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Surface and Air Sampling of Key Areas of the Hospital in a Tertiary Care Hospital in Bihar

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ABSTRACT

Background: Operation theatres (OT) and intensive care units (ICU) are considered vulnerable areas for the evolution and spread of healthcare-associated infections (HAI) as well as microbial resistance. The confined environment present in the OTs and ICU facilitates aerosol formation and further builds up infection levels. Surveillance, one of the integral parts of an effective infection control programme, aids in reducing the burden of health care-associated infections and antimicrobial resistance.

Methods: This prospective observational study was conducted from September 2022 to February 2024 in the Microbiology laboratory at Katihar Medical College, Katihar. An estimated 768 pre-fumigation surface samples 178 settle plates and the same number of post-fumigation samples were taken from various OTs and ICUs during the entire study period.

Results: In total 1892 samples (including surface swabs and settle plates) were processed within 18 months. Growth was detected in 17.8% (137/768) of pre-fumigation swabs and 4.2% (32/768) of post-fumigation swabs. ICU II was found to be most contaminated with 36/137 (26.3%) isolates. Gynae OT was found to be the least contaminated with 3/137(2.1%) isolates. The most common isolate in pre-fumigation surface sample was *S. aureus* 38/137 (27.7%). The pre-fumigation air bio-load of all OTs and ICUs ranged between 6.16 to 23.28 colonies/90 mm diameter plate/hr. The post-fumigation air bio-load in all OTs and ICUs ranged between 3.00-10.21 colonies/90mm diameter plate/hr.

Conclusion: There was a significant reduction in the growth in surface samples following fumigation. The mean air bio-load of all OTs and ICUs during pre-fumigation and post-fumigation was within normal limits as per the IMA index.

Key-words: Healthcare-associated infection (HAI), healthcare facility (HCF), Air Sampling of Key Areas, Nosocomial infection

INTRODUCTION

Healthcare-associated infection (HAI), previously referred to as "nosocomial" or "hospital-acquired" infection, occurs in a patient during the process of care in a hospital or other healthcare facility (HCF), but was not present or incubating at the time of admission. HAIs include occupational infections among healthcare providers.[1]

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Surveillance, one of the integral parts of an effective infection control program is responsible for reducing the burden of antimicrobial resistance and healthcare-acquired infection (HAI).[2]

Microbiological contamination of air in the operation theatre and ICUs is a major risk factor for HAI-like surgical site infections (SSI). Closed environment like OT and ICU enhances aerosol formation and further build up infection levels. Infected patients, movement of medical personnel and high visitor loads are important sources of aerosol generation.[3]

Monitoring of the hospital environment is essential for the control of HAI. The risk level of HAI can be ascertained by microbiological monitoring of hospital surfaces and air sampling.[4] The present study was undertaken to ascertain the microbiological ecosystem in

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our hospital with special reference to key areas like OT and ICU to prevent HAI.

MATERIALS AND METHODS

This prospective observational study was undertaken for surface and air sampling of key areas of a tertiary care hospital for the identification of organisms present in these areas and to determine the antibiogram of these isolates. The study was conducted in the Department of Microbiology, Katihar Medical College, Katihar from September 2022 to February 2024.

Study design - Prospective observational study

Inclusion criteria- Samples from six Operation theatres (OTs) and two Medical Intensive Care Units (ICUs)

Exclusion criteria- Samples were not collected from the Neonatal intensive care unit (NICU), Postnatal intensive care unit (PICU), Surgical intensive care unit (SICU) and other areas of the hospital.

Samples were collected from the OTs on a fortnightly basis before and after terminal cleaning and fogging of the OT. Four surface samples were collected and one settle plate was placed in each OT. An estimated 600 pre-fumigation surface samples and 150 pre-fumigation settle plates and the same number of post-fumigation samples were collected during the entire study period.
Samples from the two ICUs were collected every month before and after the fogging of the ICU. Six surface samples were collected and one settle plate was placed in each ICU. An estimated 168 pre-fumigation swabs and 28 settle plates and the same number of post-fumigation samples were collected from the ICUs during the entire study period.

