THE DEGREE OF BACTERIAL CONTAMINATION OF OPERATING ROOMS AND SURGICAL WARDS ENVIRONMENT AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ISOLATES AT JIMMA UNIVERSITY SPECIALIZED HOSPITAL, SOUTH WEST ETHIOPIA

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ABSTRACT

Surgical site infection (SSI) stands in the second position accounting one-third of total health care associated infection (HAI). One of the risk factor for SSI is bacterial contamination of indoor air and environmental surfaces in Operating rooms (ORs) and surgical wards (SWs). Different measures are undergoing to reduce these contaminations so as to reduce the risk of SSI of which determining the degree of bacterial contamination and antibiotic susceptibility pattern of the isolates in the environment is one measure. These will help to evaluate the bacterial load of the environments, select appropriate antibiotics as well as to revises and design appropriate hospital infection prevention protocols. Thus, the aim of this study was to determine the degree of bacterial contamination and antibiotic susceptibility pattern of isolates in ORs and SWs of Jimma University Specialized Hospital (JUSH). Laboratory based cross sectional study was conducted taking 252 environmental (108 indoor air and 144 housekeeping surface) samples using purposive sampling technique. The settle plate and sample rinse methods were used for the collection of air and housekeeping surface samples respectively. Then samples were processed with standard bacteriological techniques from October to January 2009/2010 and the data was analyzed using SPSS version 16 for descriptive statistics. The mean indoor air aerobic colony count (ACC) of most SWs and ORs of JUSH were beyond acceptable range being Critical Zone of ORs exceptionally with highest count. Similarly, the mean ACC in housekeeping surfaces (Floor and Table top surfaces) of both ORs and SWs were also higher when compaired with the recommended standard level. Staphylococcus aureus was a predominant isolate 44.4% and 33% from both indoor air and housekeeping surfaces respectively. Among potential pathogenic Gram negative bacteria identified from housekeeping surfaces of ORs and SWs, Klebsiella spp and Escherichia coli were frequently isolated with a rate of 11% each followed by Citrobacter spp (7%) and P. aeruginosa (6.3%). Regarding to the antimicrobial susceptibility pattern of isolates, S. aureus showed 100% (70 isolates tested) resistance to methicillin and 82.3% (96 isolates tested) to ampicillin. Similarly, more than 87% of Klebsiella spp and E. coli were resistant for ampicillin and cephalothin. Unexpectedly, multidrug resistant Enterobacteriaceae for the antibiotics tested was seen in more than 90 % of isolates. Since, the indoor air and housekeeping surface of ORs and SWs of JUSH revealed high degree of aerobic bacterial load being most of the isolates are a potential pathogens and multi drug resistant; an appropriate measures on the infection prevention system need to be implemented to reduce the risk of SSIs.

Key words: Bacterial contamination, indoor air, housekeeping surface, surgical site infection

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OPERATIONAL DEFINITION

Bacterial isolates:

Refers to bacteria that can be found from indoor air and housekeeping surfaces in OR and surgical ward units when incubated at 37c^{o} in aerobic condition for 24 hours

Colony forming unit:

Either one or an aggregate of bacterial cells which, when cultivated on solid media, will develop into a single visual colony.

Critical Zone:

Immediate working zone where the actual surgical procedure is performed and where surgical instruments come into direct contact with work surfaces (includes OR-1 and OR-2)

Degree of bacterial contamination:

To what extent high or low is ACC from indoor air and housekeeping surfaces with respect to the standards and other studies as well as what type and how much potential pathogenic bacterial isolates are there in OR and SW of JUSH

Environmental surfaces:

Inanimate environmental surfaces in OR and SW units
(Housekeeping surfaces)

Female clothing room:

The room in the Intermediate Zone where females change surgical cloths

General surgical department

Refers to the three operating room zones (Critical, Intermediate and Non-critical Zones) and surgical ward units (SW-female rooms and SW-male rooms) of JUSH

Health-care associated infection:

An infection, that occurs in a client (patients) of any health-care facility during receiving medical services after 48 hour of admission.

Hospital environment:

Refers to indoor air and environmental surfaces of ORs and SWs environments unless it is stated as "the overall hospital environment" to express the whole hospital environment

Housekeeping surfaces:

Environmental surfaces (floors and tabletops) that are not involved in direct delivery of patient care in general surgical department of JUSH.

Intermediate:

When bacteria isolated from hospital environment is partially inhibited (with a predetermined zone of inhibition for a particular antibiotics and bacterium) when grown in a Muller-Hinton media containing antibiotics.

Intermediate zone:

The area in the operating room where surgeons and their assistants change their surgical cloths and shoes as well as sterilized cloth storing rooms (includes SCS, FCR and MCR)

Male clothing room:

The room in the Intermediate Zone where males change surgical cloths

Multi drug resistance:

A bacterium that cannot be inhibited or killed by two or more antibiotics with their corresponding inhibitory or lethal dose

Non-critical zone:

The area in the operating room where surgical and other instruments are sterilized, packed and stored in a clean support room (includes SR, PR and SMS)

OR units (ORs):

Operating room units includes three zones; Critical (OR-1, OR-2 and minor OR), Intermediate Zone (SCS, FCR and MCR) and Non-Critical Zone (SR, PR and SMS)

Packing room:

The room in the non Critical Zone where sterilized surgical equipments and surgical Cloths are packed

Potential pathogenic bacteria:

Bacteria that were isolated from air and housekeeping samples in OR and SW including: *S. aureus*, *S. pyogenes*, *Klebsiella* spp, *E. coli*, *P. aeruginosa*, *Citrobacter* spp, *Enterobacter* spp, *Acinetobacter* spp, *Providencia* spp and *Serratia* spp

Resistance

When bacteria isolated from hospital environment grow with in the inhibition zone (with in a predetermined zone of inhibition for a particular antibiotics and bacterium) of antibiotics when cultured in a Muller-Hinton media.

Sterilized cloth store:

The room in the Intermediate Zone where sterilized surgical clothes are stored

Susceptible

When bacteria isolated from hospital environment cannot grow in the inhibition zone (with a predetermined zone of inhibition for a particular antibiotics and bacterium) of antibiotics when inoculated in a Muller-Hinton media.

SW units (SWs):

Refers to Female room-1, Male room-1, Female room-2 and Male room-2

ABBREVIATION

ACC → Aerobic Colony Count

AST → Antimicrobial Susceptibility Testing

CDC → Center for Diseases Control and prevention

CFU→ Colony Forming Unit

FCR→ Female clothing room

FR-1→ Female room one

FR-2→ Female room two

HAI → Health care Associated Infection

HE→ Hospital Environments

JUSH→ Jimma University Specialized Hospital

MCR→ Male clothing room

MRSA→ Methicillin resistant *S. aureus*

MR-1→ Male room one

MR-2→ Male room two

OR→ Operating Room

OR-1→ Operating room one

OR-2→ Operating room two

PR→ Packing room

SCS→ Sterilized cloth store

SMS→ Sterilized material store

SR→ Sterilizing room

SSI → Surgical Site Infection

SW → Surgical Ward

WHO → World Health Organization

CHAPTER 1: INTRODUCTION

1.1. Background

Health care associated infection (HAI) also called nosocomial infection is a localized or systemic condition occurring in a patient during receiving medical services which results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) that was not present or incubating at the time of admission to the health care institutions (1, 2, 3).

In an average, depending on the population surveyed and definitions used, studies have shown that the prevalence of HAI is varied in different countries. In developed continents like Europe and North America, it occurs in 5%-10% of all hospitalizations (2, 4, 5). In countries like Greece and Spain, for example, the prevalence is 9.3% (6) and 7.9% (7) respectively. In USA, 2 million people per year develop HAIs (3). But in developing countries as in part of Asia, Latin America and Africa (especially sub-Saharan Africa), the prevalence can reach in more than 40% of hospitalizations (8). Generally the risk of HAIs in developing countries has been estimated to be 2–20 times higher than that in developed countries (9). This higher prevalence can partly be attributed to poor infrastructure, over-crowding, inadequate health personnel and management in most hospitals (10). The prevalence in African countries like Morocco, Tunisia and Ethiopia is 17.8 % (4), 17.9% (5) and 13 % (11) respectively.

Microorganism which causes HAI is called health care acquired pathogens and almost any microbe (like bacteria, fungi and viruses) can cause the infection. HAIs due to bacterial agent can be caused by a wide variety of bacterial species. Some of these include *Staphylococcus* species, *Streptococcus* species, *Enterococcus* species, *Pseudomonas* species, *Klebsiella* species, *Escherichia coli, Enterobacter* spp, *Citrobacter* spp, *Acinetobacter* spp, *Proteus* species, *Serratia* spp and *Salmonella* species of which *Staphylococcus* species, *Enterococcus* species, *P. aeruginosa, E. coli, Klebsiella* spp and *Streptococcus* species being the most frequently reported (10, 12, 13, 14).

The HAI pathogens can come either from endogenous or exogenous sources. Endogenous sources are those that are from the patient's own microbial flora following surgical, diagnostic, therapeutic procedures and other factors which translocate the bacteria from their normal habitat

and/or suppresses the natural defense of the patients. While exogenous sources are from the surrounding which includes hospital staffs and inanimate environmental sources like contaminated surfaces, air, food or water. Environmental reservoirs like housekeeping surfaces can act directly as a source of infection for the patients when there is direct contact or may contaminate health personnel acting indirectly [for example, around four in ten of a health personnel entering to environment contaminated with *S. aureus* will take the bacterium though they do not make any contact with patients in the ward (15)] as a source of infection (10).

