

## A study on Microbial contamination and susceptibility of Hospital Theatres -at Tertiary care centre

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### Abstract

**Background:** Microbial contamination of hospital environment especially the operating theatre has continued to increase the prevalence of nosocomial infection; it leads to the effect of high morbidity and mortality rate among patients admitted for surgery

**Aim & Objective:** To study the level of contamination of air, surfaces and equipment in Operation Theatres of Osmania General Hospital - a tertiary care hospital in Hyderabad

**Methodology:** The present study was done in all operation theatres of OGH.

**Results:** Total number of samples collected was 360, of which 120 air samples and 240 surface samples. Twenty one open plates (17.5%) were found bacterial load (TVC) above the acceptable limit. Gram positive organisms were predominant (78.33%). CoNS was the most common isolate (28.84%). Most of the Gram positive organisms were sensitive to vancomycin. Total number of Enterococci isolated were 7(1.82%), of which vancomycin resistant Enterococci were 2(0.53%). Gram negative organisms were *E.coli*, *P.aeruginosa*, *Acinetobacter* and *Klebsiella* species. All of them were sensitive to imipenem.

**Conclusion:** Finally our study concluded that, there was a microbial contamination of hospital environment, In order to reduce it suggestions were given to the theatre personnel to reduce the foot traffic in and out of the operating rooms. And Sensitization programmes for medical, nursing and sanitary personnel every week at different timings were suggested.

**Keywords:** Contamination, Enterococci, Acinetobacter, *E.coli*, Imipenem

## INTRODUCTION

Microbial contamination of hospital environment especially the operating theatre has continued to increase the prevalence of nosocomial infection, <sup>[1],[2],[3]</sup> with resultant effect of high morbidity and mortality rate among patients admitted for surgery.<sup>[4]</sup>

In hospital setting, reduction of microbial contamination in fact depends primarily on improved cleaning and proper disinfection of the hospital environment, especially high risk areas, as these measures are crucial to stemming down dissemination of these microbial contaminations. <sup>[5]</sup> Important bacterial pathogens can be transmitted by air, droplet and direct contact with contaminated surfaces, also the patients, the equipment and operation team, gowns, gloves with holes and contaminated tools.<sup>[6],[7]</sup>

Multiple reservoirs have been reported as being responsible for hospital contamination particularly the operating theatre including unfiltered air, ventilation systems and antiseptic solutions. Medical staff still represents an exogenous contaminant source in operating theatres and personnel move back and forth between the operating theatres and other parts of the hospital without changing their gowns or slippers. Moreover, patients are not consistently cleaned or shaved before coming to the operating theatre. All these factors play a role in contamination of operating theatres and consequent post operative infections<sup>[8]</sup>

The impact of these sources on the degree of bacterial contamination differs depending on the numbers of bacterial pathogens involved. Similarly the level of contamination can be reduced, if high level of hospital hygiene is adhered to by health care worker.

Microbiological testing methods are a simple and reliable means of identifying the microbial flora that inhabit or contaminate theatre air, surfaces and equipment. Although sampling is only used as-needed basis in theatres, regular sampling is generally not recommended by regulatory agencies. The microbiological air quality in operating rooms may be considered a mirror of the hygienic condition of the operating room.<sup>[9]</sup>

The present study was conducted to determine the degree of bacterial contamination of operating rooms in Osmania General Hospital, with respect to acceptable bacterial load standards and measure antimicrobial susceptibility pattern of the isolates.

To study the level of contamination of air, surfaces and equipment in operation theatres of Osmania General Hospital - at tertiary care hospital in Hyderabad.

## **MATERIAL AND METHODS**

### **Study Centre:**

The study was conducted in all operation theatres in the Osmania General Hospital, Afzalgunj, Hyderabad. It is a tertiary care hospital and major referral centre for other hospitals in Hyderabad. It has 1000 bed capacity.

### **Study Period:**

January to July 2012.