Surface sampling- Swabs from OT were collected fortnightly both before and after terminal cleaning and fogging of the OT. Swabs from the Medical ICU were collected once a month before and after cleaning and fogging of the ICU. Sterile swabs moistened in peptone water were used to collect samples from 4 sites in each of the 6 OTs (table, light, instrument trolly, hand wash area) and 6 sites in each of the 2 Medical ICU (patients' bed, cardiac monitors, air-conditioner vents, walls, instrument/medicine trolly and hand wash area). Following collection, each swab was inserted in previously labelled sterile test tubes and immediately transported to the Microbiology department for processing. Swabs taken from the different sites were inoculated on blood agar (BA) and MacConkey's agar (MAC) plates followed by incubation at 37^{o}C for 18-24 hrs under aerobic conditions. After incubation, the isolates were identified by colony characteristics, Gram stain reactions and standard biochemical tests.[5]

Air sampling- The settle plate method was used for air sampling. Blood agar plates were placed in the OT and ICU before and after terminal cleaning and fogging which was done once every 15 days in the OT and once a month in the ICU. Air sampling was done while the OT was in use. BA plates were placed at different locations in the OT and ICU one metre above the ground, one metre away from the wall/any obstacles and for one hour. The plates were then transported to the laboratory in zip-lock plastic bags and incubated at 37^{o} C for 18-24 hours under aerobic conditions. After incubation, plates were observed for growth.
The number of colonies on each plate was counted and expressed as TVC in CFU/m^3 using the Omeliansky formula:[6]

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

N= Colony forming unit per cubic meter of air (CFU/m^3)
a= No. of colonies forming unit per petri plate (CFU)
b= The surface measurement of plate used in cm^2
t= The time of the exposure of the petri plates in minutes

Statistical Analysis- Statistical analysis was done by using online statistical software. The mean TVC of pre- and post-fumigation swabs was analysed by paired students by using online software https://www.graphpad.com accessed on 24.05.24.

Ethical Committee- Institutional Ethical Committee clearance was obtained before conducting the study.

RESULTS

In total, 1,892 samples (including surface swabs and settle plates) were processed within 18 months. In the present study, a total of 768 pre-fumigation and 768 post-fumigation surface samples from surface and articles of various OTs and ICUs were collected and processed. Growth was detected in 17.8 % (137/768) of pre-fumigation swabs and 4.2 % (32/768) of post-fumigation swabs.

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Table 1 presents the pre-fumigation distribution of isolates in OTs and ICUs. ICU II was identified as the most contaminated, accounting for 36 out of 137 isolates (26.3%), while the Gynae OT was the least contaminated, with only 3 out of 137 isolates (2.1%).

Table 1: Pre-fumigation distribution of isolates in OTs and ICUs

Isolates	OT-1 (Surgery I)	OT-2 (Ortho)	OT-3 (Surgery	OT-4 (Gynae)	OT-5 (Eye)	OT-6 (ENT)	ICU I	ICU II	Total
Klebsiella	0	1	1	0	0	0	0	2	4
Pseudomonas	2	4	0	0	2	1	6	4	19
Acinetobacter	1	0	0	0	1	0	3	1	6
E. coli	0	4	1	0	0	0	1	1	7
S. aureus	4	5	5	0	0	2	12	10	38
CONS	4	1	2	0	0	0	4	5	16
GPB (diphtheroids)	1	2	2	2	4	3	3	9	26
ASB (*Bacillus* sp)	3	0	0	1	5	3	1	4	17
Micrococcus	2	1	1	0	0	0	0	0	4
Total (%)	17 (12.4)	18 (13.1)	12 (8.8)	3 (2.1)	12 (8.8)	9 (6.6)	30 (21.9	36 (26.3	137 (100)

Table 2 presents the post-fumigation distribution of isolates in OTs and ICUs. ICU I was the most contaminated, with 10 out of 32 isolates (31.3%), while the ENT OT was the least contaminated, with only 1 out of 32 isolates (3.1%).