HAIs are worldwide public health problems affecting developed and developing countries. They have a multi directional impact constituting an important cause of mortality, morbidity and increase treatment costs (2, 5, 6). In USA, HAIs were responsible for 0.7% to 10.1% of all deaths occurred in the early 1990s and 1,737, 125 infections in 2002 (16, 17). On average, around 90,000 deaths occur in USA annually as a result of HAIs (3). In 2001, 21.3% of deaths in Spain hospital were due to HAI (17). Whereas, the population-attributable risk of death due to HAI in France ranges from 2.1% to 4.0% (18). HAIs has also (directly or indirectly) a great economic impact as a result of increased length of hospital stay, need for isolation of patients, increased antibiotic and diagnostic test use (2). HAI is responsible for more than 4.5 billion dollar (3, 19) and 2 billion dollar (20) excess health care costs annually in USA and England respectively.

Eventhough there is limited data on the morbidity, mortality and excess costs attributed to HAI (16), the impact is more severe on the cost-effectiveness of health care provision in developing countries. It is generally recognized that the lower the economic status of a given country, the greater HAI impact in mortality and compromise quality of life (4, 19). Some data in developing countries indicates that HAI is greatly exploiting the weak economy of different countries. In Thailand, it has been estimated that the infections cost more than 40 million US dollar every year (21).

There are 13 major categories of HAIs depending on the site involved (1) but the most frequent ones are surgical site infection (SSI), urinary tract infections and lower respiratory tract infections (2). Surgical site infections, which is an important clinical indicator for quality of patient care and infection control (22), is the second most common type of HAI and accounts for almost one-third of all health care associated infection. The prevalence of SSI varies from one country to another country depending on different factors (for example level of infection prevention and control practice). Africa, which have low infection prevention practice measures, have high rate of SSI compared to developed countries. The infection rate in developed countries, for example, like Greece and Turkey is 5.3% (23) and 4.53% (24) respectively. But the rate in African countries is higher than developed countries (4). The prevalence of SSIs in Morocco and Tanzania is 19.5% (4) and 23.5% (25) respectively. Where as a study in Ethiopia indicates that from the total HAIs, wound infection (including surgical wounds) ranks first accounting around 49% (11).

Over all, SSI rate is determined by the bacterial contamination level of hospital environment together with the surgeon's technique during the operation, patient's degree of susceptibility, insertion of foreign material or implants, appropriateness of surgical preparation, adequacy and timing of antimicrobial prophylaxis (24, 26, 27).

1.2. Statement of the problem

Surgical site infection (whose rate is related with bacterial contamination of the environment) to occur, there must be susceptible host, a pathogen and its source (reservoir). Patient's normal flora, health personnel and hospital environment can act as a source of infection. Thus patients under gone some surgical procedures can develop SSI by acquiring the agent (in this case-bacteria), directly or indirectly, in OR's and/or SW's environment. Due to contaminated hospital environments (HE) and rapid progress in the development of antibiotic resistance in a wide variety of health care acquired bacterial pathogens particularly contaminating the HE along with high number of immunocompromised patients and increasing variety of medical procedures and invasive techniques (creating potential routes of infection), SSIs are still a major problem in modern medicine posing a substantial public health impact interms of morbidity, mortality and extra health care cost (2,19, 27, 28). SSI lengthens the hospitalization stay by an average of 7.4 days and raises the cost of hospitalization by more than 800 dollars (29). From the extra health care associated cost due to nosocomial infection in USA (for example), the highest proportion is due to SSIs which accounts 42% from the total extra cost (26).

Now days, the surgical interventions are complex and time consuming that demands intensive activities, a large team of professionals and the use of many different instruments. These situations make the effective control of variables that interfere in the health, wellbeing and comfort of patients increasingly important. Thus to achieve acceptable performance, operating rooms (ORs) and surgical wards (SWs) should accomplish a complex range of infection prevention measures by considering different contamination risks for SSI. One of the risk factor for the development of SSI is bacterial contamination of indoor air and environmental surfaces in ORs and SWs (30). Though the extent to which hospital environment contributes to the development of SSI is not fully exploited because of measurement difference between studies and variability in institutional cleaning qualities (which act as a confounder) (12, 31, 32), the role of the environment as a reservoir of potential pathogens has also received increasing attention.

One study on environmental contamination by methicillin resistant *S. aureus* (MRSA) indicates that 73% of the hospital rooms containing patients infected with MRSA and 69% of the rooms containing patients colonized with MRSA had some environmental contamination (33). In some occasions, the HE can become contaminated extensively that the infection control system which is under implementation by the hospital may not be effective needing more enhanced cleaning. For example, studies indicate that conventional cleaning of surfaces in hospital rooms of patients with vancomycin resistant *Enterococcus* spp was inadequate on 16% of occasions but these surfaces were uniformly free of vancomycin resistant Enterococcus (VRE) after more enhanced cleaning. In another occasion, enhancing environmental cleaning results control of outbreaks due to vancomycin resistant *Enterococcus* spp which was indicated by a decrease in environmental culture positives of the isolates from 29% to 1% (34).

Over all, a favorable niche for bacterial pathogens (for resistant and non resistant strains) can quickly be found in the environment and retained unless disturbed by some appropriate infection prevention process. It is indicated that when the environment is heavily contaminated by bacterial pathogens (despite routine cleaning), different Organisms such as VRE, MRSA and other bacteria survive for long period on surfaces (35, 36). Even exemplary hand hygiene cannot completely remove nosocomial bacterial pathogens thus it cannot prevent the environment from acting as a source of SSIs (32, 37). Thus to minimize the extra burden like prolonged morbidity, mortality and associated extra healthcare costs in the community due to SSI (HAI as well), identifying these risk factors and implementing appropriate infection preventive measures based on the local guidelines established is the best way (4, 17, 19, 28, 38). Since a well implemented infection control program can reduce the incidence of HAIs by around one-third (though eradication is impossible) (5) as it is done in countries like USA (19).

So, minimize the contribution of hospital environment due to bacterial contamination (which is one of the risk factor) acting as a reservoir for bacterial pathogens causing SSIs, cleaning of environmental surfaces (in addition to hand hygiene) should be implemented in a cost effective manner and its effectiveness should be assessed based on microbiological basis rather than using subjective criteria like visual assessment which is poor indicator of cleaning. In one study, 82% of ward sites were assessed as visually clean yet only 30% were considered clean using microbiological techniques. Reduction of airborne bacteria in the operating room by about 13-fold, for example, would reduce the wound contamination by about 50% (39). Thus cleaning should be investigated as a scientific process with measurable outcome with integrated approach. This would include from simple preliminary visual assessment (as the first stage in an integrated monitoring programme) to specific microbiological investigations (32, 40).

Therefore the present study was intended to measure the degree of bacterial contamination of OR and SW environments and determine antimicrobial susceptibility pattern of the isolates as part of microbiological investigation.

CHAPTER 2: LITERATURE REVIEW AND SIGNIFICANCE OF THE STUDY

2.1. Literature review

Wide variety of microorganisms including potential pathogenic bacteria are found in hospital environment where different people with different types of diseases with various pathogenic organisms aggregate. There are several reports demonstrating bacterial contamination of different environmental sites in ORs and SWs including indoor air, tables, floors and other furniture surfaces with the potential to lead to nosocomial spread (27, 35, 41, 42, 43, 44).

2.1.1. Indoor air

Indoor air can be polluted with potential bacterial pathogens released in to the air from different sources. Environmental surface reservoirs like housekeeping surfaces (more than 15% of the bacteria of OR air comes from floor (43)), patients, carrier health personnel, construction activities and delayed maintenance can act as a source for bacterial air pollution through shedding and environmental disturbance during different activities (45, 37). Factors like number of visitors, number of carrier health personnel, extent of indoor traffic, time of day and the amount of materials brought in from outside aggravate the extent of air bacterial contamination. Airborne dispersal of *S. aureus*, for example, is directly associated with the concentration of the bacterium in the anterior nares. Approximately 10% of healthy carriers are known to disseminate *S. aureus* into the air. So that microbiological quality of indoor air can be considered as a mirror of the hygienic conditions of the operating room (45, 46, 47).

There are a few standards set for the acceptable indoor air bacterial load in different hospital environment. A bacteriological standard of indoor air in ORs at different condition and in SWs initially suggested by Fisher *et al* in the 1970s (48) [its English version is also found on review article published by Pasquarella *et al* (49)] set different aerobic bacterial load levels. According to this standard, the total aerobic bacterial count of samples obtained from the indoor air when ORs at rest, ORs in activity and SWs should be in the range of 0-4, 0-60 and 0-250 CFU//hr respectively to be optimal. The bacterial load is acceptable in these rooms when it is in the range of 5-8, 61-90 and 251-450 CFU//hr respectively. Whereas when the bacterial load is greater than these limits, it is considered as unacceptable. In other words, the bacterial load of these rooms become unacceptable when the load is greater than 9, 91 and 451 CFU/hr for ORs at rest, ORs active and SWs in general respectively.

Studies have been conducted to determine the bacterial load of indoor air in ORs and SWs of hospital environments. A study by Suzuki A. *et al* (1984) in Japan was conducted to determine airborne contamination in a conventional operating rooms for five years using settle plate methods and other methods indicates that the rooms when active showed a higher bacterial load than when they were at rest (empty) showing a 12 times increase in mean total aerobic bacterial count. In the same study, it is also shown that the bacterial count in the Critical Zone doubled in the Intermediate Zone and further doubled in the Non-Critical Zone (50).

Another study conducted in Nigeria state hospital taking indoor air samples from Female Surgical Ward, Male Surgical Ward and major OR using settle plate method indicated that a mean aerobic colony count (ACC) was 42.25, 30.5, 3.25 CFU respectively (51).

2.1.2. Housekeeping surfaces

Hospital environments like housekeeping surfaces could become contaminated by different reasons like by settlement of airborne bacteria, by contact with shoes, trolley tables, other solid objects and occasionally by fecal contamination, spilling of urine, pus, sputum and other fluids (44).

