are a definitive risk factor for the indoor patients. Routine sampling to assess bioburden in high risk areas of hospital should be done regularly for identification & correction of the weakness of ongoing infection control protocol.

Background

Monitoring of the hospital environment is an essential element in the control of healthcare associated infections (HCAIs) as it contains diverse population of microorganisms and act as highest dissemination reservoir of pathogenic microbes¹. Among the whole hospital environment, Operating rooms and Intensive care units are regarded as the high risk areas for acquiring HCAIs as intimate handling and care of critically ill patients are given there.

The risk of HCAIs has been estimated to be two to twenty times higher in the developing countries than that of resource-rich countries with the percentage of infected patients exceeding 25%^{2,3,4,5}. The rate of HCAIs in Bangladesh may exceed 30% in some hospitals⁶.

Till the date, only one study conducted in New York University School of Medicine explored the entire microbiome of an Operating Room (OR) by using molecular techniques performed by next generation sequencing of the microbial communities present in three OR environments (found in two different hospitals), and proved that the OR dust contained a microbial community similar to

Correspondence:

Adeeba Khanduker

Department of Microbiology

Labaid Cancer Hospital & Super Speciality Center

Dhaka, Bangladesh.

E-mail: adeebakhanduker@gmail.com

the one found on human skin (dominated by *Staphylococcus* and *Corynebacterium*)⁷. Up to 30% of all surgical site infections (SSIs) are known to be caused by *Staphylococcus aureus*, especially the methicillin-resistant strains⁸. Besides *Staphylococcus* spp., other microorganisms which were isolated from ORs are as follows: *Enterobacter* spp., *Micrococcus* spp., *Acinetobacter* spp., *Brevibacterium* spp., *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp. and *Escherichia coli*⁹⁻¹³.

It was found that, among the hospital-acquired infections that develop within the intensive therapy unit, 40-60% were due to endogenous flora, 20-40% were due to the contaminated hands of healthcare workers (HCWs), 20-25% were due to antibiotic-driven change and 20% were potentially due to environmental contamination¹⁴. Due to the extreme selective pressure that confinement and cleaning practices induce, microorganisms living in ICUs develop or acquire resistance mechanisms that allow them to survive in the presence of a vast range of antimicrobial agents used in cleaning and antibiotic treatment, to adapt to extremely low nutrient content and to persist on dry surfaces for a long time¹⁵.

In a study on Microbiology of HCAs in tertiary hospitals in Dhaka, Bangladesh, the majority of infections were caused by *Escherichia coli* followed by *Pseudomonas*, *Proteus species*, *Staphylococcus aureus*, *Klebsiella species*, and *Acinetobacter*¹⁶. All infections were found antibiotic resistant which is thought to be due to the widespread use of antibiotics.

The hand contact surfaces, floors, water and air of the hospital environments are the main source of different pathogens that can cause HCAs^{14,17}. Transmission of potential pathogens inside the hospital is complex and involves contaminated hands of healthcare workers, contaminated equipment or contamination of the room from a prior patient. Pathogenic microorganisms persist in the hospital environment from hours to months, which depends on factors such as number, location, biofilm formation, intrinsic resistance of organisms to various cleaning products as well as local conditions. This has been well documented for *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus* (VRE), *Clostridium difficile*, *Pseudomonas aeruginosa*, *Acinetobacter* spp¹⁸.

The main factors associated with the ability of a nosocomial pathogen to survive on inanimate surfaces and equipment

are the specific microorganism characteristics (such as genus, species, specific strain, ability to form biofilm and microorganism concentration) and the environmental factors (such as UV radiation, temperature, humidity, presence of organic materials and surface type)¹⁹⁻²¹.

Microbes have an immanent ability to colonize any surface. Studies have shown that microbes persist for weeks on stainless steel surfaces and polymeric materials which are used to fabricate touch surfaces in hospitals²². Frequently touched surfaces such as doorknobs, push plates, bed rails, faucet handles and poles supporting intravenous fluid supplies (IV poles) have been identified as reservoirs for the transmission of pathogenic microbes^{23,24}. This can easily contaminate hands and equipment of health care workers, who, in turn, can transmit these pathogens to patients during routine care. This proffer is a call for bacteriological standards with which to assess clinical surface hygiene in hospitals, based on those used by the food industry. The first standard concerns any finding of a specific 'indicator' organism, presence of which suggests a requirement for increased cleaning. Indicators include *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, Vancomycin-resistant *Enterococci* and various gram-negative bacilli. The second standard concerns about quantitative aerobic colony count of 5 CFU/cm² on frequent hand touch surfaces in hospitals. The finding of ≥ 5 CFU/cm² from hand contact surface, whatever the identity of the organisms, indicates that there might be an increased risk of infection for the patient in that environment²⁵. This study was conducted for quantitative and qualitative assessment of microbial burden on hospital environmental surfaces and their antimicrobial susceptibility pattern in high risk areas (Operation theatres and Intensive care units) of three hospitals of Dhaka city.