Table 2: Post-fumigation distribution of isolates in OTs and ICUs

Isolates	OT-1 (Surgery I)	OT-2 (Ortho)	OT-3 (Surgery II)	OT-4 (Gynae)	OT-5 (Eye)	OT-6 (ENT)	ICU I	ICU II	Total
Klebsiella	0	0	0	0	0	0	0	0	0
Pseudomonas	1	1	1	1	1	0	2	0	7
Acinetobacter	0	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0	0
S. aureus	0	1	0	0	0	0	3	2	6
CONS	0	0	0	0	0	0	0	0	0
GPB (diphtheroids)	1	4	1	1	1	1	4	2	15
ASB (*Bacillus* sp)	1	0	0	0	1	0	1	0	3
Micrococcus	1	0	0	0	0	0	0	0	1
Total (%)	4 (12.5)	6 (18.8)	2 (6.3)	2 (6.3)	3 (9.4)	1 (3.1)	10 (31.3)	4 (12.5)	32 (100)

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Fig. 1 illustrates the species-wise distribution of isolates in pre-fumigation surface samples. The most frequently isolated species was *Staphylococcus aureus* (38/137, 27.7%), while *Klebsiella* species and *Micrococcus* species were the least common, each with 4 out of 137 isolates (2.9%).

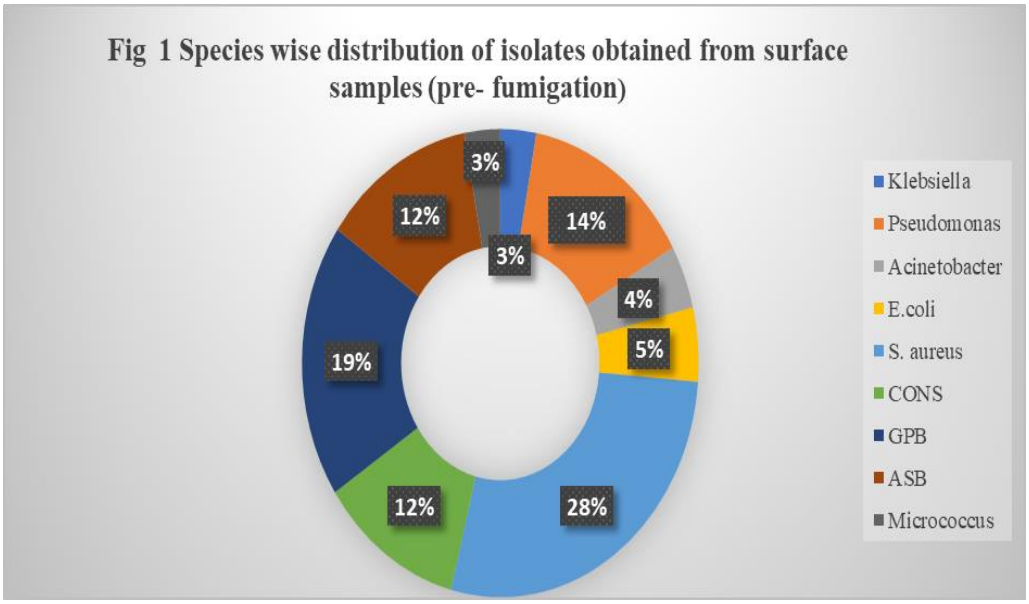

Fig. 1: Distribution of isolates in pre-fumigation surface samples

Fig. 2 depicts the species-wise distribution of isolates in post-fumigation surface samples. GPB was the most common isolate, accounting for 15 out of 32 isolates (46.9%), while *Micrococcus* was the least common, with 1 out of 32 isolates (3.1%). Notably, *Klebsiella* species, *E. coli*, *Acinetobacter* species, and CoNS were absent in the post-fumigation samples (0/32).