There are a few proposed bacteriological standards to evaluate the load and distribution of potential pathogenic bacteria in hospital environmental surfaces as that of indoor air bacterial load standards.

The proposed standard states that the total bacterial load and the species isolated in samples taken from housekeeping surfaces from different units of targeted environment should be comparable with the stated values [which is supported and sited by different guideline like British Society for Antimicrobial Chemotherapy (52), CDC (53), Scottish Infection Standards and Strategy (54) and literatures (15, 55, 56, 57)]. The standard recommends the acceptable aerobic bacterial load of environmental surface in hospitals based on two criteria: indicator organisms and quantitative aerobic colony count. The first criteria is finding of a specific 'indicator' organism, the presence of which suggests a requirement for increased cleaning. Indicator organisms include *S. aureus*, vancomycin resistant *Enterococcus* (VRE) and multiple resistant Gram-negative bacilli. The second criterion is quantitative ACC which is the total number of aerobic organisms from a sampled area. The ACC from a hand contact surface should be < 5 CFU/cm^2. The finding of ≥ 5 CFU/cm^2 from a hand contact surface, whatever the identity of the organisms, indicates that there might be an increased risk of infection for the patient in that environment. This should generate an evaluation of the cleaning/disinfection practices and frequencies for that surface (32).

A study was conducted to determine the bacterial load of housekeeping surfaces taking floor samples from Operating Rooms by Hambraeus A *et al* (1978) in Sweden. In the study, the highest aerobic bacteria count was seen in the Dressing Room of the Intermediate Zone with 7 CFU/cm^2 where as the count was 0.72 CFU/cm^2 in Critical Zone (58).

2.1.3. Potential pathogenic bacteria in hospital environment

Different potential pathogenic bacteria are isolated colonizing environmental surfaces of hospital environment (35). Some of them which are frequently isolated include *S. aureus*, *Enterococcus* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* species, *Streptococcus* species, *Citrobacter* species, *Enterobacter* spp, *Acinetobacter* spp and other bacteria (12, 59, 60, 61). Most of these bacteria persist on dry environmental surfaces for months. All members of the Staphylococcal family have the ability to survive in the environment over a wide range of temperatures, humidity and exposure to sunlight (62). In a study, *Staphylococcus* species and *Enterococcus* species shows maximum survival time of more than 60 days (31). When mixed with hospital dust, MRSA can still be viable even for more than one year after inoculation (33). Gram negative bacteria like *Klebsiella* species, *P. aeruginosa* and *E. coli* show a maximum survival time of 27, 25, 20 days respectively in different hospital environment surfaces (31). The other gram negative bacteria *Acinetobacter* spp have long mean survival time on dry surface approximately 27 days – a year depending on the species and strain types (63).

Studies also indicated that bacteria which are found in the environment are responsible for increased risk to acquire HAI (e.g. SSI) compared to non- colonized environment. In one study, a positive culture result for VRE in samples taken from environmental surface of patient's room (like tables and floors) before admission increases the risk of acquisition of the bacterium by more than 3-fold when compared with culture negative rooms. Similarly different bacteria were isolated from hands of health care workers after contact with environmental surfaces near hospitalized patients. Some of the bacteria include *S. aureus*, *Enterococcus* spp. and *K. pneumoniae* implying that hand contamination is, in part, from touching contaminated surfaces or equipments (33, 35, 36, 42, 45, 60, 64).

Moreover, different investigations using molecular epidemiology and experimental study designs indicate that bacteria which are found in the environment are responsible for different outbreaks (60). An outbreak in surgical ward was occurred by a multidrug resistant *P. aeruginosa* in USA hospital around 1977. Based on the investigation undertaken by the help of serological, pyocin and phage typing, it was found that the source of *P. aeruginosa* was the hospital environment. Finally, the outbreak by the bacterium was concluded that it was due to cross contamination from

surgical ward environment (65). Similarly, multiple-resistant *K. pneumoniae* outbreak in Scotland was occurred in 1992-1995. In the outbreak, the bacteria expressing expanded spectrum β-lactamase (ESBL) activity was described and environmental screening revealed extensive ward contamination. Though there was a decline following the implementation of standard infection control procedures, difficulty in controlling the outbreak was encountered due to heavy environmental contamination (66). In one study, it is also found that the *Acinetobacter* spp. isolates from housekeeping surfaces were indistinguishable from isolates from patients (13) indicating environment as a potential source of the bacteria. Similarly, *S. aureus* is frequently responsible for SSI through air born transmission in SW and operating theater (67).

Another reason which makes the contribution of hospital environment to SSI development more sever is that a significant proportion of potential pathogenic bacteria isolated from the environment develop a multi-resistant to different antibiotics. The occurrence of multi-drug resistance in these pathogens has resulted in the emergence and reemergence of difficult to treat HAIs in patients. Examples of bacteria possessing such drug resistance are methicillin-resistant *S. aureus*, penicillin resistant *Pneumococcus*, vancomycin-resistant enterococci, vancomycin resistant *S. aureus*, multi drug resistance Enterobacteriaceae, multi drug resistant *P. aeruginosa* and *Acinetobacter* spp (61, 68, 69, 70, 71). As a result, from more than 2 million HAIs occurring each year 50 to 60% are caused by antimicrobial-resistant strains of bacteria in USA (72).

According to a study conducted by Wolde Tenssay Z. on "Multiple antimicrobial resistance in bacterial isolates from clinical and environmental sources of Jimma Hospital, Southwest Ethiopia" in 2002 showed that the most frequent bacterial isolates were *S. aureus*, *Proteus* species and *Klebsiella* species with a frequency of 17, 10 and 8% respectively. Whereas *E. coli* and *Enterobacter* speciese together accounts 14%. In the study, the antimicrobial susceptibility test result showed that *E. coli*, *Klebsiella* species and *Enterobacter* species were 100% resistant for ampicillin where as *S. aureus* was 93% resistant to the same antibiotic. On the other hand, almost all the isolates were found to be multiple resistant to ampicillin, tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole which are the commonly used antimicrobials (73).

2.1.4. Measures of controlling bacterial contamination of hospital environment

Despite improvements in hand hygiene and ongoing efforts to optimize isolation practices, reducing the spread of health care–associated pathogens to patients constitutes one of the most challenging unless appropriate infection control measure is implemented on the hospital environment (74, 75). There are different measures which are known to reduce the total bacterial load and potential pathogenic bacteria in hospital environment to the optimal level. Some of these measures include reducing foot trafficking in and out of the OR, improving the ventilation system, appropriate educational intervention on routine cleaning which is under implementation, programmatic (arrangement) intervention on the existing cleaning system and enhancing the existing cleaning system (50, 57, 76, 77). If these intervention measures are appropriately implemented, significant improvement in cleaning of hospital environment will be achieved (57) as confirmed by culture (57, 76, 74) and non-culture based (57, 75) cleaning evaluation studies following implementation of the measures.

Different studies directed in educational and programmatic intervention on the existing cleaning systems in different hospitals indicate improvement in the cleaning of hospital environment is possible. In a study by Carling CP *et al* (75) indicated that sustained improvement in cleaning of housekeeping surfaces was achieved following educational intervention by more than 2-fold when compared with cleaning before the intervention. Similarly, culture based study indicated that the rate of environmental contamination of hospital environment with potential pathogenic bacteria (with Vancomycin resistant Enterococcus and *Clostridium difficile*) fall from 71% to 0-20% after routine cleaning following educational and programmatic interventions (57).

In the same way, enhancing the existing cleaning system improves the contamination of hospital environments. A study by Dancer JS *et al* in surgical ward shows that one extra cleaner was associated with a 32.5% reduction in levels of microbial contamination and reduced recovery of MRSA from 16 to 12 hand touch sites as well as decreased ward acquired MRSA infection from 9 to 4 after intervention (76). Similarly, another study by Rampling A *et al* indicated that the aquestation of environmental MRSA was reduced greatly by increasing the domestic cleaning hour of housekeeping surfaces and medical equipments. The study also indicated that as a result of enhanced cleaning, it was also possible to effectively control MRSA outbreak (in which promoting hand hygiene and isolation of patients alone was not effective) (74) as well as different outbreaks by *Acinetobacter* spp (15).

Thus, a single or a combination of infection control measures explained were found to be effective in reducing the bacterial contamination level of hospital environment as confirmed by different studies.

2.1.5. Method of indoor air and housekeeping surface sample collection

Air specimen for bacteriological investigation can be collected by different methods which can generally be classified into four methods: counting of colony forming units per cubic meter of air (cfu/m3), the count of CFU on settle plates, measurement of a chemical component of the microbial cells/m3 of air and the count under the microscope. The measurement of chemical components of microbial cells (DNA, enzymes) has not yet produced practical and reliable methods for the study of airborne micro-organisms. Counts under the microscope or by automatic counters in fluorescence have limited applications and are still under study. At the moment, the only effective means of quantifying airborne microbes is limited to the count of CFU. The CFU count is the most important parameter as it measures the live micro-organisms which can multiply. The method designed for counting CFU, which is widely used, can be performed in two ways: by active air samplers or by passive air sampling (the settle plate method). Active air sampling could be performed by different methods including impingers, impactors (slit-type), impactors (sieve type), filtration samplers, centrifugal samplers and electrostatic precipitation samplers. Whereas passive air sampling is performed using settle plates. Petri dishes containing a solid nutrient medium are left open to air for a given period of

time. Though both active and passive sampling has its own advantage and disadvantages, the passive sampling is economical, reproducible and feasible (readily available). It allows the evaluation of surface contamination settling from the air and does not disturb the natural trend of the microbial population in the air during the sampling time. It also measure microbial fallout rather than air suspended microbes which is more practical and a more relevant indicator of actual wound contamination rate if it is used specially in Operating rooms and Surgical wards. The Settle Plate Method, for example, was used for bacteriological monitoring by 49% of 147 centers with 438 Operating Theaters assessed in Great Britain and Ireland (49, 78).

