

Microbial contamination of operating Theatre at Ayder Referral Hospital, Northern Ethiopia

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Abstract

Background: Microbial contamination of the Operating Theatre (OT) had continued to increase the prevalence of nosocomial infections. **Aim:** This study was conducted to assess the level of microbial contamination and to determine the antimicrobial resistance of the bacterial isolates. Settle plate's method was used for air sample collection while swab method was used to collect samples from surfaces and other articles in the major OT. Collected samples were transported and microbiologically processed using standard procedures. **Findings:** One hundred twenty air, 36 article and 12 surface samples were taken for microbiological evaluation. The highest level of microbial contamination was detected in the OT air before proper cleaning-fumigation as compared to after the intervention. Moreover, microbial growth was found on surfaces and semi-critical articles. On the other hand articles which were sterilized by autoclave showed no microbial growth. There were five types of bacteria isolated with the highest prevalence of coagulase negative *Staphylococci* (68; 53.5%) followed by *Staphylococcus aureus* (42; 33.1%). Methicillin resistance *S. aureus* (MRSA) account for 7.7% of the *S. aureus* isolates. The highest resistance was found against penicillin G and ampicillin with a resistance rate of 52.7%, and 44.5% respectively. Multidrug resistance was observed among 23(36.5%) of the bacterial isolates. **Conclusion:** In general, the results indicate proper cleaning-fumigation of operating theatre significantly reduced the microbial contamination, and bacterial strains such as CoNS, and *S. aureus* have a greater propensity to cause contamination in OT. Therefore, efforts should be made to ensure strict infection control practices in the OT.

Keywords: Microbial contamination, operating Theatre, surfaces, antimicrobial resistance.

Introduction

Microbial contamination of the operating theatre (OT) had continued to increase the prevalence of nosocomial infections (NIs) [1-4,5]. Its clinical implication is enormous on both the patient and the caring surgical team [2,4,6]. Approximately, 10% of all infections can have serious consequences in terms of increased patient mortality, morbidity and length of hospital stay and overall costs among patient admitted for post-operative surgery [5,7]; with multidrug resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) [5]. Antimicrobial resistance results in increased illness, deaths and health care costs. The emergence of multidrug resistant strains in a hospital environment, particularly in developing countries is an increasing infection control problem and associated with a high frequency of NIs and antibiotic resistance rate [8].

Multiple reservoirs have been reported as being responsible for the contamination of the OT, including unfiltered air, ventilation systems and antiseptic solutions, drainage of the wounds, transportation of patients and collection bags, surgical team, extent of indoor traffic, theatre gown, foot wares, gloves and hands, use of inadequately sterilized equipment, contaminated environment and grossly contaminated surfaces [2,4]. The impact of these sources on the degree of microbial contamination differs, depending on the numbers of pathogens involved. *Staphylococcus aureus* and coagulase negative *staphylococci* (CoNS), for example, are the major pathogens associated with infection of implantable biomedical devices [9].

Microbial contamination of the OT can be prevented through adequate application of infection control practices. Reduction of airborne bacteria in the OT by about 13-fold, for example, would reduce the wound contamination by about 50% [2]. This depends primarily on improving cleaning, proper disinfection and regular fumigation of OT [2,4,5]. In Ethiopia, data on the microbial contamination of OT is scarce. Therefore, this study was

conducted to assess the level of microbial contamination and to determine the antimicrobial resistance of the bacterial isolates from major OT at Ayder Referral Hospital, Northern Ethiopia.

Methods and materials

After getting full approval from College of Health Sciences Ethical Review Committee, Mekelle University, the study was conducted to evaluate the microbial contamination of major operating theatre at the Ayder Referral Hospital, Northern Ethiopia. Air and surface samples were collected from five major operating rooms without prior discussion with the cleaning staff. Air sampling (before and after cleaning-fumigation) was performed with passive air sampling (settle plate's methods) according to the 1/1/1 scheme (a Petri dish with a diameter of 9 cm was placed for 1 hour, 1 meter above the floor, about 1 meter away from the walls or any major obstacles) [10]. Passive air sampling provides a valid risk assessment as it measures the harmful part of the urban population, which falls onto a critical surface, such as in the surgical cut or on the instruments in major OT (critical zone) [11]. Sealed Petri dishes containing 5% sheep blood agar (Oxoid, UK) was transported to major OT in a sealed plastic bag. The plates were labeled with sample number, set within theatre, time and date of sample collection. Then, they were placed at four chosen places in the operating room at about 1 meter above the ground, and exposed for 1 hour. Each day, the air samples were collected three times: in the morning between 10 a.m. and 11 a.m., lunch between noon and 2 a.m. and in the evening between 5 p.m. and 6 p.m. After this exposure, the plates were covered with their lids and then sealed. To check the sterility of articles and surfaces in the major OT, a sterile swab moistened with sterile normal saline was used to collect samples. All the plates were labeled properly and then, the swab was immediately streaked on to 5% sheep blood agar (Oxoid, UK). Upon inoculation, and plates were sealed and transported along with those exposed in the air to the Ayder hospital microbiology laboratory in sealed plastic bags and incubated at 37°C under aerobic conditions for 24 hours.