Materials And Methods

Study Setting, Design, and Period. A hospital-based cross-sectional observational study was conducted from 15 March 2019 to 30 February 2020 at Department of Microbiology, BIRDEM General Hospital, Dhaka. Three hospitals were included in the present study which were selected by purposive sampling. Those hospitals were designated as hospital A, hospital B and hospital C in order to maintain the confidentiality of the hospitals. Hospital A is a private general hospital. It is a multidisciplinary hospital complex consisting 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. It consists of 78 beds for in patient management including 8 beds in ICU and 2 Operation theatres. Hospital C is a tertiary level government general hospital consisting of around 1700 beds, 36 ICU beds in 3 ICUs and 12 Operation theatres in the main OT complex. The samples were collected in the following manner.

Operation theatre

In Hospital 'A' 5 OT were selected out of 8 OT.

In Hospital 'B' samples were taken from 2 OT as it consists 2 OT.

In Hospital 'C' 5 OT were selected randomly as every second or third out of 12 OT in the main OT complex in order to keep the sample size same with Hospital 'A'.

So, in total samples were taken from 12 (5+2+5) OTs of three hospitals. From these 12 OTs, samples were taken from floor, walls, door handle, OT light, IV pole, OT table, sucker, anesthesia machine and diathermy machine. OT sandal, instrument trolley, a sterile instrument, fine scissor, sterile OT gown, sterile draping sheet and sterile kidney tray were collected as single sample from each hospital.

As OT floor, light source and table were routinely cleaned, here the samples were taken half an hour before and after routine cleaning.

Intensive Care Unit

Sites for sample collection were chosen as frequently or high touch surfaces, such as bedrail, over bed table, IV pole and stethoscope. As Hospital 'A' and 'C' ICU were 21 and 20 bedded respectively so samples were taken from every 3rd bed to cover the whole ICU.

Along with the above mentioned 4 site samples were also taken from wall, floor and sink. As the floor was only routinely cleaned, here the samples were taken half an hour before and after routine cleaning. Hand and nasal swabs were also taken from the on duty ICU staffs to see the rate of colonization.

Bacteriological Sampling and Culture. A total 318 bacteriological samples were collected for analysis from three tertiary care hospitals. Among those 232 were

inanimate surface samples and 86 human samples from ICU staffs only. Out of 232 inanimate surface samples 91, 50 & 91 samples each were taken from Hospital 'A', 'B' and 'C' respectively. Among the 86 human samples 43 were hand swabs and 43 were nasal swabs. Sixteen hand swabs and 16 nasal swabs were taken from Hospital 'A', 12 hand and 12 nasal swabs were taken from Hospital 'B' and 15 hand and 15 nasal swabs were taken from Hospital 'C'.

Inanimate surfaces to be sampled were screened by sterile cotton swabs by two methods: (I) Non-enrichment method (II) Enrichment method.

Collection of hand and nasal swabs

For hand and nasal swab of ICU staffs, sterile cotton swabs were moistened in 5 ml of 0.9% sterile normal saline solution and rubbed over the hand (specially the finger webs and nails) and anterior nares. Then brought to the laboratory and inoculated in Blood & MacConkey agar media and incubated overnight into an incubator at 37°C. The isolated organisms from different samples were identified by standard microbiological techniques such as colony morphology, microscopic features and standard phenotypic characters.

Antimicrobial Susceptibility Testing. Antimicrobial susceptibility profile of the isolates was performed based on the Kirby-Bauer agar disc diffusion method. The suspension of the identified test organism was prepared from similar colonies. The densities of suspension were determined by comparing with McFarland 0.5 Barium sulfate solutions²⁶.

Data Analysis. All data were entered, cleaned, and analyzed using Statistical Software Package for Social Sciences (SPSS) version 23 (SPSS Inc., Chicago, IL, USA) for Windows. Generated data were compiled and presented using descriptive statistics.

Results

Table 3.1 shows the distribution of surface samples according to the collection sites in ICUs and OTs respectively. From the total 232 inanimate surface samples 87 were collected from the three ICUs and 145 were collected from the three OTs.

Table 3.1: Distribution of inanimate surface samples taken from three ICUs and three OTs according to collection site.