Environmental surface sample can be performed by different methods of which contact plate method and sample/rinse method are the common one. Compared with contact plate method, Sample/Rinse Method is frequently chosen. This is because sample/rinse method is widely used, simple, easy to use and most accurate for enumerating micro-organisms on a surface (79, 80).

2.2. Significance of the study

Despite a relative advancement of HAI prevention in developed countries, still there is no well established measure in developing countries that could be applied based on the local situation in the countries. In Ethiopia, as part of developing countries, there is no well established infection prevention measure in health care institutes even though the country has prepared national guide line in 2004 (81). The problem is compounded by environmental contamination of hospital environment in the health care setting which acts as significant source of HAI causing a magnificent morbidity and mortality. Thus it creates a public health problem mainly due to lack of data indicating the level of environmental contamination in the hospital setting which is very important to devise appropriate infection prevention measures (32).

Many HAIs have been demonstrated to be due to cross transmission of bacterial pathogens from hospital environment. From different hospital environments, OR and SW environments are one of the workplaces that need the highest hygiene standards (64). Most of the infections arising from these environments could potentially be prevented through adequate application of infection prevention practices (82). Different methods are implemented of which measuring the degree of bacterial contamination of hospital environment as well as determining the susceptibility pattern of the isolates to commonly used antibiotics in the area is the main one. Such kind of study will help to evaluate infection prevention measures under implementation (to some extent), to select appropriate antibiotics for empirical therapy (which requires understanding of local susceptibility patterns of bacterial pathogens) as well as to revises and, if necessary, design appropriate hospital infection prevention and control protocols in an effort to minimize the incidence of SSI (3). By taking these facts into consideration, the present study was conducted to determine the degree of bacterial contamination of ORs and SWs environment to measure antimicrobial susceptibility profile of the isolates at JUSH in Ethiopia area where there is no such studies has been conducted. The study was also intended to provide a base line information for whom interested to make further studies.

CHAPTER 3: OBJECTIVE

3.1. General objective

- To determine the degree of bacterial contamination of ORs & SWs environment and antibiotic susceptibility pattern of isolates at JUSH

3.2. Specific objectives

- To determine the total bacterial load of indoor air and housekeeping surfaces in ORs and SWs
- To isolate potential pathogenic bacteria from the indoor air in OR and SW units
- To identify the distribution of bacterial pathogens from housekeeping surfaces in ORs and SW units
- To describe antibiotic susceptibility pattern of bacterial isolates from different sites
- To generate base line information and forward appropriate recommendations based on the findings

CHAPTER 4: MATERIALS AND METHODS

4.1. Study area & study period

Study area:

The study was carried out in JUSH. The hospital which is found in Jimma town located south west Ethiopia 335 km from the capital-city, Addis Ababa. The town is located: 07°39' Lat and 36°50' Long, at an altitude of 1700-1750m above sea level (83). JUTH is the only hospital in the town with 300 beds. General surgical department is one of the departments in the hospital.

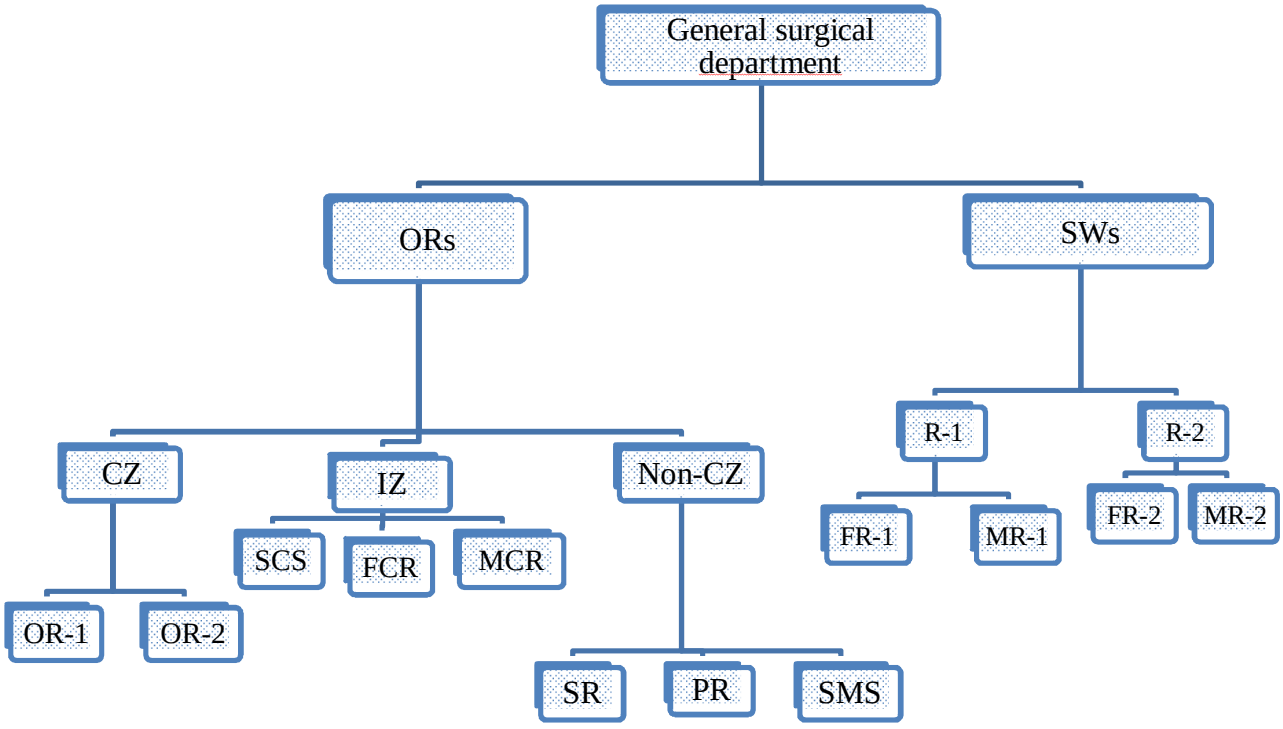

Figure 1: A diagram showing the rooms of General Surgical Department of Jimma University Specialized Hospital.

ORs: Operating Rooms
CZ: Critical Zone
IZ: Intermediate Zone
Non-CZ: Non Critical Zone
OR-1: Operating Room-1
OR-2: Operating Room-2
SCS: Sterilized Cloth Store
FCR: Female Clothing Room
MCR: Male Clothing Room
SR: Sterilizing Room
PR: Packing Room
SMS
SWs: Surgical Wards
R-1: Room-1
R-2: Room-2
FR-1: Female Room-1
MR-1: Male Room-1
FR-2: Female Room-2
MR-2: Male Room-2

The general surgical department of JUSH consists of ORs which is divided in to three zones and four SW units. The four SW units (FR-1, MR-1, FR-2 and MR-2) have a total of 53 beds. The surgical ward rooms (R-1 and R-2), which is separated by long corridor, are served by two ORs (OR-1 and OR-2) where the actual surgical procedure is performed and is considered as Critical Zone. The other two zones of ORs are called Intermediate (includes SCS, FCR and MCR) Zone and non-Critical Zone (includes SR, PR and SMS). Both ORs and SWs are situated at ground level. The ORs are connected to SWs by a long corridor.

The environmental samples were collected from the general surgical department of JUSH and processed in Microbiology Laboratory of Jimma University. This well equipped laboratory performs different microbiological investigations including parasitological and serological tests for academic and research purposes. It also gives service (culture and susceptibility testing) for needing community.

Study period

The study was conducted from October to January, 2009/2010.

4.2. Study design

Cross sectional study

4.3. Population

4.3.1. Source population

General Surgical (SWs and ORs) department environment

4.3.2. Sample population

Sample population of the study was indoor air and housekeeping surface (Tabletop and Floor) of ORs and SWs.

Sample population was selected by considering different factors like their representativeness of the environment targeted, the study objective and its significance.

4.4. Sample size & sampling technique

4.4.1. Sample size

The sample size was determined by taking into account the factors such as sampling site, time, number (frequency) and interval as it was recommended by CDC (84) and Pasquarella *et al* (49) in an attempt to make the data representative. Accordingly a total of 252 samples were taken for the study by considering additional different guidelines prepared by CDC (45) and Scottish quality assurance specialist interest group (79).

4.4.1.1. Number of indoor air samples:

The bacterial concentration in indoor air may be affected by factors like indoor traffic, visitors entering the facility, time of a day and width of the room to be sampled. To be meaningful, CDC and quality assurance specialist interest group recommends considering such factors in conducting microbiological study (45, 79). Thus to make the samples taken from indoor air in SWs and ORs representative of the environment targeted, the sample size was determined by considering these factors as well as different literatures (39, 51).

Sampling of ORs indoor air was performed from three zones (Critical Zone, Intermediate Zone and non-Critical Zone). In Critical Zone, sampling was done at two different rooms using two separate culture plate for two rounds and one separate culture plate for the remaining two rounds of sample collection in each rooms. In Intermediate Zone and non-Critical Zone, sampling was done using one separate culture plate in three rooms of each zone for four rounds. Thus, from these OR units a total of 36 air samples were taken.

Air sampling of the four SW units (FR-1, MR-1, FR-2 and MR-2) were performed in two different sites (center and corner of the units) at three different time of a day using one separate culture plates in each sampling sites. During the study, each room is sampled three times. Thus a total of 72 air samples from surgical ward units were taken. The data collection period, sampling frequency (interval) and number of sites for air samples was determined by considering the recommendation of CDC (45), other studies by Ekhaise OF *et al* (46), Sarica S *et al* (85) and the resource available for the study.