The criteria of selection of the study sites were based on the fact that these units (OTs) are expected to be sterile. The main operating theatre consists of two operating rooms, one recovery room, patient reception room and staff change room.

The aim of this study was to assess the degree of contamination of the hospital operation theatres.

Total number of operation theatres in OGH - 8

1. General surgery OT
2. Gastroenterology OT
3. Urology OT
4. Orthopaedic OT
5. Cardiothoracic OT
6. Neurology OT
7. Skin and plastic surgery OT

## 8. Emergency OT

Of these theatres identified for the study, air, the floors, walls, operating tables, airvents /conditioners and the equipments present in the theatres were sampled for analysis.

In each room sampling was performed at the time of beginning of surgical activity i.e 9 am to 10 am. Samples were collected once in a week from each theatre i.e every Monday [Fumigation was done on saturday evening]. From each theatre samples were collected once in two months. Sampling was done repeatedly 4 times in General surgery, Gastroenterology, Urology, Orthopedics, Cardiothoracic and Neurology operation theatres and 3 times in Skin and Plastic surgery, Emergency operation theatres.

### **Sample collection:**

Two sampling procedures used in the study were Open plate method and Swabbing as described by Javed et al., (2008).<sup>[10]</sup>

### **Settle Plate/Open Plate method /Passive sampling:**

Passive sampling was performed to determine the Index of Microbial Air contamination (IMA). This index corresponds to the number of Colony Forming Unit (CFU) counted on a Petri dish with a diameter of 9 cm placed according to the 1/1/1 scheme (1m above from the floor, 1m away from the wall and for 1 hour). In the present study the IMA plates (one Blood Agar Plate for Total Viable Count and one plain Sabourads Dextrose Agar plate with chloramphenicol for filamentous fungi) were placed in each site of the operating theatre. Total 4 air samples were collected at each time. One from 1m distance from the operating table, second from near the entrance and the remaining two samples from 1m away from the wall of two corners.

After exposure for one hour which allows sufficient time for settling of particles in natural and efficient manner, plates were covered with their lids and taken to laboratory in sealed plastic bags and incubated at 37<sup>0</sup>c for 48 hours. After incubation, the colonies were counted, colony characters were observed and further identification and confirmation was done by standard identification tests [11].The results were expressed in CFU/dm<sup>2</sup>/hr.

### **Swabbing method:**

Sterile cotton swab sticks (Hi media) were used to swab the surface of equipment/ materials used in operating theatres.

In the present study 8 sites were chosen for surface swabbing

1. Head end of the Operation Table
2. Boyle's apparatus
3. Floor at head end of the table
4. Head lamp
5. Suction apparatus
6. Instrument Trolley
7. Fan
8. Wall near to the electric switches

All surface swabs are collected in duplicate. Immediately after collection, one swab is dipped into Brain Heart Infusion broth for aerobic organisms and another swab into the Robertson's Cooked Meat

medium for anaerobic spores.

### Processing of samples:

#### Swab:

1. Tubes containing BHI broth with surface swabs were incubated at 37<sup>0</sup>c for 24 hours.
2. Any turbidity indicated the growth of organisms.
3. Samples found turbid were streaked on Blood agar and MacConkey agar and incubated the plates at 37<sup>0</sup>c for 24 hours.
4. After streaking, the swab was used for preparation of direct smear and stained by Jenson's modification of Gram's Method. After allowing it to air dry, the smears were screened under oil immersion objective to note the morphological features of the bacteria.
5. After overnight incubation, surface swabbing plates, were examined for bacterial growth.
6. Further identification and confirmation of organisms was done by the standard identification tests.

[11]

## RESULTS

The present study was undertaken in various operation theatres in Osmania General Hospital, Hyderabad to evaluate the level of contamination, rate of isolation of bacteria and fungi.