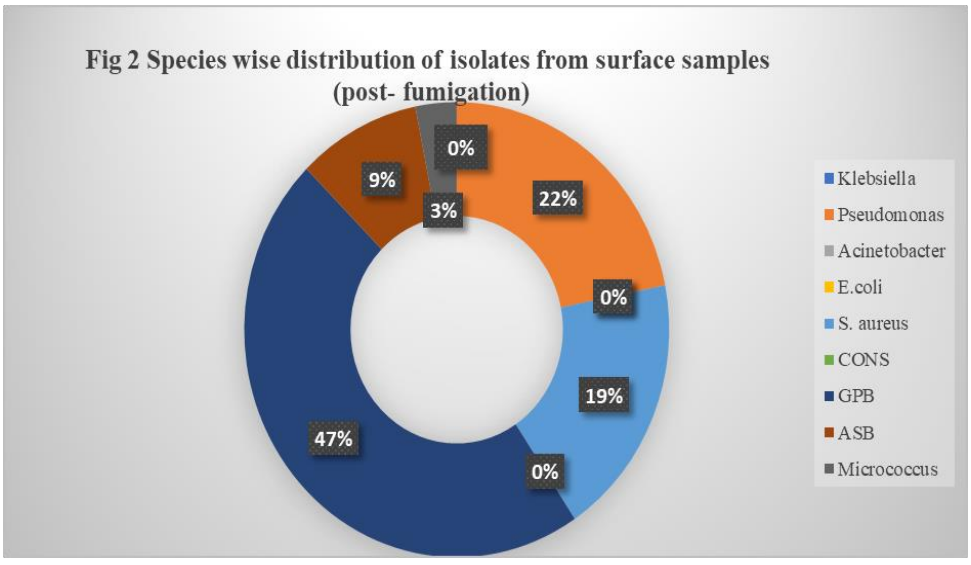

Fig. 2: Species-wise distribution of isolates in post-fumigation surface samples

Table 3 presents the antibiotic susceptibility pattern of *S. aureus* (n=44). All isolates (44/44) were fully susceptible to teicoplanin, tetracycline, vancomycin, and linezolid. Resistance was most observed to ampicillin (77.3%, 34/44), followed by levofloxacin (68.2%, 30/44). Notably, none of the strains were identified as MRSA.

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Table 3: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	9 (20.4)	1 (2.3)	34 (77.3)
Amoxiclav	21 (47.7)	-	23 (52.3)
Amikacin	28 (63.6)	10 (22.7)	6 (13.6)
Cefoxitin	44(100)	-	-
Gentamicin	21 (47.7)	3 (6.8)	20 (45.5)
Ciprofloxacin	22 (50.0)	6 (13.6)	16 (36.4)
Teicoplanin	44 (100)	-	-
Tetracycline	44 (100)	-	-
Levofloxacin	13 (29.5)	1 (2.3)	30 (68.2)
Vancomycin	44 (100)	-	-
Linezolid	44 (100)	-	-
Clindamycin	28 (63.6)	-	16 (36.4)

Table 4 summarizes the antibiotic susceptibility pattern of CoNS (n=16). All CoNS isolates (16/16) were fully susceptible to teicoplanin, tetracycline, vancomycin, and linezolid. Resistance was most frequently observed to ampicillin (81.3%, 13/16), followed by levofloxacin (75.0%, 12/16).

Table 4: Antibiotic susceptibility pattern of CoNS

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	3 (18.7)	-	13 (81.3)
Amoxiclav	7 (43.7)	1 (6.3)	8 (50.0)
Amikacin	12 (75.0)	4 (25)	-
Cefoxitin	16 (100)	-	-
Gentamicin	12 (75.0)	-	4 (25.0)
Ciprofloxacin	6 (37.5)	2 (12.5)	8 (50.0)
Teicoplanin	16 (100)	-	-
Tetracycline	16 (100)	-	-
Levofloxacin	4 (25.0)	-	12 (75.0)
Vancomycin	16 (100)	-	-
Linezolid	16 (100)	-	-
Clindamycin	9 (56.3)	-	7 (43.7)

Table 5 presents the antibiotic susceptibility pattern of *Pseudomonas* species (n=26). The isolates demonstrated the highest susceptibility to ceftazidime/clavulanic acid (88.4%, 23/26), followed by amikacin (84.6%, 22/26). The highest resistance was observed to gentamicin (73.07%, 19/26), followed by ciprofloxacin (69.2%, 18/26).