4.4.1.2. Number of housekeeping surface samples:

To make the sample representative, CDC recommends deciding location of surface to be sampled and number of replicate samples based on guidelines and available literatures (45, 36). Thus based on literature search and guidelines (79), 144 housekeeping surface samples were taken.

Housekeeping surfaces in ORs were sampled from three zones (Critical Zone, Intermediate Zone and non-Critical Zone). In Critical Zone, two housekeeping surfaces (floor and table top surfaces) were sampled twice for two sampling rounds and once for the remaining two rounds at two different rooms. In Intermediate Zone and non-Critical Zone, sampling of two housekeeping surfaces was done at three different rooms in each zone for four rounds (once in each round). Thus in the study period, 72 samples were taken from ORs. Similarly, two housekeeping surfaces in four SW units (FR-1, MR-1, FR-2 and MR-2) were sampled at three different time of a day. During the study, each room is sampled three times. Thus a total of 72 housekeeping surfaces were taken from SWs. Hence, the total of housekeeping surface samples taken from ORs and SWs were 144 (i.e. 72 samples each).

4.4.2. Sampling technique

The sampling technique used for study subject selection to collect bacteriological samples was purposive sampling technique.

4.5. Variables

4.5.1. Independent variables

- Housekeeping surfaces
- Indoor air
- OR units
- SW units
- Site of indoor air collection in SWs
- Time of indoor air collection in SWs
- Time of housekeeping surface collection in SWs
- Antibiotics tested

4.5.2. Dependant variables

- Aerobic colony count
- Bacterial isolate
- Susceptibility results

4.6. Data collection

4.6.1. Sample collection

4.6.1.1. Bacteriological Sampling of hospital Environment

Bacteriological samples of indoor air and housekeeping surfaces were taken from different OR and SW units. During sample collection, a survey format was field at the time of collection which contains code number, collection time, type of specimen and other parameters (Annex-I).

There were twelve rounds of sampling in SW (for both air and housekeeping surface) for the three month of sample collection period. Each sample collection rounds were every week. In each sample collection, one SW unit was sampled by taking six air and six housekeeping samples. Thus a total of 144 air and housekeeping samples were taken at different site and time from SW units. On the other hand, OR units were sampled four times in the study period. In each round of sampling, 18 housekeeping surface and 9 air samples were taken. Thus in OR units, 72 housekeeping and 36 air samples were taken at different site and time during the study period.

4.6.1.2. Method of indoor air samples collection in ORs and SWs:

In the present study, air sample was collected by the Settle Plate Method which is economical, reproducible and feasible (readily available) (49).

4.6.1.2.1. Sites of indoor air sampling in ORs

There were three sites in each zone of ORs (i.e. Critical, Intermediate and non-Critical Zones). Thus there were a total of nine sampling sites in ORs. Each site was sampled in all of the four rounds using one separate Plate in each site at the same time.

4.6.1.2.2. Time of indoor air sampling in ORs

In the two round of sampling, samples were taken from Critical Zone when OR were active (surgical procedures were undergoing) where as in the other two round of sampling, the samples were taken when ORs were passive. On the other units of ORs (Intermediate and non-Critical Zones), samples were collected in the morning for two round of sampling and afternoon for the other two round of sampling periods. To increase the representativeness, sampling were taken alternatively (i.e. in the first round, samples were taken when ORs were active and in the second round, samples were taken when ORs were passive for critical zone. For intermediate and non-critical zone, samples were taken alternatively in the morning for the first round and in the afternoon for the second round and so on.

4.6.1.2.3. Sites of indoor air sampling in SWs

There were two sites in each unit of SWs. These were corners and center of the room selected based on recommendations by Scottish quality assurance specialist interest group (79). Since there are four units of SW, there were eight site of sample collection in which one separate plate was used for each site.

4.6.1.2.4. Time of indoor air sampling in SWs

Air samples in surgical ward units were collected at three different time of a day: at 8 am to 9 am, at 11am to 12 am and at 4 pm to 5 pm in each round of sampling. In each round of sample collection, the corners in which the plates were placed were rotated to increase the representative of the samples taken from the surgical ward units.

4.6.1.2.5. Indoor air sampling procedure in brief:

The schedule 1/1/1 (a method devised by Fisher G *et al* (48) which can also be found on a review article by Pasquarella C *et al* (49)) was used in collection. A 9 cm in diameter sterile blood agar plate (labeled with code number at the back side) was left open to the air in OR and SW units for 1 hour, 1meter above the floor and 1meter from the wall (48, 49). Stepwise protocol of indoor air sample collection is explained in Annex-II.

4.6.1.3. Method of housekeeping surface sampling:

The house keeping surfaces were Floor and Table top surfaces.

Environmental surface samples were collected from housekeeping surfaces using Sample/Rinse Method which is frequently chosen. Sterile cotton swab was used for specimen collection and it was rubbed over the surfaces during collection to insure better capture (45, 79, 80).

4.6.1.3.1. Sites of housekeeping surface sampling in ORs

There were three sites for floor and three for table top surfaces in each zone of ORs (i.e. Critical, Intermediate and non-Critical Zones). Thus there were a total of 18 sampling sites in one round of sample collection for ORs. So, there were a total of 72 sampling sites in four round of sample collection since each site is sampled once in the study.

4.6.1.3.2. Time of housekeeping surface sampling in ORs

Samples from selected housekeeping surfaces in OR units were collected when ORs were active for one round and when ORs were passive for the other round alternatively (in Critical Zone) as well as in the morning for one round and afternoon for the other round (in Intermediate and non-Critical Zones).

4.6.1.3.3. Sites of housekeeping surface sampling in SWs

There were a total of 76 sampling sites in 12 round (in one round, one SW unit was sampled) of sample collection: 36 for floor and 36 for table top surfaces. For six rounds of sampling the floor surfaces in SW units, 18 floor sampling sites which are under patient bed were selected. In each of these six rounds of sampling, different under patient bed floor sampling sites was selected. For the remaining six rounds of floor sampling, 18 floor sampling sites which were not under patient

bed in SW were selected. To increase the representativeness, sampling was taken alternatively (i.e. in the first round, samples were taken from floors which is under patient bed and in the second round, samples were taken from floor which was not under patient bed and so on). For table top sampling in SW units, a total of 36 different sampling sites from different bed side tables in 12 rounds were sampled.

4.6.1.3.4. Time of housekeeping surface sampling in SWs

Samples from selected floor and table top surfaces in SW units in each round, samples were collected at 8 AM, 11 AM and 4 PM on the same day.

4.6.1.3.5. Surface sampling procedure in brief:

During sample collection from housekeeping surfaces (from Table and Floor), nutrient broth moisten cotton swab was used to swab the selected sites by rubbing over 25cm^2 areas (86). To make the sample size reproducible, the area of sample collection was marked out with sterile template of known dimensions (a flat square stainless steel) with a 5 cm x 5 cm hole cut out in which the inner edges of the hole was swabbed. Then immediately, the tip of the swab was aseptically placed into a tube containing a 5ml sterile elution solution of nutrient broth for elution of bacteria from the swab used to sample the surface. To facilitate the elution process and suspend material from the sampling device and break up clumps of organisms (if any) for a more accurate count, the swab within the elution solution was mixed by a mechanical mixing using a vortex mixer for around 60 seconds (45, 79). Stepwise protocol of housekeeping surface sample collection is explained in annex-III.

Note: The sample collection time (for air and housekeeping surfaces) is selected based on the study objective and different literature search (45, 46, 79, 87, 88).

4.6.2. Specimen transportation

Air specimens after being collected by settle plate method, it was transported immediately from the hospital by using clean container to Microbiology Laboratory for processing. Environmental surface specimens were also transported in to Microbiology Laboratory for processing by placing the swabs in to the test tubes having elusion solution. As soon as it reaches the laboratory, it was processed.

4.6.3. Isolation of bacteria

For the initial aerobic colony counting of the bacteria, growth medium with low selectivity like plat count agar (Oxoid, UK) for housekeeping surface samples and blood agar (Oxoid, UK) for air samples were used. To detect and isolate a particular type of bacterial species, 7 % sheep blood agar, mannitol salt agar (MSA) (Oxoid, UK) and MacConkey agar (Oxoid, UK) were used in the study accordingly (48, 49, 79, 88).

The blood agar plate on which air sample collected was incubated for 24 hours at 37c^{o}. Suspected possible pathogenic bacteria (if any) were sub cultured in to nutrient broth to get pure culture. Moreover, further sub culture in to Blood agar, MSA and MacConkey agar were made to make presumptive identification of bacteria based on colony texture and Gram stain.

Additionally, part of the rinse fluid of housekeeping surface sample was directly inoculated onto Blood agar, MSA and MacConkey agar soon after the sample was reached to the Microbiology laboratory for possible pathogenic bacteria identification after incubating these media at 37 c^{o} for 24 hours aerobically.

For accurate quantification of the total bacteria, the specimen should be normally diluted appropriately to make the cells within the range of 30-300 CFU bacteria per Petri dish. In the present study, a 1ml housekeeping surface sample diluted with 5ml rinse fluid was directly inoculated in to the culture media (without further dilution) using pour plat technique (Annex VI) for total colony count in plate count agar. This was estimated based on an assumption of a short microbiological study conducted in JUSH pediatric ward (unpublished data) by the department of Microbiology, Immunology and Parasitology, Jimma University to assess the bacterial contamination level in the milk preparation room environment of the ward where the total number of colonies did not exceed from the range of 30-300 CFU in a plate (89, 90).

4.6.3.1. Colony enumeration for indoor air samples

After incubation of air samples at 37c^{o} for 24 hours, culture plates that showed discrete macroscopic colonies was counted using plat colony counter (48, 49, 79).