**Table 1 : Total Bacterial Samples**

Total number of samples collected	360
Air samples	120
surface samples	240

**Table 2 : Aerobic bacterial load in operation theatres from air samples**

No.	Theatre	Samples Collected	No of settle plates which have found			
			<25 cfu		>25 cfu	
			No	%	No	%
1	General Surgery	16	10	62.5	6	37.5
2	Gastro enterology	16	12	75	4	25
3	Urology	16	15	93.75	1	6.25
4	Orthopedics	16	12	75	4	25

5	Cardiothoracic	16	16	100	0	0
6	Neurology	16	16	100	0	0
7	Skin&Plastic Surgery	12	11	91.67	1	8.34
8	Emergency	12	7	58.34	5	41.67

According to C.Pasquarella et al, 2000 [12] maximum acceptable level of colony forming unit in a conventional operation theatre on settle plate of 9cm diameter exposed to air for 1 hr is 25 cfu/dm<sup>2</sup>/hour.

Bacterial load was above the acceptable level in 21 plates (17.5%) and within the acceptable level in 99 plates (82.5%).

**Table 3 : Bacterial isolates in air samples of various theatres**

No.	Theatre	Samples Collected	Organisms Isolated															
			CoNS		S.aureus		E.coli		P.aeruginosa		Acinetobacter sp		Enterococci		Klebsiella pneumoniae		Bacillus spp	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
1	General Surgery	16	13	81.25	-	-	7	43.7	3	18.75	-	-	-	-	-	-	4	25
2	Gastro enterology	16	8	50	9	56.25	4	25	4	25	-	-	-	-	-	-	-	-
3	Urology	16	5	31.25	4	25	3	18.75	7	43.75	2	12.5	-	-	-	-	9	56.25
4	Orthopedics	16	7	43.75	-	-	4	25	8	50	2	12.5	-	-	-	-	3	18.75
5	Cardiothoracic	16	8	50	-	-	-	-	-	-	-	-	2	12.5	-	-	5	31.25
6	Neurology	16	1	6.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Skin Plastic Surgery	12	10	83.34	8	66.67	3	25	-	-	-	-	-	-	-	-	9	75
8	Emergency	12	1	8.34	7	58.34	1	8.34	3	25	9	75	-	-	2	16.67	-	-
	Total	120	53	44.16	28	23.34	12	18.34	25	20.83	13	10.83	2	1.67	2	1.67	30	25

**Table 4 : Rate of isolation of bacteria in air samples**

Organisms	No of Isolates	Rate of Isolation(x/120)	% among Isolates(x/175)
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CoNS	53	44.16	30.28
S.aureus	28	23.34	16
Bacillus spp	30	25	17.14
P.aeruginosa	25	20.83	14.28
E.coli	22	18.34	12.57
Acinetobacter sp	13	10.83	7.42
Klebsiella pneumoniae	2	1.67	1.14
Enterococci	2	1.67	1.14

Total number of air samples collected - 120

Total number organisms isolated - 175

Rate of isolation = organisms isolated/total no. of isolates X 100

Highest rate of isolation was found in CoNS (44.16%). This was followed by S.aureus (23.34%).

**Table 5 : Fungal isolates in air samples of various theaters**

No.	Theatre	Samples Collected	Organisms Isolated							
			A. niger		A. flavus		A. fumigatus		A.terreus	
	Organisms isolated		No	%	No	%	No	%	No	%
1	General Surgery	16	15	93.75	11	68.75	-	-	3	18.75
2	Gastro-enterology	16	16	100	8	50	-	-	9	56.25
3	Urology	16	13	81.25	12	75	-	-	8	50
4	Orthopedics	16	14	87.5	3	18.75	3	18.75	-	-
5	Cardiothoracic	16	15	93.75	-	-	-	-	-	-
6	Neurology	16	1	6.25	-	-	-	-	-	-
7	Skin Plastic Surgery	12	10	83.34	3	25	-	-	-	-
8	Emergency	12	12	100	4	33.34	10	83.34	-	-

Total	120	96	80	41	34.16	13	10.83	20	16.67
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Fungal isolates were found only in air samples. *A.niger* was frequently isolated in all operation theatres. The rate of isolation was 80%. *A.fumigatus* was less frequently isolated. The rate of isolation was 10.83%, which were found only in orthopedics and emergency operation theatres.