Table 5: Antibiotic susceptibility pattern of *Pseudomonas* species

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	22 (84.6)	1 (3.8)	3 (11.5)
Gentamicin	7 (26.9)	-	19 (73.0)
Ciprofloxacin	8 (30.7)	-	18 (69.2)
Piperacillin/Tazobactam	13 (50.0)	4 (15.3)	9 (34.6)
Ceftazidime/Clavulanic acid	23 (88.4)	-	3 (11.5)

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Imipenem	11 (42.3)	-	15 (57.6)
Meropenem	14 (53.8)	-	12 (46.1)
Levofloxacin	15 (57.6)	-	11 (42.3)

Table 6 presents the antibiotic susceptibility pattern of *Acinetobacter* species (n=6). The isolates showed the highest susceptibility to ceftazidime/clavulanic acid, piperacillin/tazobactam, and amikacin (66.7%, 4/6). Resistance was most observed to ciprofloxacin and imipenem (66.7%, 4/6).

Table 6: Antibiotic susceptibility pattern of *Acinetobacter* species

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	4 (66.7)	-	2 (33.3)
Gentamicin	3 (50.0)	-	3 (50.0)
Ciprofloxacin	2 (33.3)	-	4 (66.7)
Piperacillin/Tazobactam	4 (66.7)	2 (33.3)	-
Ceftazidime/Clavulanic acid	4 (66.7)	1 (16.6)	1 (16.6)
Imipenem	2 (33.3)	-	4 (66.7)
Meropenem	3 (50.0)	-	3 (50.0)
Levofloxacin	3 (50.0)	-	3 (50.0)

Table 7 presents the antibiotic susceptibility pattern of *E. coli* (n=7). The highest susceptibility was observed to imipenem (85.7%, 6/7), followed by amikacin (71.4%, 5/7). Resistance was most seen to cefuroxime and ceftriaxone (85.7%, 6/7), followed by ampicillin and amoxiclav (71.4%, 5/7).

Table 7: Antibiotic susceptibility pattern of *E. coli*

Antibiotics	Sensitive (%)	Intermediate (I%)	Resistance (%)
Ampicillin	2 (28.6)	-	5 (71.4)
Amoxiclav	2 (28.6)	-	5 (71.4)
Amikacin	5 (71.4)	-	2 (28.6)
Gentamicin	4 (57.1)	1 (14.3)	2 (28.6)
Ciprofloxacin	2 (28.6)	1 (14.3)	4 (57.1)
Cefuroxime	1 (14.3)	-	6 (85.7)
Piperacillin/Tazobactam	3 (42.9)	-	4 (57.1)
Cefoperazone/sulbactam	4(57.1)	-	3(42.9)
Ceftriaxone	1(14.3)	-	6(85.7)
Imipenem	6 (85.7)	-	1 (14.3)
Meropenem	4 ((57.1)	-	3 (42.9)

Table 8 presents the antibiotic susceptibility pattern of *Klebsiella* (n=4). All *Klebsiella* isolates (4/4) were susceptible to cefuroxime, piperacillin/tazobactam, ceftriaxone, cefoperazone/sulbactam, imipenem, and meropenem. Resistance was observed to ampicillin, amoxiclav, amikacin, gentamicin, and ciprofloxacin in 25% of isolates (1/4).

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Table 8: Antibiotic susceptibility pattern of *Klebsiella*

Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
Ampicillin	3 (75.0)	-	1 (25.0)
Amoxiclav	3 (75.0)	-	1 (25.0)
Amikacin	3 (75.0)	-	1 (25.0)
Gentamicin	3 (75.0)	-	1 (25.0)
Ciprofloxacin	3 (75.0)	-	1 (25.0)
Cefuroxime	4 (100)	-	-
Piperacillin/Tazobactam	4 (100)	-	-
Cefoperazone/sulbactam	4 (100)	-	-
Ceftriaxone	4 (100)	-	-
Imipenem	4 (100)	-	-
Meropenem	4 (100)	-	-

Air sampling result pre-fumigation A total of 178 pre-fumigation air samples of various OTs and ICUs were taken by the settle plate method. The average air bio-load of all OTs and ICUs ranged between 6.16 to 23.28 colonies/90 mm diameter plate/hr.