4.6.3.2. Identification of bacteria in indoor air samples

After colony count, the colonies were assessed for the growth of potential pathogenic bacteria. These were done initially by colony morphology, hemolysis pattern shown on settle plate blood agar and microscopic examination using Gram stain. These suspected colonies were sub cultured on to appropriate media (MSA, MacConkey and blood agar) accordingly for further identification following the standard bacteriological procedure and different biochemical tests indicated in medical laboratory manual for tropical countries in volume two (microbiology) (89) and WHO document on basic laboratory procedures in clinical bacteriology (90).

4.6.3.3. Colony enumeration from environmental surface samples

After appropriate incubation, culture plates that show discrete macroscopic colonies were enumerated using plat colony counter. The number of discrete colony forming units (CFU) was calculated interms of CFU/cm^2 for samples taken for environmental surfaces (32).

4.6.3.4 Identification of bacteria from environmental surface samples

Presumptive identification of the colonies were done by observing their individual colony morphology and hemolysis pattern on blood agar as well as their growth in different culture media like MSA, MacConkey and blood agar. Further identification was made by carrying out different biochemical tests following the standard bacteriological procedure indicated in medical laboratory manual for tropical countries in volume two (microbiology) (89) and WHO document on basic laboratory procedures in clinical bacteriology (90).

4.6.3.5. Biochemical and other identification tests

Different biochemical tests were used to identify the genus or/and species of bacterial isolates from both housekeeping surfaces and indoor air. These testes includ catalases test, coagulase test, lactose fermentation, glucose fermentation, gas production, lysine decarboxylation, hydrogen sulfide production, motility test, indole production, citrate utilization, mannitol fermentation, urea production, optochin susceptibility, bacitracin susceptibility and oxidase test as described by Cheesbrough (89) and WHO document on basic laboratory procedures in clinical bacteriology (90). All of the biochemical media were from Oxoid, UK.

4.6.4. Antibiotic susceptibility testing

Antimicrobial susceptibility testing (AST) was performed by disk diffusion method. This method is one of the most commonly used AST methods among diagnostic laboratories (91, 92). In a survey conducted in New York state laboratories, more than 50% of the laboratories used disk diffusion AST. Kirby-Bauer and Stokes methods are usually used for antimicrobial susceptibility testing in disc diffusion type, with the Kirby-Bauer method being recommended by the Clinical and laboratory standards institute (CLSI) (93).

A McFarland 0.5 turbidity standard was used to adjust the inoculum. The test organisms (bacterial isolates) was grown in nutrient broth and incubated for 4-6 hours at 37^0C until the turbidity was matched with the prepared McFarland standard.

The AST for all potential pathogenic bacteria isolated from the air and environmental surfaces was performed using different antibiotics discs panels. The selection of antibiotic disks was based on clinical relevance (94), representativeness of antibiotics for the group, literature search (73, 95) as well as epidemiological information (their usage in the study area by contacting health personnel in surgical ward). A total of 13 different antibiotics were used which include: ampicillin (AML, 10 µg), ceftriaxone (CRO, 30 µg), cephalothin (KF, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (Cf, 5 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), methicillin (Met, 5 µg), penicillin G (P, 10 IU), tetracycline (T, 30 µg), trimethoprim-sulphamethoxazole (SXT, 1.25/23.75 µg), oxacillin (Ox, 1 µg) and vancomycin (VA, 30 µg) (Annex-IV).

After matching the inoculum with a prepared 0.5 McFarland standard, it was inoculated in to Muller Hinton agar (Oxoid, UK) using sterile cotton swab. The appropriate antibiotic discs were aseptically placed on the inoculated Muller Hinton agar using sterile forceps. The plates were then incubated at $37c^o$ for 18-24 h. The degree of susceptibility of the test isolate to each antibiotic was interpreted according to the principles established by CLSI as sensitive (S), intermediate (I) or resistant (R) by measuring the zone diameter of inhibition in millimeter using ruler (94).

4.7. Quality control issues

4.7.1. During culture media preparation

Culture media was prepared strictly following manufacturer's instructions or according to approved formulations. The prepared media was placed in refrigerator (if not immediately used) and to cheek the sterility of the preparation, one plate (prior to inoculation) in each batch of media prepared was incubated at $37c^{o}$ for 24 hour to look for any growth.

4.7.2. During sample collection

The air sampling was performed by wearing sterile gloves, mouth masks and protective gown to prevent self contamination of the samples. The blood agar plate was cheeked visually for any bacterial growth before collection in order to prevent contamination of the media.

4.7.3. During sample processing

The incubation temperature was monitored using thermometer and the incubation time was followed. In general, aseptic bacteriological procedures were strictly followed at each stapes in sample processing.

In order to monitor miss identification (false positive and false negative culture results) and in order to cheek antibiotic disc potency, control organisms were used. Control organisms that were used include *S. aureus* (ATCC25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) which were brought from Ethiopian Health and Nutrition Research Institute - Addis Ababa; Ethiopia.

4.8. Data processing & analysis

The data was processed for descriptive statistics using SPSS version 16 and Microsoft Excel.

In the analysis and interpretation of results obtained from air samples taken from SW and OR units, scientifically determined baseline values (annex-V) initially suggested by Fisher in the 1970s and now widely adopted by different organizations like the European cooperation for Accreditation of Laboratories (48, 49) together with different literatures was used.

The analysis and interpretation of results obtained from environmental surface samples, in the same way to air samples, a proposed microbiologic standard values for hospitals by S.J. Dancer (32) and different literatures was used.

4.9. Ethical issue

The study was commenced after obtaining separate permission from Jimma University ethical review board and from the management of Jimma university specialized hospital.

CHAPTER FIVE: RESULTS

5.1. Bacterial load of indoor air

The bacterial load of 108 indoor air samples (36 from ORs and 72 from SWs) showed wide range of variation. The highest mean aerobic colony count was observed from FR-1 and FR-2 of SWs which were 465 and 461 CFU/hr respectively (Table-1)

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Table 1. Total aerobic bacterial load of indoor air from SW and Critical Zone of ORs in JUSH; October -January, 2009/2010

	Rooms sampled	**Aerobic colony count/hr; No♥. (mean value)**	**Standard (cfu/hr)48, 49**		
			Optimal	Acceptable	Unacceptable
SW units	FR-1	18 (465)	0-250	251-450	>450
	MR-1	18 (416)	0-250	251-450	>450
	FR-2	18 (461)	0-250	251-450	>450
	MR-2	18 (352)	0-250	251-450	>450
OR units (Critical Zone)	OR1-A	2 (43)	0-60	61-90	>91
	OR1-P	4 (46)	0-4	5-8	>9
	OR2-A	4 (92)	0-60	61-90	>91
	OR2-P	2 (28)	0-4	5-8	>9

FR-1: Female room-1 FR-2: Female room-2 OR1-A: OR1- active MR-1: Male room-1 MR-2 Male room-2 OR2-A: OR2- active OR1-P: OR1- passive OR2-P: OR2- passive

♥: Number of samples taken

Similarly, the highest colony count was observed in OR-2 especially when the room was active (Table-1). Moreover, both FCR (Intermediate Zone) and PR (Non- Critical Zone) of ORs showed abnormally increased colony counts. The colony counts in all ORs except SCS were higher in the afternoon than in the morning (Figure-2).

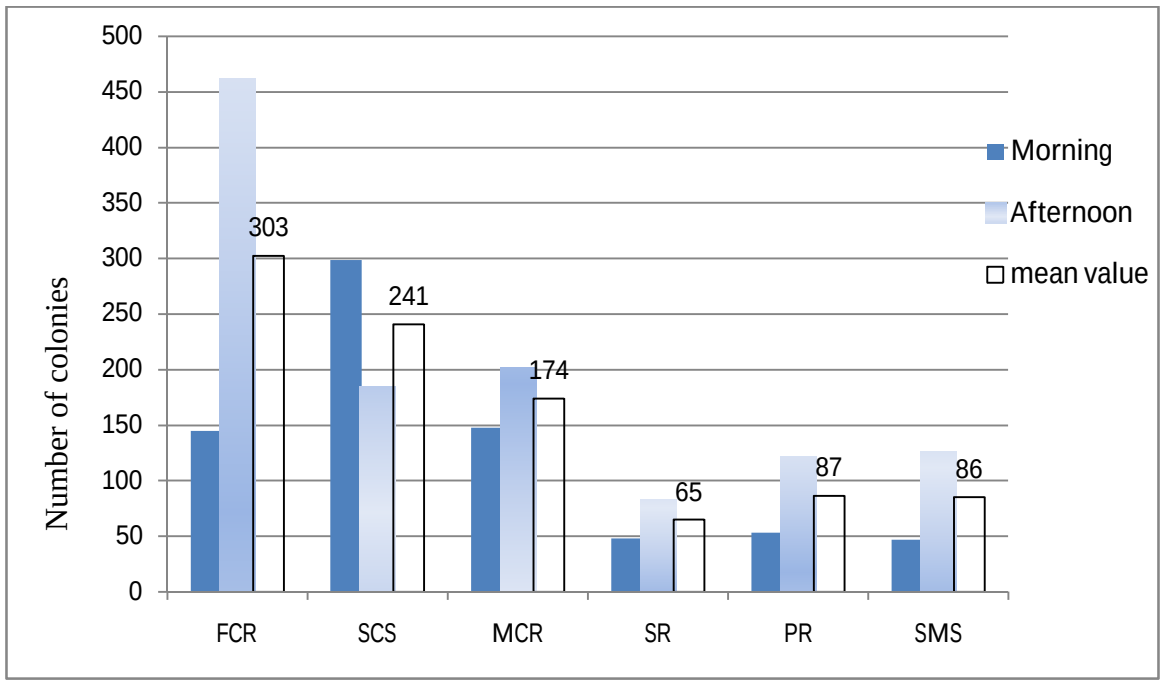

Figure 2. Total aerobic bacterial load of indoor air from Intermediate Zone and non-Critical Zone of OR in JUSH; October -January, 2009/2010

Intermediate Zone ------▶ (SR: Sterilizing room, PR: Packing room, SMS: Sterilized material store)

Non- Critical Zone -----▶ (FCR: Female clothing room, MCR: Male clothing room, SCS: Sterilized cloth store)

5.2. Potential pathogenic bacteria isolated from indoor air

Out of 108 air samples collected, 3 isolates of *S. pyogenes* from SWs and 48 isolates of *S. aureus* (70.8 % from SWs and 29.2 % from ORs) were identified. From ORs, the isolation rate (66.7%) of *S. aureus* was significantly higher in Critical Zone than Intermediate and non Critical Zone ($p<0.001$). Additionally, *S. aureus* was frequently isolated in FR-2 and MR-2 of SW units (Figure-3).

