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.
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, 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

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

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, 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.

Acknowledgements

The authors would like to thank the College of Health Sciences and Ayder Referral Hospital Mekelle University for providing us the facility and funding support.

References