**TABLE 6 : Species of fungal isoltes from air samples**

Fungal Isolates	No o f Isolates	Rate of Isolation X/120	% among Isolates X/175
<i>A. niger</i>	96	80	56.47
<i>A. flavus</i>	41	34.16	24.11
<i>A. fumigatus</i>	13	10.83	7.64
<i>A.terreus</i>	20	16.67	11.76

Total number of air samples collected - 120

Total number of Fungi isolated - 175

The rate of isolation was maximum in *A.niger* (80%)

**Table 7: Bacterial isolates in surface samples of various theatres**

No.	Theatre	Samples Collected	Organisms Isolated													
			CoNS		S.aureus		Bacillus		E.coli		Klebsiella pneumoniae		Enterococci		Clostridium sp	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%
1	General Surgery	32	8	25	7	21.87	9	28.12	5	15.62	-	-	-	-	1	3.12
2	Gastroenterology	32	9	28.12	8	25	8	25	5	15.62	-	-	2	6.25	-	-

3	Urology	32	6	18.75	6	18.75	5	15.62	1	34.37	-	-	1	3.12	-	-
4	Orthopedics	32	8	25	6	18.75	1	34.37	1	3.125	-	-	-	-	-	-
5	Cardiothoracic	32	5	15.62	5	15.62	6	18.75	-	-	-	-	-	-	-	-
6	Neurology	32	5	15.62	3	9.37	1	0	-	-	-	-	-	-	-	-
7	Skin Plastic Surgery	24	6	25	5	20.84	5	-	-	-	-	-	-	-	-	-
8	Emergency	24	7	29.17	7	29.17	9	37.5	3	12.5	1	4.17	2	1.34	-	-
	Total	240	54	22.5	47	19.58	63	26.25	25	10.4	1	0.41	5	2.08	1	0.41

**Table 8: Surface wise distribution of isolates**

No	Surface	No of isolates (N=196)	Percentage
1	Operation table	13	6.63
2	Boyle's apparatus	16	8.16
3	Floor at the head end	19	9.69
4	Head lamp	32	16.3
5	Suction apparatus	40	20.40
6	Instrument trolley	0	0
7	Fan	38	19.38
8	Wall near to the electric switches	38	19.38

N= total number of isolates

Highest no of organisms isolated from suction apparatus (20.40%)



**Table 9 : Rate of isolation of bacteria in surface samples**

Organisms	No of Isolates	Rate of Isolation	% Among the Isolates
Bacillus spp	63	26.25	32.14
CoNS	54	22.5	27.55
S.aureus	47	19.5	23.9
E.coli	25	10.41	12.75
Enterococci	5	2.08	2.55
Klebsiella pneumoniae	2	0.83	1.02
Clostridium sp	1	0.41	0.51

Total number of surface samples collected - 240

Total number of positive cultures - 196

Rate of isolation in surfaces was maximum in Bacillus species (26.25%). This was followed by CoNS (22.5%).

**Table 10: Sensitivity pattern of gram positive organisms**

Antibiotic	CoNS (n=107)		S.aureus (n=75)		Bacillus spp (n=93)		Enterococci (n=7)	
	No	%	No	%	No	%	No	%
Amoxicillin	91	85	25	34	90	97	2	29
Amoxyclav	102	95	51	68	93	100	5	71
Ciprofloxacin	96	90	36	48	85	91	4	57
Cefaclor	81	76	49	66	82	88	NT	-
Cefoxitin	79	74	51	68	NT	-	NT	-

Gentamicin	94	88	30	40	80	86	NT	-
Vancomycin	107	100	75	100	93	100	5	71
Ceftazidime	94	88	50	67	93	100	NT	-

All the Gram positive organisms showed 100% sensitive to vancomycin except Enterococci, 29% of Enterococcal isolates showed resistant to vancomycin. In *S. aureus* 68% of isolates were sensitive to amoxyclav, 67% of isolates were sensitive to ceftazime, 67% were sensitive to Cefaclor.