For air samples, the total number of colony forming units (CFU) was enumerated using colony counter and results were expressed in CFU/dm²/hour [11]. Then, bacterial colonies were initially characterized by morphology and microscopic examination [12]. Then, they were Gram-stained and sub-cultured onto appropriate medium based on the Gram-reaction; i.e. Gram positive *Staphylococci* were cultured on to mannitol salt agar (Oxoid, UK) and 5% Sheep's blood agar (Oxoid, UK), and gram negative rods onto macConkey agar (Oxoid, UK). Further identification was done by biochemical tests using the standard bacteriological techniques [12]. Screening of MRSA was done using oxacillin Screen Agar (Mueller Hinton Agar with 6 µg/ml oxacillin and 4% NaCl). The efficacy of the disinfectants used in the OT was also tested by qualitative suspension tests [24].

The antimicrobial susceptibility testing of the bacterial species identified before proper cleaning-fumigation species was done on Mueller-Hinton agar (Oxoid, UK) against nine antibiotics by Kirby-Bauer disk diffusion method matching the test organism to 0.5 McFarland turbidity standards. Then, the susceptibility result was interpreted according to the Clinical Laboratory Standards Institute (CLSI) methodology (CLSI M100) [13]. *S. aureus* ATCC25923, *P. aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were used as control bacterial strains to monitor the whole bacteriological procedures. Data obtained was subjected to statistical analysis using SPSS16.0 and Microsoft Office Excel 2007. A significant difference among the mean microbial air counts was tested Kruskal-Wallis test.

Results and discussion

Microbial contamination of the operating theatre (OT) had contributed significantly to high prevalence of nosocomial infections [1-4, 5].

One hundred twenty air (before and after cleaning-fumigation), 36 article and 12 surface samples were taken from the major OT for microbiological evaluation. The mean microbial count of the major OT air before cleaning-fumigation during morning, lunch, and evening time was 91.8 (SD 54.3), 44.9 (SD 29.3), and 17.2 (SD 17.1) CFU/dm²/h, respectively, however, after proper cleaning-fumigation this was significantly reduced to 42.6, 31.3, and 14.7 CFU/dm²/h respectively (figure 1). The difference among the means was statistically significant (p=0.000). According to Fisher's index of microbial air contamination [10], air microbial count of OT at rest and inactivity should not exceed 9.0 CFU/dm²/h and 91.0 CFU/dm²/h, respectively. However, the results obtained before proper cleaning-fumigation indicates higher means air microbial count; i.e. at rest (evening) 17.2 CFU/dm²/h and in activity (morning) 91.8 CFU/dm²/h. Nevertheless, the microbial load reduced significantly to acceptable levels after proper cleaning-fumigation of the OT. This dictates proper infection control practices in the OT.

The critical articles which were sterilized by autoclave showed no microbial growth, whereas articles like endotracheal tubes and laryngoscope which were heat labile and disinfected by chemicals and surfaces like floors, operation room (OR) table and OR light showed heavy growth of pathogens. The *in vitro* disinfectants efficacy tests indicate that the disinfectants were effective against the bacterial isolates and control bacterial strains, hence the microbial growth on the OR articles may be due to improper preparation and/or application of these chemicals.