Table 9 presents the results of pre-fumigation air sampling using the settle plate method. The highest air bio-load was observed in ICU I with 23.28 colonies/90mm diameter plate/hr, followed by ICU II with 18.57 colonies/90mm diameter plate/hr. The lowest air bio-load was found in OT5 (Ophthalmology) with 6.16 colonies/90mm diameter plate/hr. The mean total viable count (TVC) in CFU/m^3 from all OTs and ICUs ranged from 87.3 in OT5 (Ophthalmology) to 305.04 in ICU I.

Table 9: Pre-fumigation air sampling by settle plate method

Location	No of settle plates collected	No. of colonies (mean)	Mean TVC CFU/m^3
ICU I	14	23.28	305.04
ICU II	14	18.57	243.28
OT-1(Surgery I)	25	16.92	213.26
OT-2 (Ortho)	25	17.32	227.01
OT-1(Surgery II)	25	12.20	159.80
OT-4 (Gynae)	25	7.64	99.90
OT-5 (Eye)	25	6.16	87.30
OT-6 (ENT)	25	7.40	103.54

Table 10 presents the results of post-fumigation air sampling using the settle plate method. Following fumigation, there was a significant reduction in air bio-load. The average air bio-load across all OTs and ICUs ranged from 3.00 to 10.21 colonies/90mm diameter plate/hr.

Table 10: Post-fumigation air sampling by settle plate method

Location	No of settle plates collected	No. of colonies (mean)	Mean TVC CFU/m^3
ICU I	14	10.21	154.16
ICU II	14	7.42	107.25
OT-1(Surgery I)	25	7.12	93.20

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OT-2 (Ortho)	25	8.36	109.50
OT-1(Surgery II)	25	6.12	80.17
OT-4 (Gynae)	25	3.32	43.49
OT-5 (Eye)	25	3.00	38.25
OT-6 (ENT)	25	3.68	48.20

DISCUSSION

In pre-fumigation surface sampling *S. aureus* 27.7 % (38/137) was found to be the most common isolate followed by GPB 19.0 % (26/137). Deepa *et al*. [7] reported among 158 isolates obtained from ICUs and OTs Staphylococcus was the most common 51(32.3 %). Among 51 isolates of *Staphylococcus*, 36 (70.5 %) were *S. aureus* and 15 (29.5%) isolates were CoNS.

Anjali *et al.* [8] found CoNS was the most common isolate 4 (5.8%) followed by klebsiella and bacillus species 3 (4.4 %) each. Matinyi *et al*. [9] reported most of the microbial pathogens were isolated from Ophthalmology theatre, 38.9% (91/234). The major pathogens in this theatre were pseudomonas species 22.0% (20/91) and bacillus species 18.7% (17/91). General OT was mainly contaminated by pseudomonas species 19.6% (9/46) and CoNS 15.2% (7/46).

Matthew *et al*. [10] reported that amongst 393 bacterial pathogens, 245(62.3%) were Gram-positive and 148(37.7%) were Gram-negative. Among Gram-positive isolates, *S. aureus* was predominant (69.4%). Bhattacharjee *et al.* [11] in 2021 reported that in their study *Bacillus* species 45% and *Micrococci* species, 33% were the most common isolates followed by *Klebsiella* species (9%).

All (44/44) isolates were susceptible to cefoxitin, teicoplanin, tetracycline, vancomycin and linezolid. Resistance was observed mostly to ampicillin followed by levofloxacin. MRSA and VRSA were not encountered in the present study in both pre- and post-fumigation samples. All (44/44) isolates were susceptible to cefoxitin, teicoplanin, tetracycline, vancomycin and linezolid. Resistance was observed mostly to ampicillin followed by levofloxacin.

A similar study conducted by Singh *et al*. [12] reported that all Gram-positive bacterial isolates were 100% sensitive to linezolid and vancomycin. Methicillin and vancomycin resistance was not found in CoNS isolates. The isolates showed maximum susceptibility to ceftazidime/ clavulanic acid followed by amikacin.

Maximum resistance was observed to gentamicin followed by ciprofloxacin. Carbapenem resistance was common with 57.6% and 46.1% of pseudomonas strains being resistant to imipenem and meropenem respectively.