Sample collection site	Frequency of samples No. (%)		
	Hospital 'A'	Hospital 'B'	Hospital 'C'
ICU samples			
Bedrail (n = 21)	8 (38.1)	5 (23.8)	8 (38.1)
Over bed table (n = 19)	7 (36.8)	5 (26.3)	7 (36.8)
IV pole (n = 18)	7 (38.9)	4 (22.2)	7 (38.9)
Stethoscope (n = 14)	5 (35.7)	4 (28.6)	5 (35.7)
Floor (n = 8)	3 (37.5)	2 (25.0)	3 (37.5)
Wall (n = 4)	1 (25.0)	2 (50.0)	1 (25.0)
Sink (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
Total (N = 87)	31 (35.6)	25 (28.7)	31 (35.6)
OT samples			
Wall (n = 12)	5 (41.7)	2 (16.6)	5 (41.7)
Floor (n = 24)	10 (41.7)	4 (16.6)	10 (41.7)
OT light (n = 12)	5 (41.7)	2 (16.6)	5 (41.7)
IV pole (n = 12)	5 (41.7)	2 (16.6)	5 (41.7)
OT table (n=12)	5 (41.7)	2 (16.6)	5 (41.7)
Door handle (n =12)	5 (41.7)	2 (16.6)	5 (41.7)
Sucker (n =12)	5 (41.7)	2 (16.6)	5 (41.7)
Anaesthesia machine (n =12)	5 (41.7)	2 (16.6)	5 (41.7)
Diathermy machine (n =12)	5 (41.7)	2 (16.6)	5 (41.7)
Sink (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
OT sandal (n =3)	1 (33.33)	1 (33.33)	1 (33.33)
Instrument trolley (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
Sterile instrument (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
Fine scissor (n = 2)	1 (50.0)	Nil	1 (50.0)
OT gown (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
Sterile draping sheet (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
Sterile kidney tray (n = 3)	1 (33.33)	1 (33.33)	1 (33.33)
0.2% Glutaraldehyde (n = 2)	1 (50.0)	Nil	1 (50.0)
Total (N = 145)	59 (40.7)	27 (18.6)	59 (40.7)

Table 3.2 shows the mean (\pm SD) bacterial load from the inanimate surface samples in the three ICUs and OTs. Among the ICUs, the highest bacterial load was found on the sink of Hospital 'A' (155.00 ± 0.00 CFU/cm²) and the lowest load was on the stethoscope of Hospital 'B' (1.10 ± 0.26 CFU/cm²). Among the OTs, highest load was found on the OT sandal of Hospital 'A' (68.00 ± 0.00 CFU/cm²) and the lowest load was found on the IV pole of Hospital 'B' (0.70 ± 0.28 CFU/cm²). No growth was found on the sterilized equipment in all the OTs.

Table 3.2: Mean bacterial load & hygiene status of inanimate surface samples in three ICUs and OTs.

Sample sites	Mean(\pm SD) of Bacterial load in CFU/cm ² (Hygiene status)			ANOVA test p-value
	Hospital 'A'	Hospital 'B'	Hospital 'C'	
ICU samples				
Bed rail	6.40 \pm 0.97 (UA)	4.72 \pm 0.37 (A)	7.56 \pm 0.76 (UA)	A vs B = 0.005 A vs C = 0.027 B vs C = <0.001
Over bed table	6.62 \pm 0.53 (UA)	4.94 \pm 0.57 (A)	5.84 \pm 2.22 (UA)	A vs B = 0.182 A vs C = <0.001 B vs C = <0.001
IV pole	6.06 \pm 0.46 (UA)	3.00 \pm 0.63 (A)	6.21 \pm 0.39 (UA)	A vs B = 0.013
Stethoscope	2.84 \pm 0.30 (A)	1.10 \pm 0.26 (A)	2.12 \pm 1.15 (A)	Not done
Wall *	5.40 (UA)	3.80 (A)	5.90 (UA)	Not done
Sink *	155.00 (UA)	110.00 (UA)	120.00 (UA)	Not done
OT samples				
Wall	4.16 \pm 0.48 (A)	4.50 \pm 0.42 (A)	6.30 \pm 0.59 (UA)	A vs C = <0.001 B vs C = 0.008
IV pole	4.92 \pm 0.99 (A)	0.70 \pm 0.28 (A)	6.50 \pm 1.41 (UA)	A vs B = 0.005 B vs C = 0.001
Door handle	4.92 \pm 0.99 (A)	3.50 \pm 0.71 (A)	7.64 \pm 0.95 (UA)	A vs C = 0.004 B vs C = 0.002
Sucker	5.40 \pm 0.94 (UA)	0.00 (A)	16.40 \pm 12.79 (UA)	B vs C = 0.143
Anaesthesia machine	10.28 \pm 14.45 (UA)	2.00 \pm 0.57 (A)	7.18 \pm 0.72 (UA)	A vs B = 0.995
Diathermy machine	6.64 \pm 0.74 (UA)	4.85 \pm 1.06 (A)	7.54 \pm 1.06 (UA)	A vs B = 0.995
Sink *	48.00 (UA)	33.10 (UA)	66.00 (UA)	Not done
OT Sandal *	68.00 (UA)	48.00 (UA)	60.00 (UA)	Not done
Instrument trolley *	3.20 (A)	0.00 (A)	5.80 (UA)	Not done
Sterile instrument *	0	0	0	Not done
Fine scissors *	0	-	0	Not done
OT gown *	0	0	0	Not done
Sterile draping sheet *	0	0	0	Not done
Sterile kidney tray *	0	0	0	Not done
0.2% Glutaraldehyde *	0	-	0	Not done