**Table 11: Sensitivity pattern of gram negative organisms**

Antibiotic	E.coli(47) (n=47)		Klebsiella pneumoniae (n=4)		P.aeruginosa (n=25)		Acinetobacter sp (n=13)	
	No	%	No	%	No	%	No	%
Amoxicillin	30	64	0	0	5	20	3	23
Amoxyclav	40	85	2	50	15	60	8	62
Piperacillin	43	91	3	75	20	80	5	38
Gentamicin	6	13	2	50	10	40	0	0
Ceftazidime	40	85	1	25	10	40	3	23
Ciprofloxacin	35	74	2	50	10	40	0	0
Imipenem	47	100	4	100	25	100	13	100

## DISCUSSION

Microbiological contamination of operation theatre environment is generally considered to be a risk factor for surgical site infections. According to Pasquarella et al, 2004 (9), microbiological quality of air may be considered as mirror of the hygienic condition of the operation theatres.

Observations of the number of bacteria carrying particles in air of surgical theatres and its premises may be required for safe working. Because it depends on the air content of bacteria being kept at very low level. <sup>[13]</sup> The evaluation of count, type and diversity of bio-contamination in hospital rooms especially operation theatres is very important to control and prevent hospital associated infections

(HAI).

The present study was undertaken to evaluate the microbiological contamination in environment of various operation theatres of Osmania General Hospital. Total 360 samples were collected repeatedly in 8 different operation theatres, in which 120 air samples and 240 surface samples.

According to C.Pasquarella et al 2000<sup>[12]</sup>, maximum acceptable level of colony forming unit in a conventional operation theatre on a settle plate of 9cm diameter exposed to air for one hour is 25 cfu/dm<sup>2</sup>/hr. In the present study out of 120 air samples, the bacterial load was above the acceptable level in 21 plates (17.5%) and within the acceptable level in 99 plates (82.5%).

Out of 120 air samples, the total number of organisms isolated was 175. Among which, Coagulase negative Staphylococci 53(44.16%), Bacillus species 30(25%),

Staphylococcus aureus 28(23.34%), Pseudomonas areuginosa 25(20.83%), E.coli 22(18.34%), Acinetobacter sp 13(10.83%), Klebsiella pneumoniae 2(1.67%) and Enterococci 2(1.67%) were isolated. The predominant organism was CoNS (44.16%) in all OTs. This was followed by Staphylococcus aureus (23.34%). The similar findings, CoNS (39.1%), Staphylococcus aureus (17.4%) were reported by S. Ensayel et al.<sup>[8]</sup>

Fungal isolates were found only in air samples. Aspergillus niger 96(80%), A.flavus 41(34.16%), A.terreus 20(16.67%), A.fumigatus 13(10.83%) were isolated. Similar highest rate of isolation of A.niger was found in the study of Ismail et al, 2008.<sup>[14]</sup> Aspergillus species remain in uncommon cause of disease. These fungi are only occasionally associated with colonizing syndromes and rarely cause invasive infection. Individuals at particular risk for invasive infection include those with protein-calorie malnutrition, patients receiving chemotherapy for malignancy, recipients of bone marrow, solid organ transplants and persons with congenital or acquired immune disorders. Predisposing host factors include defects in alveolar macrophages, which kill conidia and neutrophils, which kill hyphae. Although immunocompromised patients are predisposed to invasive infections these infections are distinctly uncommon in immunocompetent persons, accounting for less than 10% of all cases in recent large series.<sup>[15]</sup>

Among all Gram Negative bacteria, P. areuginosa (25%) were frequently isolated in Urology and Gastroenterology operation theatres, Acinetobacter(10.83%) was frequent in Emergency operation theatre. Least bacterial isolates were found in Cardio thoracic and Neurology operation theatres. Hambraeus 1995<sup>[16]</sup> mention that Gram negative rods have characteristic ability to be resistant for unfavorable environmental conditions.