Although the direct involvement of these fomites in disease transmission was not investigated in this study, the isolation of coagulase negative *Staphylococci* (CoNS) (68; 53.5%) *S. aureus* (42; 33.1%), *P. aeruginosa* (13; 10.2%), *Bacillus* Spp. (2; 1.6%), and *E. coli* (2; 1.6%) presents a serious concern for possible nosocomial transmission. Among the bacterial pathogens isolated CoNS (65; 55.1%) and *S. aureus* (38; 32.2%) had the highest percentage of occurrence in air samples. This finding was comparable with studies conducted in Jimma [14], India [5], Pakistan [15], and Iraq [3]. In these cases the contamination source is usually endogenously from the normal skin flora of patients or exogenously from surgical staff, especially as CoNS was the main isolate in the OT air [3]. It was observed that OR table, floor and OR light were heavily contaminated with *S. aureus*. This finding was in line with similar studies in India [8]. However, this was contradicted with a study conducted in Pakistan [14] which reported *Bacillus* Spp. This might be due to variation in methodology and sample size. Among the *S. aureus* isolated only 5(7.7%) were methicillin resistant (MRSA).

The antimicrobial susceptibility pattern of bacterial isolates revealed that the most effective antimicrobials were vancomycin, amikacin, ciprofloxacin, and gentamicin with a resistance rate of 0.0%, 3.2%, 75.6% and 9.6% respectively. However, the highest resistance was against penicillin G and ampicillin with a resistance rate of 46.4%, and 39.2% respectively. On the other hand, *Staphylococcus* Spp. was found to be highly resistant against penicillin G and ampicillin (52.7%, 44.5% respectively). Multidrug resistance was observed among 23(36.5%) of the bacterial isolates.

Table 1: Antimicrobial resistance of bacterial isolates from major operation theatre (OT) at the Ayder Referral Hospital, Northern Ethiopia

Antibiotic (disc potency)	CoNS (n=68)	<i>S. aureus</i> (n=42)	<i>P. aeruginosa</i> (n=13)	<i>E. coli</i> (n=2)	Total (N= 125)
	Number (%) of resistance				
penicillin G (10IU)	32(47.1)	26(61.9)	NT	NT	58(46.4)
ampicillin (10µg)	30(44.1)	19(45.2)	NT	NT	49(39.2)
chloramphenicol (30µg)	11(16.2)	10(23.8)	NT	1	22(17.6)
amikacin (30µg)	2(2.9)	2(4.8)	0	0	4(3.2)
gentamicin (10µg)	9(13.2)	3(7.1)	0	0	12(9.6)
ciprofloxacin (5µg)	7(10.3)	0	0	0	7(5.6)
trimethoprim-Sulphamethoxazole (1.25/23.75 µg)	8(11.8)	0	NT	1	9(7.2)
tetracycline (30µg)	10(11.7)	16(38.1)	NT	1	27(21.6)
vancomycin (30µg)	0	0	NT	NT	0

NT: not tested, CoNS: Coagulase negative *S. aureus*

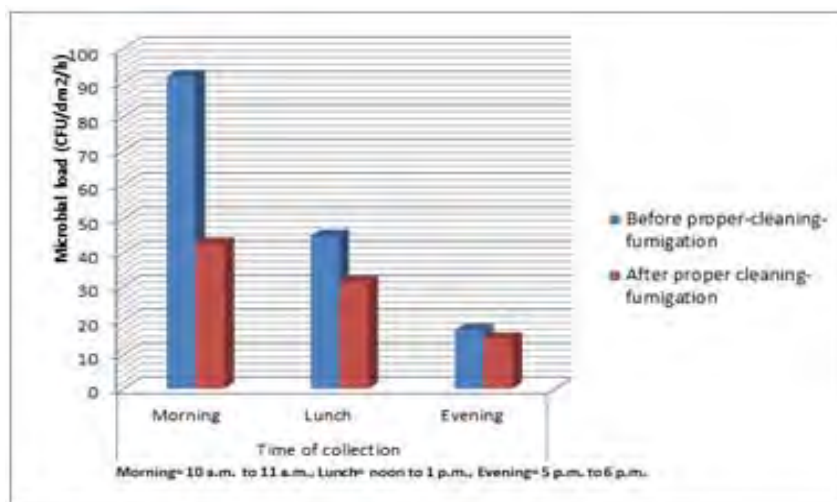


Figure 1: Bar chart showing microbial count of air in major operation theatre (OT) at the Ayder Referral Hospital Ayder Referral Hospital, Northern Ethiopia

Conclusions

In general the results indicate proper cleaning-fumigation of operating theatre significantly reduced the microbial contamination, and bacterial strains such as CoNS, *S. aureus*, and *P. aeruginosa* have a greater propensity to cause contamination in OT. The microbiological quality of air and surfaces in OT may be considered a mirror image of the hygienic conditions of the OT. Therefore, regular microbiological surveillance of the OT mandatory in reducing microbial contamination consequently postoperative infectious episodes can be reduced considerably. Furthermore, efforts should be also be made to ensure strict infection control practices in the OT.