Isolates were mostly susceptible to ceftazidime/ clavulanic acid, piperacillin/tazobactam and amikacin. Maximum resistance was observed mostly to ciprofloxacin and imipenem. Carbapenem resistance was common with 66.7% and 50.0% of strains being resistant to imipenem and meropenem respectively. Carbapenem resistance in both pseudomonas and *Acinetobacter* species with 50.0% or more strains being resistant is worrisome and calls for the use of alternative antibiotics to spare carbapenem antibiotics. Maximum susceptibility was seen to imipenem with 85.7% of strains being sensitive. Resistance was observed mostly to cefuroxime and ceftriaxone 85.7% followed by ampicillin and amoxiclav 71.4%.

All isolates were susceptible to cefuroxime, piperacillin/tazobactam, ceftriaxone, cefoperazone /sulbactam, imipenem and meropenem 100%. Resistance was observed in ampicillin, amoxiclav, amikacin, gentamicin and ciprofloxacin with 25.0% of strains being resistant.

Okon K. O et al in 2012 reported the antibiotic resistance pattern of isolates and found that strains were highly resistant to commonly used antibiotics like cotrimoxazole, ampicillin and gentamicin, CoNS showed the highest resistance to ampicillin cloxacillin combination 75 (55.9%), *K. pneumoniae* showed the highest resistant to ampicillin 3 (75.0%), and the most resistant organism that was isolated was *P. aeruginosa.*[13] Coliforms, *E. coli*, and *Proteus* species showed the highest resistance to cotrimoxazole.[14]

Currently, there is no international consensus (neither in the Italian ISPESL guidelines nor in the ISO standards) on the most suitable method for air sampling or any precise guidelines for obtaining TVC values. Passive air sampling by the settle plate method is the most extensively used

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air sampling technique. Settle plates are simple to use, economical, do not disturb the movement of the microbial population in the air during sampling and do not interrupt the laminar airflow in any way.[15]

In the present study, passive air sampling by settle plate was done. The average air bio-load of all OTs and ICUs before fumigation ranged between 6.16 to 23.28 colonies/ 90 mm diameter plate/hr. The highest air bio-load was found in ICU I with 23.28 colonies/ 90mm diameter plate/hr followed by ICU II with 18.57 /90mm diameter plate/hr. The lowest air bio-load was found in OT5 (Ophthalmology) at 6.16 colonies /90mm diameter plate/hr.

The mean TVC CFU/m^3 counts of air from all OTs and ICUs ranged from 87.3 in OT 5 (Ophthalmology) to 305.04 in ICU I. Deepa *et al*. [7] reported that bio load was 688.3 CFU/m^3 in NICU, in MICU 2401.8 CFU/m^3, in SICU 4766.2 CFU/m^3 and 991.5 CFU/m^3 in OT. Yadav *et al*. [16] reported that bacterial counts during OT ranged from 31-200 CFU/ mm^3. The highest air bio-load was from an emergency department at 200 CFU/ mm^3 and the lowest bio-load was from the Ophthalmology department at 31 CFU/ mm^3.

CONCLUSIONS

The study highlights the significant reduction in microbial contamination following fumigation in OTs and ICUs, as evidenced by decreased surface and air bio-loads. Pre-fumigation samples showed higher contamination, with Staphylococcus aureus and GPB being the most common isolates, while post-fumigation samples exhibited a marked decline in microbial growth. Resistance patterns emphasized susceptibility to key antibiotics and the absence of MRSA and VRSA. Air bio-load and total viable counts (TVC) significantly decreased post-fumigation, with improved indices of microbiological air contamination. The findings underscore the importance of periodic decontamination, environmental monitoring through cost-effective settle plates, and implementing robust infection control measures to minimize surgical site infections and reduce unnecessary antibiotic use.

CONTRIBUTION OF AUTHORS

Research concept- Dr(s). Nibha Bharti, Sangeeta Dey, Kahkashan Akhter

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Materials- Dr(s). Nibha Bharti, Sangeeta Dey, Kahkashan Akhter

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