Out of 240 surface samples, positive cultures were obtained in 196(81.66%), of which Bacillus species 63(26.25%), CoNS 54(22.5%), Staphylococcus aureus 47(19.5%), E.coli 25(10.41%), Enterococci 5(2.08%), Klebsiella pneumoniae 2(0.83%), Clostridium species 1(0.41%). Bacillus species were predominant organisms isolated from various surfaces and articles in theatres of General surgery (28.12%), Orthopedics (34.37%) and Emergency OT (37.5%). CoNS was predominant isolate in theatres of Gastroenterology (28.12%), Cardio Thoracic (15.62%), Neurology (15.62%) and Skin & Plastic surgery (25%); and E.coli was predominant in Urology operation theatre(34.37%). Maximum number of organisms isolated from suction apparatus (20.40%).

In our study Gram positive bacteria like Coagulase Negative Staphylococci (44.16%), Staphylococcus aureus (23.34%) and Bacillus species (25%) were obtained in higher level from the open culture plate technique. This level of bacterial contamination simply revealed the quality of air with in the sampled units. Primarily, the quality of indoor air depends on external and internal factors. The external factors are surgical / medical team and the degree of activity; and the internal factors are the type of ventilation systems and cleaning procedures.<sup>[17]</sup> The effect of external factors can be explained by the presence of Coagulase negative Staphylococci isolates that predominate in sampled units by open plate method. The primary ecology niche of Staphylococci is anterior nares, axilla and groin, and small numbers of individuals are carriers in which relatively large number of these Staphylococci

could be shed in the air in high activity state. <sup>[18],[17]</sup>

Out of 240 surface swabs 196(81.66%) positive cultures were obtained from swabbing method. This value might not be surprising, as high traffic density of personnel and students are involved in the day to day surgical/clinical procedures in the operating theatre or other care units, which could serve as source of bacterial contamination evident in our findings. Duguid and Wallace 1948, [19] reported that increased activity enhanced the dispersion of bacteria. Also movement can shed up to 10,000 skin scales per minute of which 10% carry cluster of microorganisms. <sup>[18]</sup>

The breakdown of bacterial pathogens isolated showed the CoNS (28.84%) predominate in all operation theatres which is consistent

with similar reported studies.<sup>[10],[8],[17]</sup> CoNS are known exogenous organism, often referred to as contaminant.<sup>[8]</sup> However, clinical implication of CoNS is more pronounced in immunocompromised patients, as entry into systemic environment could initiates infection. <sup>[17]</sup>

All Gram positive organisms showed 100% sensitive to vancomycin, except Enterococci, 29% of Enterococcal species showed resistant to vancomycin. In *S.aureus* isolates 40% were sensitive to gentamicin, 34% were sensitive to amoxicillin.

All Gram negative organisms (100%) showed sensitivity to imipenem. In *E.coli*, 91% of isolates were sensitive to piperacillin, followed by 85% to amoxyclav, ceftazidime. In *Klebsiella* species 75% of isolates were sensitive to piperacillin, 50% to amoxyclav and gentamicin. *P.aeruginosa* showed high resistant to antibiotics. In this species, 80% of isolates were resistant to amoxicillin and 60% were resistant to gentamicin, ceftazidime, ciprofloxacin. *Acinetobacter* showed 100% resistant to gentamicin and ciprofloxacin. This pattern of antibiotic sensitivity was comparable with the study of Okon K.O. et al., 2012. <sup>[17]</sup>

## CONCLUSION

As conclusion these findings demonstrate that the microbiological quality of air and surfaces in operation theatre may be considered a mirror image of the hygienic conditions of the operating theatre. Settle plate method for air and swabbing technique for surfaces proved to be valuable in detecting the contamination level.

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## Conflict of Interest

None

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