- Acceptable bacterial load on inanimate surface sample is <5 CFU/cm² [28]
- UA = Unacceptable, A = Acceptable
- * Statistical analysis of bacterial load were not done due to single sample size from each hospital.

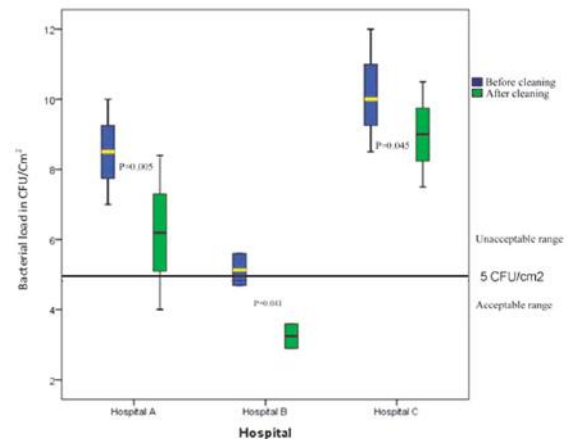


Figure 3.1: Box and Whisker plot shows the observation of bacterial load on ICU floor before and after routine cleaning. In all three hospitals, reduction of bacterial load was found statistically significant ($p < 0.05$) but the burden remained in unacceptable range (> 5 CFU/cm²) in Hospital 'A' & 'C'.

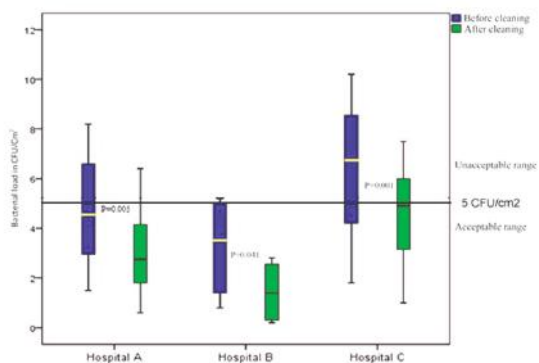


Figure 3.2: Box and Whisker plot shows the observation of bacterial load on inanimate surfaces (floor, light and table) before and after routine cleaning. Here reduction of bacterial load was found statistically significant ($p < 0.05$) and in acceptable range ($< 5 \text{ CFU/cm}^2$) in all three hospitals.

Out of 87 ICU inanimate surfaces, growth was found in 74.7% (65) samples. Highest growth positive inanimate surfaces were floor, wall & sink (100%). And out of 145 OT inanimate surfaces, growth was found in 70.3% (102) samples. Highest growth positive inanimate surfaces were OT sandal & sink (100%). *Micrococcus* spp. was found highest among the ICUs and OTs. Details of specimen and bacterial isolates are depicted in Table 3.3 & 3.4. Mixed bacterial flora were isolated from various sites. No organisms were found on sterile instruments, fine scissor, sterile kidney tray, sterile OT gown, draping sheet and from in use 0.2% Glutaraldehyde. Isolation rate of gram negative organism was significantly higher in ICU of Hospital ‘A’ & ‘C’, whereas frequency of gram positive bacteria was significantly more in OTs of Hospital ‘A’ ($p < 0.001$) & Hospital ‘C’ ($p < 0.001$) (Table 3.5). Potentially pathogenic organisms were found significantly more ($p < 0.001$) in Hospital ‘A’ & ‘C’ ICU, where in all OTs non-pathogenic organisms were found more (Table 3.6)

Table 3.3: Frequency of organisms isolated from different collection sites of ICU environments in three hospitals