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References

- [1] Al-Benna S. Infection control in operating theatres. *J Perioper Pract* 2012;22:318-322.
- [2] Fleischer M, Bober-Gheek B, Bortkiewicz O, Rusiecka-Ziółkowska J. Microbiological control of airborne contamination in hospitals. *Indoor Build Environ* 2006;15:53-56.
- [3] Ensayef S, Al-Shalchi S, Sabbar M. Microbial contamination in the operating theatre: A study in a hospital in Baghdad. *East Mediter Health J* 2009;15:219-223.
- [4] Okon KO, Osundi S, Dibal J, Ngbale T, Bello M, Akuhwa RT, et al. Bacterial contamination of operating theatre and other specialized care unit in a tertiary hospital in Northeastern Nigeria. *Afr J Microbiol Res* 2012;6:3092-3096.
- [5] Chacko L, Jose S, Isac A, Bhat KG. Survival of nosocomial bacteria on hospital fabrics. *Indian J Med Microbiol* 2003;21:291.
- [6] Reichman DE, Greenberg JA. Reducing surgical site infections: A review. *Rev Obstet Gynecol* 2009;2:212-221.
- [7] Reddy BR. Management of culture-negative surgical site infections. *J Med Allied Sci* 2012;2:2-6.
- [8] De Lissovoy G, Fraeman K, Hutchins V, Murphy D, Song D, Vaughn BB. Surgical site infection: Incidence and impact on hospital utilization and treatment costs. *Am J Infect Control* 2009;37:387-397.
- [9] Edmiston CE Jr, Seabrook GR, Cambria RA, Brown KR, Lewis BD, Sommers JR, et al. Molecular epidemiology of microbial contamination in the operating room environment: Is there a risk for infection? *Surgery* 2005;138:573-579.
- [10] Ekhaie OF, Ighosewo UO, Ajakpovi DO. Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria. *World J Med Sci* 2008;3:19-23.
- [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 Infect Control* 2012;40:750-755.
- [12] Chow TT, Yang XY. Ventilation performance in operating theatres against airborne infection: Review of research activities and practical guidance. *J Hosp Infect* 2004;56:85-92.
- [13] Patwardhan N, Kelkar U. Disinfection, sterilization and operation theater guidelines for dermatosurgical practitioners in India. *Indian J Dermatol Venereol Leprol* 2011;77:83-93.
- [14] 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;21:9-17.
- [15] Saadoun I, Al Tayyar IA, Elnasser Z. Concentrations of airborne fungal contamination in the medical surgery operation theaters of different hospitals in Northern Jordan. *Jordan J Biol Sci* 2008;1:181-4.
- [16] Pasquarella S, Pitzurra O, Savino A. The index of microbial air contamination. *J Hosp Infect* 2000;46:241-256.
- [17] Cheesbrough M. District laboratory practice in tropical countries. New York: Cambridge University Press; 2006.
- [18] Napoli C, Marcotrigiano V, Montagna MT. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. *BMC Public Health* 2012;12:594.
- [19] Singh K, Dar FA, Kishor K. Bacterial contamination in operating theatres of district hospital Budgam in Kashmir division. *IJMHS* 2013;3:62-63.
- [20] Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-second information supplement. *CLSI* 2012;3:1-183.
- [21] Kelkar U, Kelkar S, Bal AM, Kulkarni S, Kulkarni S. Microbiological evaluation of various parameters in ophthalmic operating rooms. The need to establish guidelines. *Indian J Ophthalmol* 2003;51:171-176.
- [22] Javed I, Hafeez R, Zubair M, Anwar MS, Tayyib M, Husnain S. Microbiological surveillance of operation theatres and ICUs of a tertiary care hospital, Lahore. *Biomedica* 2008;24:99-102.
- [23] Al Laham NA: Distribution and Antimicrobial Resistance Pattern of Bacteria Isolated from Operation Theaters at Gaza Strip. *Journal of Al Azhar University-Gaza* 2012;14:19-34.
- [24] Siridhar RPN: Testing of disinfectants. Available at: http://www.microrao.com/micronotes/pg/testing_of_disinfectants.pdf. Accessed: January 10/2014.