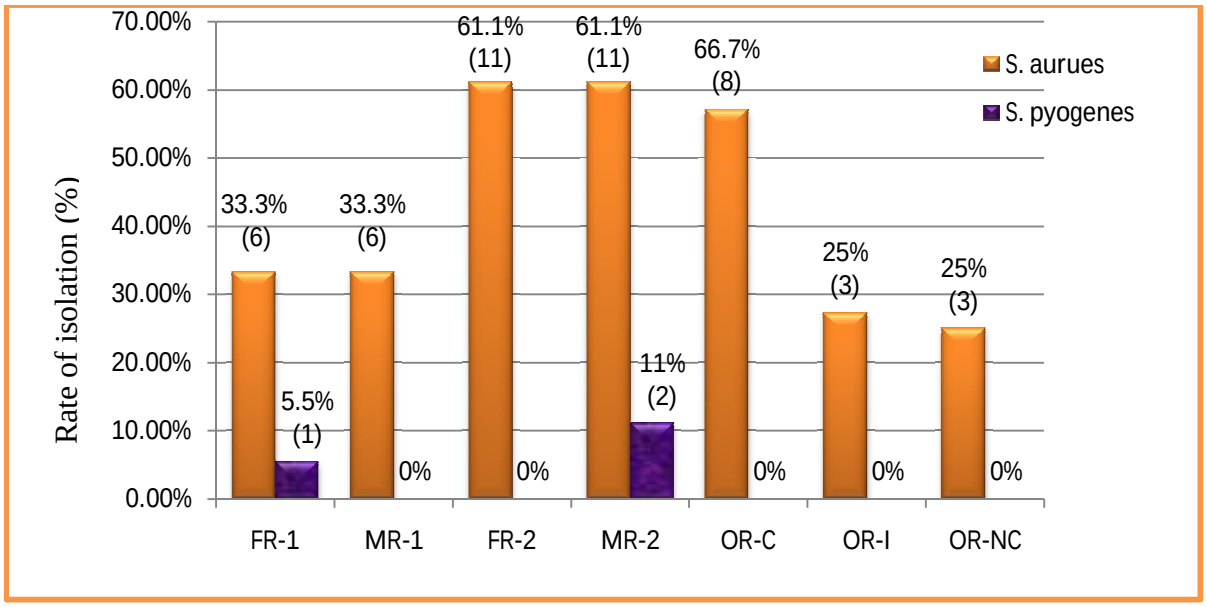

Figure 3. Isolation rates of potential pathogenic bacteria from indoor air in SW and OR units at JUSH; October -January, 2009/2010: % of isolation (No. of isolates)

FR-1: Female room-1 FR-2: Female room-2 OR-C: OR-critical zone

MR-1: Male room-1 MR-2 Male room-2 OR-I: OR-intermediate zone

OR-NC: OR-non critical zone

5.3. Bacterial load in the housekeeping surfaces

The bacterial load of 144 housekeeping surfaces (72 from ORs and SWs each) showed that the mean aerobic colony count of Floor and Table Top Surfaces were 10 and 6 cfu/cm^2 for ORs whereas 19 and 34 cfu/cm^2 for SWs respectively. Samples taken from Floors of the Intermediate Zone of ORs and MR-2 of SWs showed the highest colony counts which were 14.8 and 26.4 CFU/ cm^2 respectively. On the other hand, the Table Top Surface samples from FR-1 of SWs and Intermediate Zone of ORs revealed the highest colony count which account 40.7 and 7.3 CFU/ cm^2 respectively (Figure-4).

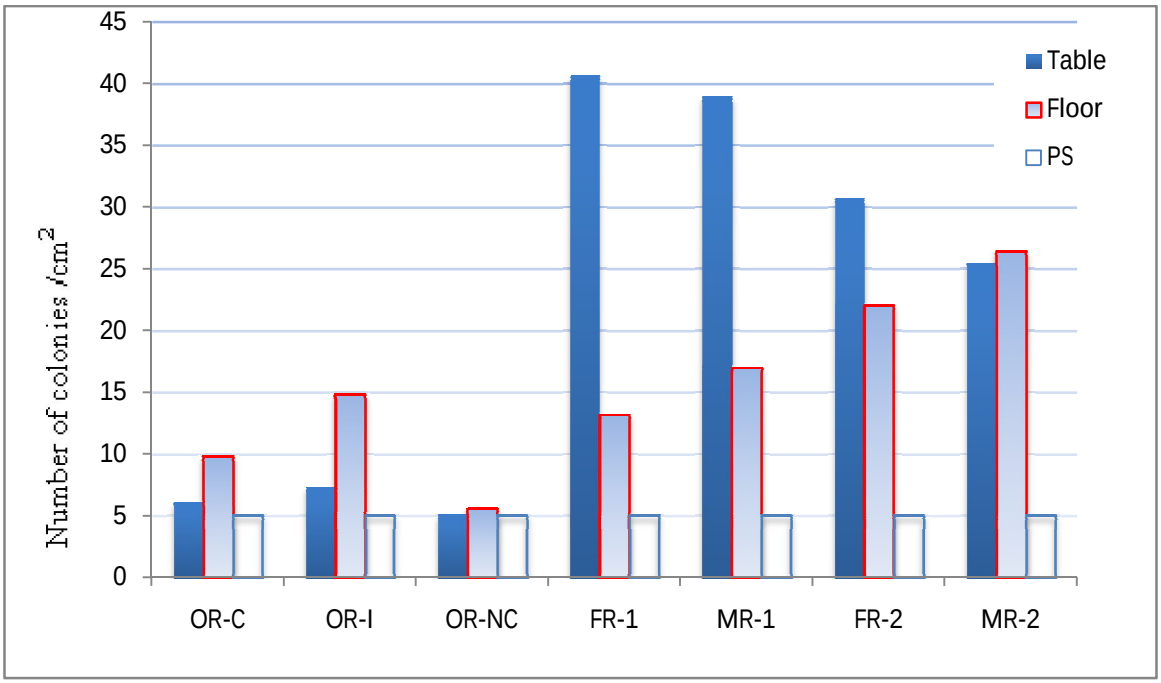

Figure 4. Aerobic colony count from housekeeping surfaces of OR and SW units in JUSH; October -January, 2009/2010

PS: Proposed standard

5.4. Potential pathogenic bacteria isolated from housekeeping surfaces

Potential pathogenic bacteria were isolated from 83 of the 144 housekeeping surface samples of which 29 were from ORs and 54 were from SWs. The isolation rates of potential pathogenic bacteria were 40.3% in ORs and 75% in SWs.

Among gram negative bacterial isolates in ORs, *E. coli* was the leading one accounting 38.9% of the isolates followed by *P. aeruginosa* which is 22.2%. On the other hand, *Klebsiella* spp. was most frequently isolated bacteria accounting 27.1% followed by *E. coli* accounting 18.6% from samples taken from housekeeping surfaces of SWs (Figure-5).

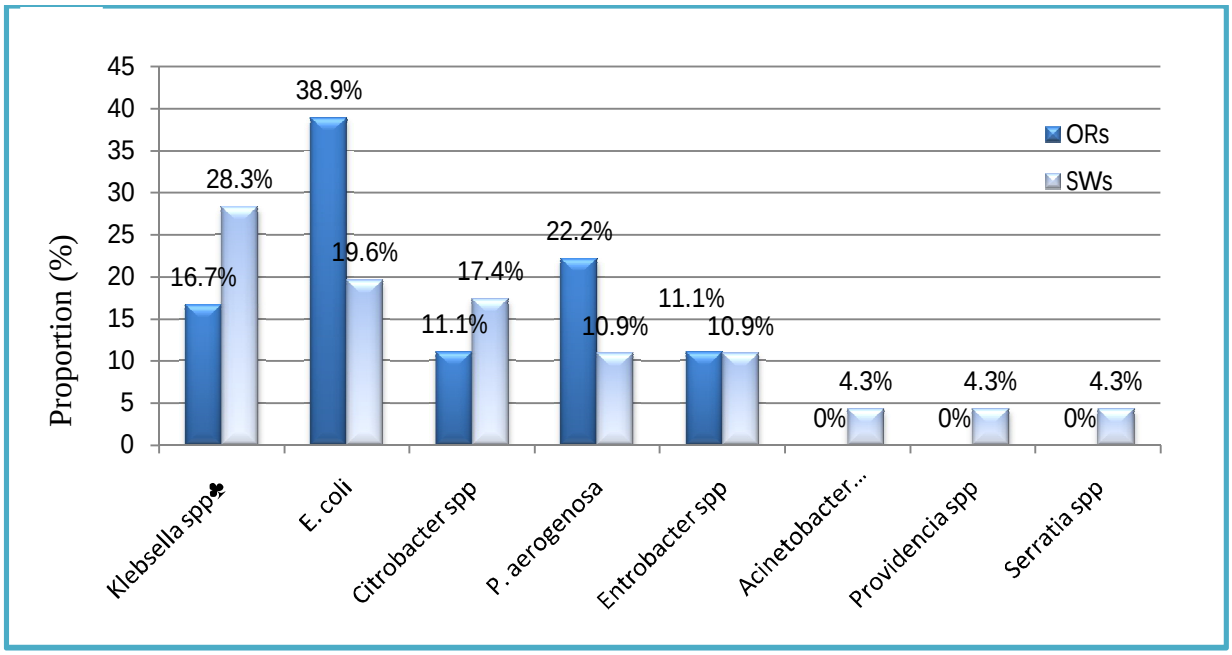

Figure 5. Potential pathogenic Gram negative bacterial isolates from housekeeping surfaces of ORs and SWs in JUSH; October -January, 2009/2010

♣ Includes: *K. pneumoniae*, *K. oxytoca*, *K. rhinose* and *K. ozenae*

Additionally, potential pathogenic Gram positive bacteria were also isolated in 49 of these surface samples (33 isolates from SWs and 16 isolates from ORs). Unexpectedly, all of these isolates (except one isolates of *S. pyogenes* in SWs) were *S. aureus* in both ORs and SWs.