Sampling Sites	No. of isolates (n)	No. (%) of gram positive isolates						No. (%) of gram negative isolates					
		MSSA	MRSA	CoNS	<i>Bacillus</i> spp.	<i>Micrococcus</i> spp.	<i>Gr. D</i> <i>Enterococcus</i>	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Enterobacter</i> spp.	<i>Pseudomonas</i> spp.	<i>Flavobacterium</i> spp.	<i>Acinetobacter</i> spp.
Inanimate surfaces													
Bedrail	46	1 (2.2)	2 (4.3)	2 (4.3)	5 (10.9)	6 (13.04)	1 (2.2)	2 (4.3)	4 (8.7)	3 (6.52)	9 (19.6)	2 (4.3)	9 (19.6)
Over bed table	34	0	0	0	4 (11.8)	7 (20.6)	0	0	5 (14.7)	0	10 (29.4)	0	8 (23.5)
IV pole	21	1 (4.76)	0	0	2 (9.5)	5 (23.8)	0	2 (9.5)	2 (9.5)	0	2 (9.5)	1 (4.76)	6 (28.6)
Stethoscope	15	1 (6.67)	0	0	2 (13.3)	4 (26.7)	0	1 (6.67)	0	0	2 (13.3)	0	5 (23.8)
Floor	16	1 (6.25)	1 (6.25)	0	1 (6.25)	5 (31.25)	0	0	0	0	2 (12.5)	1 (6.25)	5 (31.25)
Wall	13	0	0	0	3 (23.1)	3 (23.1)	0	0	0	0	2 (15.4)	5 (38.5)	0
Sink	11	0	0	0	1 (9.1)	4 (36.4)	0	2 (18.2)	1 (9.1)	0	3 (27.8)	0	0
Total	N = 156	4 (2.56)	3 (1.92)	2 (1.28)	18 (11.5)	34 (21.8)	1 (0.6)	7 (4.49)	12 (7.69)	3 (1.92)	30 (19.2)	9 (5.76)	33 (21.2)

- CoNS = Coagulase negative *Staphylococcus*, MRSA = Methicillin resistant *Staphylococcus aureus*, MSSA = Methicillin sensitive *Staphylococcus aureus*

Table 3.4: Frequency of organisms isolated from different collection sites of OT environments in three hospitals

Sampling Sites	No. of isolates (n)	No. (%) of gram positive isolates						No. (%) of gram negative isolates					
		MSSA	MRSA	CoNS	<i>Bacillus</i> spp.	<i>Micrococ</i> spp.	<i>Gr. D</i> <i>Entero</i> <i>coccus</i>	<i>E.coli</i>	<i>Klebsie</i> <i>lla</i> spp.	<i>Entero</i> <i>bacter</i> spp.	<i>Pseudo</i> <i>monas</i> spp.	<i>Flavobac</i> <i>terium</i> spp.	<i>Acinet</i> <i>obacter</i> spp.
Inanimate surface													
Wall	21	2 (9.5)	0	1 (4.76)	7 (33.33)	8 (38.1)	0	0	0	0	1 (4.76)	2 (9.5)	0
Floor	26	0	0	1 (3.85)	5 (19.23)	6 (23.1)	0	2 (7.7)	0	5 (19.2)	1 (3.85)	2 (7.7)	4 (15.4)
IV stand	17	0	0	0	3 (17.6)	10 (58.8)	0	0	0	0	0	1 (5.9)	3 (17.6)
OT table	14	2 (14.3)	0	0	3 (21.4)	7 (50.0)	0	0	0	0	1 (7.14)	1 (7.14)	0
Door handle	22	3 (13.6)	0	0	5 (22.7)	9 (40.9)	0	0	0	0	1 (4.54)	4 (18.2)	0
Sucker	21	0	0	0	3 (14.3)	6 (28.6)	2 (9.5)	0	0	0	2 (9.5)	5 (23.8)	3 (14.3)
Anaesthesia machine	18	0	0	0	5 (27.8)	8 (44.4)	2 (11.1)	0	0	0	0	3 (16.6)	0
Diathermy machine	17	0	0	0	5 (29.4)	8 (47.1)	0	0	0	0	2 (11.7)	2 (11.7)	0
Sink	20	0	0	0	0	4 (20)	0	3 (15)	0	0	7 (35)	6 (30)	0
OT sandal	31	1 (3.22)	0	2 (6.4)	5 (16.1)	8 (25.8)	0	1 (3.22)	4 (12.9)	4 (12.9)	3 (9.7)	3 (9.7)	0
Instrument trolley	10	0	0	0	2 (20)	8 (80)	0	0	0	0	0	0	0
Total	217	8 (3.68)	0	4 (1.84)	43 (19.8)	82 (37.8)	4 (1.84)	6 (2.76)	4 (1.84)	9 (4.14)	18 (8.3)	29 (13.4)	10 (4.6)

- CoNS = Coagulase negative *Staphylococcus*, MRSA = Methicillin resistant *S. aureus*, MSSA = Methicillin sensitive *S. aureus*
- Sterile instruments, draping sheet, kidney tray, OT gown, 0.2% Glutaraldehyde were not shown separately due to spaces & culture revealed no growth.

Table 3.5: Rate of isolation of gram reactive organisms in ICUs & OTs of different hospitals

High risk area	Rate of isolation No. (%)		Chi-square test p-value
	gram negative	gram positive	
Hospital 'A'			
ICU (n = 55)	36 (65.4)	19 (34.6)	<0.001
OT (n = 67)	19 (28.3)	48 (71.7)	<0.001
Hospital 'B'			
ICU (n = 48)	18 (37.5)	30 (62.5)	0.920
OT (n = 44)	14(31.8)	30 (68.2)	0.632
Hospital 'C'			
ICU (n = 5)	40 (75.5)	13 (24.5)	0.001
OT (n =106)	43 (40.6)	63 (59.4)	0.001

Table 3.6: Frequency of pathogenic & non-pathogenic bacteria in three ICUs & OTs of three different hospitals

High risk area	No. (%) of bacteria		Chi-square test p-value
	Potentially pathogenic	Non-pathogenic	
Hospital 'A'			
ICU (n=56)	39 (69.6)	17 (30.4)	<0.001
OT (n=68)	21 (30.9)	47 (69.1)	<0.001
Hospital 'B'			
ICU (n=46)	15 (32.6)	31 (67.4)	0.231
OT (n=43)	15 (34.8)	28 (65.2)	0.256
Hospital 'C'			
ICU (n=54)	48 (88.9)	6 (11.1)	<0.001
OT (n=106)	44 (41.5)	62 (58.5)	<0.001

Frequency of bacterial colonization among the ICU staffs are shown in table 3.7. In hand swabs, 20% *S. aureus* was found in Hospital 'A', *Enterobacteriaceae* was found 20% in Hospital 'A' and 42.9% in Hospital 'C', *Pseudomonas* spp. was found 20% in Hospital 'A'. MRSA was detected only 11.8% from nasal swab of Hospital 'C' ICU staffs.

Table 3.7: Frequency of bacterial colonization in hand & nose of ICU staffs of three hospitals

Site of sample	Category of bacteria	Frequency of bacterial isolates No. (%)		
		Hospital 'A'	Hospital 'B'	Hospital 'C'
Hand swab	<i>S. aureus</i>	1 (20.0)	0	0
	CoNS	0	0	0
	MRSA	0	0	0
	<i>Enterobacteriaceae</i>	1 (20.0)	0	3 (42.9)
	<i>Pseudomonas</i> spp.	1 (20.0)	0	0
	Non-pathogenic	2 (40.0)	6 (100)	4 (57.1)
Total		5	6	7
Nasal swab	<i>S. aureus</i>	2 (22.2)	2 (25.0)	4 (23.5)
	CoNS	2 (22.2)	2 (25.0)	3 (17.6)
	MRSA	0	0	2 (11.8)
	<i>Enterobacteriaceae</i>	2 (22.2)	0	4 (23.5)
	<i>Pseudomonas</i> spp.	0	0	1 (5.9)
	Non-pathogenic	3 (33.3)	4 (50.0)	3 (17.6)
Total		9	8	17

The antimicrobial drug resistance patterns of isolated *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas* spp. and *Staphylococcus* spp. from environment of ICUs & OTs of three hospitals are depicted in Table 3.8. Based on antibiogram of 3rd generation Cephalosporins, Amoxiclav and Aztreonam, approximately 53.3%, 40.0% & 71.4% were detected as ESBL producers in Hospital 'A', 'B' & 'C' respectively. Methicillin resistant *Staphylococcus aureus* (MRSA) were found in ICU of Hospital 'A' (16.7%) & Hospital 'C' (30.0%).

Table 3.8: Antimicrobial drug resistance patterns of isolated *Enterobacteriaceae*, *Acinetobacter* spp., *Pseudomonas* spp. and *Staphylococcus* spp. from environment of ICUs & OTs of three hospitals

Antibiotics tested	<i>Enterobacteriaceae</i>			<i>Pseudomonas</i> spp.			<i>Acinetobacter</i> spp.			<i>S. aureus</i>		
	Hos 'A'	Hos 'B'	Hos 'C'	Hos 'A'	Hos 'B'	Hos 'C'	Hos 'A'	Hos 'B'	Hos 'C'	Hos 'A'	Hos 'B'	Hos 'C'
	n = 15	n = 5	n = 21	n = 15	n = 8	n = 25	n = 18	n = 4	n = 21	n = 6	n = 5	n = 10
Ceftriaxone	8(53.33)	2(40.0)	15(71.42)	-	-	-	14(77.78)	2(50.0)	16(76.19)	-	-	-
Ceftazidime	8(53.33)	2(40.0)	15(71.42)	9(60.0)	4(50.0)	14(56.0)	14(77.78)	2(50.0)	16(76.19)	-	-	-
Cefotaxime	8(53.33)	2(40.0)	15(71.42)	-	-	-	14(77.78)	2(50.0)	16(76.19)	-	-	-
Cefixime	8(53.33)	2(40.0)	15(71.42)	-	-	-	14(77.78)	2(50.0)	16(76.19)	-	-	-
Amoxiclav	8(53.33)	2(40.0)	15(71.42)	-	-	-	14(77.78)	2(50.0)	16(76.19)	2(33.3)	1(20.0)	2(20.0)
Piperacillin-Tazobactam (PIT)	3(20.0)	0	6(28.57)	6(40.0)	3(37.5)	12(48.0)	14(77.78)	2(50.0)	15(71.42)	-	-	-
Aztreonam	8(53.33)	2(40.0)	15(71.42)	7(46.67)	2(25.0)	13(52.0)	14(77.78)	2(50.0)	16(76.19)	-	-	-
Imipenem	1(6.67)	0	4(19.05)	7(46.67)	4(50.0)	14(56.0)	14(77.78)	2(50.0)	15(71.42)	-	-	-
Amikacin	2(13.33)	0	6(28.57)	5(33.33)	2(25.0)	12(48.0)	14(77.78)	2(50.0)	15(71.42)	0	0	1(10.0)
Gentamicin	2(13.33)	0	6(28.57)	5(33.33)	2(25.0)	13(52.0)	14(77.78)	2(50.0)	15(71.42)	0	0	1(10.0)
Ciprofloxacin	4(26.67)	1(20.0)	4(19.05)	6(40.0)	3(37.5)	12(48.0)	14(77.78)	2(50.0)	15(71.42)	1(16.67)	1(20.0)	2(20.0)
Cotrimoxazole	2(13.33)	1(20.0)	5(23.81)	12(80.0)	6(75.0)	20(80.0)	14(77.78)	2(50.0)	16(76.19)	1(16.67)	1(20.0)	2(20.0)
Cefoxitin	-	-	-	-	-	-	-	-	-	1(16.67)	0	3(30.0)
Oxacillin	-	-	-	-	-	-	-	-	-	1(16.67)	0	3(30.0)

Discussion

To prevent the consequences and to reduce the microbial burden maintaining environmental hygiene is a must. Monitoring environmental hygiene by the microbiological testing of surfaces and equipment is useful to detect changing trends of types and counts of microbial flora. With this background this study was undertaken to observe environmental hygiene in three tertiary care hospitals of Dhaka city by quantitative and qualitative assessment of microbial burden.

In any hospital setting, air, water, medical devices dry surfaces as well as healthcare workers all act as potential sources of contamination and infection. In this study, sampling of the hospital environment was done from frequently touched inanimate surfaces from ICU and OT; nasal and hand swab samples from ICU staffs were also taken to identify the source of infection and carrier of MDR organism.

From the frequently or high touched inanimate surfaces, swab samples were taken from unit surface area of the three hospitals. The mean bacterial burden on inanimate surfaces of ICUs (Table 3.2) was found 481.35 CFU/cm² and of OTs was found 442.64 CFU/cm². Highest burden was observed over the sinks (117 - 385 CFU/cm²), OT sandals (176 CFU/cm²) and ICU bedrails (18.68 CFU/cm²). No published data regarding colony count from samples of sinks of ICU and OT were available to compare with the findings of the present study. Heavy contamination of bedrails was reported by Shams et al²⁷. and Dancer et al²⁸. Heavy microbial burden (1,112-5,198 CFU/100 cm²) was also reported by Schmidt et al²⁹. on plastic bed rails even after cleaning. The findings of Wirtanen et al³⁰. of high microbial burden on the OT sandals coincides with this study. Overall the findings of bacterial burden on high touched inanimate surfaces of Hospital 'A' & 'C' higher than Hospital 'B' was statistically significant. It appears that the infection control measures are in good practice in Hospital 'B' which is a private monodisciplinary specialized cardiac hospital than the other two multidisciplinary private and government general hospitals.

In this study total 232 environmental swab samples (ICU & OT) were taken, among which approximately 75% samples were found culture positive & this was close to the findings (69.6%) to a study conducted by Rozonska et al³¹. In another study, 51% of the environmental samples were found positive with different bacterial species in ICU samples³².

Among the isolates of ICU, Hospital 'A' and 'C' have predominant gram negative isolates (Table 3.5) which was statistically significant. Similar findings were reported by Jadhav et al³³ that gram negative bacteria constituted the dominant colonized bacteria compared with gram positive cocci among the bacteria isolated from respiratory devices (68.85% versus 31.14%) in Maharashtra, India. On the other hand, Hospital 'B' ICU has a predominance of gram positive organisms. Similar rates (60.7% Vs. 39.3%) was found by Tajeddin et al³². in a hospital of Tehran. However, in OTs the scenario was reverse where gram positive isolates were detected more than gram negative isolates in all the three hospitals-71.7% vs. 28.3% in Hospital 'A', 68.2% vs 31.8% in Hospital 'B' & 59.4% vs. 40.6% in Hospital 'C'. This finding correlate with Kiranmai³⁴ in Telangana, India.

In OTs of three hospitals the isolated organisms were predominantly non-pathogenic (58.7-65.9%) (Table 3.6). Among the non-pathogens (Table 3.4) 37.8% were *Micrococcus* spp. (normal flora) and 19.8% were *Bacillus* species (contaminants). The findings of this study was in agreement with the study of Kiranmai³⁴ in Telangana, India that reported predominance of non-pathogenic organisms-*Bacillus* spp. 45% (contaminants) and *Micrococcus* spp. 33% (normal flora). In this study, among the pathogens, rate of isolation was highest for *Flavobacterium* spp. (13.4%) followed by *Pseudomonas* spp. (8.3%) and *Acinetobacter* spp. (4.6%). No MRSA was detected from OT samples in this study.

In ICUs of three hospitals the isolated organisms were predominantly pathogenic. In Hospital 'A' ICU (69.6%) & Hospital 'C' ICU (88.9%) pathogenic organisms were detected (Table 3.6). On the other hand, non-pathogens were found more (67.4%) in Hospital 'B' ICU. In terms of isolation of organisms from ICU samples, the ICU of Hospital 'B' (private monodisciplinary cardiac specialized hospital) appeared less contaminated than the other two hospitals. In ICUs among the isolated pathogens, *Acinetobacter* spp. was the predominant isolate (21.2%) followed by *Pseudomonas* spp. (19.2%), *Klebsiella* spp. (7.7%) & *S.aureus* (4.5%). And among the non-pathogens, *Micrococcus* spp. was predominant (21.8%) followed by *Bacillus* spp. (11.5%) & Coagulase negative *Staphylococcus* (1.3%) (Table 3.3). This finding was in accordance with the findings of Huang et al³⁵. in Taiwan.

Health care workers' (HCW) hands are major sources of transmission of nosocomial pathogens³⁶. Culture of 41.9% hand & 79.1% nasal swab of ICU staffs yielded growth (Table 3.7). Among the hand swabs, majority were *Micrococcus* spp. (66.7%) which were non-pathogens. In Hospital 'B' no pathogens were found from the hands of the staffs. In rest two hospitals, *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Pseudomonas* spp. and *S. aureus* were found in a range of 5-11%. Waters et al³⁶. reported that 30% of hospital employees' hands were persistently colonized by Gram negative bacilli, including *Acinetobacter*, *Klebsiella*, *Enterobacter*, *S. marcescens*, and *E. coli*. In a study by Khodavaisy et al³⁷. higher rates of colonization of *Klebsiella* spp. (7.9%), *Enterobacter* spp. (4.7%), *E. coli* (3.9%), *Acinetobacter* spp. (3.1%) and *Pseudomonas* spp. (2.3%) were reported among HCWs' hands. Among the nasal swabs, most of the colonization was found in Hospital 'C' which includes 11.8% of MRSA, 5.9% of *Pseudomonas* spp. and 23.5% of *Enterobacteriaceae*. The isolation rate of *S. aureus* (22.2-25%) and CoNS (17.6-25%) was almost similar among the three hospitals. In this study the rate of nasal colonization with *S. aureus* was found in agreement with 21.7% reported by Satpathi et al³⁸. Frequency of bacterial colonization was found higher in Hospital 'C' than Hospital 'A' & 'B'.

Conclusion

The inanimate surfaces of ICU environment revealed unacceptable microbial burden in Hospital 'A' & 'C'. Nasal & hand swabs of Hospital 'C' ICU staffs' were also found moderately heavy colonization with MDR pathogens like MRSA. Unacceptable range of coliform organisms were found in all three ICUs. The OT environment was also found grossly contaminated though here majority of organisms were found non-pathogens. Antimicrobial resistance pattern was found high up among the pathogens. Highest resistance was observed among the *Acinetobacter* isolates which was found in the ICU environment.

The overall mean bacterial load & hygiene status of high risk areas (ICU & OT) were detected better in the private monodisciplinary specialized cardiac hospital (Hospital 'B') than multidisciplinary private general hospital (Hospital 'A') & multidisciplinary government general hospital (Hospital 'C'). It might be due to difference in implementation and practice of infection control protocol in respective hospitals. Using the results of this study, an initiative should be undertaken for establishing regular cleaning of the hospital environment to decrease the microbial burden especially in high risk areas.

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