The number of Gram negative bacterial isolates in ORs was comparable to their counter parts. However, there were un proportional distributions of Gram negative bacteria (unlike Gram positives) in ORs with the highest frequency being in Critical Zone (55.6%) (Figure-6).

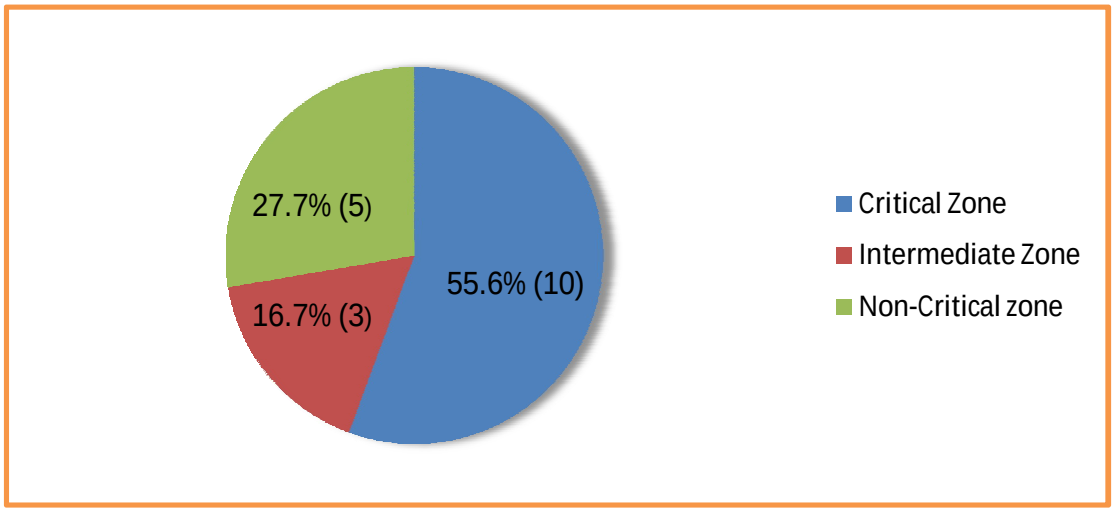

Figure 6. Frequency of Gram negative bacterial isolates from housekeeping surfaces in Critical, Intermediate and Non-Critical Zone of OR in JUSH; October -January, 2009/2010: % (Number of isolates)

5.5. Antibiotic susceptibility pattern of isolates

The antibiotic susceptibility test was done for all Gram negative and Gram positive bacterial isolates.

The susceptibility patterns of isolates revealed varying degrees of resistance and sensitivity to the antibiotics tested (Table-2 and Table-3). In the present study, *S. aureus* showed 100% resistance to methicillin, 82.3% to ampicillin, 77.1% to penicillin and the list resistance which is 9.3% was observed for ciprofloxacin. On the other hand, *S. aureus* isolates were 100% sensitive for vancomycin (Table-2).

Table 2. Antibiotic resistance pattern of Gram positive bacterial isolates from indoor air and housekeeping surfaces of OR and SW units in JUSH; October -January, 2009/2010

	Antibiotics tested												
	No.(%)of resistance												
Organisms	P	Amp	T	C	KF	Met	Ox	CN	Cf	Cro	SXT	E	Va
***S. aureus* (n=96)**	74 (77.1)	79 (82.3)	48 (50)	37 (38.5)	36 (37.5)	96 (100)	88 (91.7)	25 (26)	9 (9.3)	37 (38.5)	43 (44.8)	51 (53.1)	0 (0)
***S. pyogenes* (n=4)**	1 (25)	2 (50)	1 (25)	0 (0)	-	-	-	-	-	-	-	1 (25)	-
Total (n=100)	75 (75)	81 (81)	49 (49)	37 (37)	36 (36)	96 (96)	88 (88)	25 (25)	9 (9)	37 (37)	43 (43)	52 (52)	0 (0)

P: Penicillin
Amp: Ampicillin
T: Tetracycline
C: Chloramphenicol
KF: Cephalothin
Met: Methicillin
Ox: Oxacillin
CN: Gentamicin
Cf: Ciprofloxacin
Cro: Ceftriaxone
SXT: Trimethoprim-sulphamethoxazole
E: Erythromycin
Va: Vancomycin

The antibiotic resistance pattern of Gram negative bacterial isolates from housekeeping surfaces of OR and SWs test results showed that *Klebsiella* spp were greater than 65% resistant for all antibiotics tested except for ciprofloxacin which was 6.3%. Similarly, *E. coli*, *Citrobacter* spp and *Enterobacter* spp showed greater than 83% resistance for ampicillin and cephalothin. On the other hand, ciprofloxacin showed 100% sensitivity for *Citrobacter* spp, *P. aeruginosa*, *Enterobacter* spp and *Acinetobacter* spp (Table-3).

Table 3. Antibiotic resistance pattern of Gram negative bacterial isolates from housekeeping surfaces of OR and SW units in JUSH; October- January, 2009/2010

	Antibiotics tested							
	No.(%)of resistance							
Organisms	Amp	T	C	KF	CN	Cf	Cro	SXT
Klebsiella **spp♠ (n=16)**	16 (100)	11 (68.8)	12 (75)	16 (100)	12 (75)	1 (6.3)	11 (68.8)	14 (87.5)
E. coli **(n=16)**	15 (93.8)	8 (50)	9 (56.3)	14 (87.5)	6 (37.5)	1 (6.3)	2 (12.5)	10 (62.5)
Citrobacter **spp (n=10)**	10 (100)	3 (30)	7 (70)	10 (100)	5 (50)	0 (0)	3 (30)	6 (60)
Enterobacter **spp (n=6)**	5 (83.3)	4 (66.7)	3 (50)	6 (100)	2 (33.3)	0 (0)	2 (33.3)	3 (50)
P. aeruginosa **(n=8)**	-	1 (12.5)	6 (75)	-	3 (37.5)	0 (0)	1 (12.5)	5 (62.5)
Acinetobacter **spp (n=2)**	-	0 (0)	2 (100)	-	1 (50)	0 (0)	0 (0)	1 (50)
Total (n=58)	46 (79.3)	27 (46.6)	39 (67.2)	46 (79.3)	29 (50)	2 (3.5)	19 (32.8)	39 (67.2)

♠ Include *Klebsiella pneumonia*, K. *oxytoca*, K. *rhinose* and K. *ozenae*

Antibiotic abbreviations are explained in table 3

CHAPTER SIX: DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1. Discussion

Hospital environment may not be a place where people get well but may also be a place where sick people get sicker because of HAI; bacterial contamination of hospital environment being one factor (51).

In the present study, the mean aerobic colony counts of FR-1 and FR-2 of the SW units, OR-1 when it was passive and OR-2 when it was both passive and active were beyond the recommended acceptable range of CFU when it is compared to the bacteriological standard set by Fisher *et al* (48) and Pasquarella *et al* (49). The possible reason for this might be due to high turnover of people particularly students as it is a teaching hospital. Additionally, the age and design of the hospital and its surrounding environment might also contribute for the current high aerobic bacterial load observed in these rooms.

The aerobic bacterial load in OR (Critical Zone) air was became 1.82 times more when these rooms were active compared with when they were at rest. This finding goes in line with the study conducted by Suzuki A *et al* (50) and a standard by Fisher *et al* (48) which explained that the bacterial load of indoor air of OR is higher when OR is active than when it is passive.

The mean indoor air aerobic colony count (ACC) obtained in the present study which is 463 CFU for FSW, 384 CFU for MSW and 58 CFU for OR (Critical Zone) (Table 1) were incomparable with a study conducted in Nigeria in 2007 by Oytayo VO *et al* that was reported as the mean ACC of 42, 31 and 3 CFU in FSW, MSW and OR respectively (51). The variation observed in these two study results might include; 1) difference in representativeness of study subject where 108 indoor air samples analyzed in the present study compaired to only 12 samples processed in the former study and 2) differences in the turnover of the people of these two hospitals. However, the high mean aerobic colony count observed in these rooms might be an indication of high environmental contamination level of JUSH.

Moreover, in the present study the total indoor air aerobic bacterial load of Critical Zone was 58 CFU whereas the load in the Intermediate Zone and non Critical Zone of ORs were 246 and 76 CFU respectively (Figure 2). The finding of the present study in this regard goes in contrary to a study conducted by Suzuki A *et al* (1984) that the mean bacterial load of non Critical Zone was twice than in the Intermediate Zone (50). The high colony count shown in Intermediate Zone when compaired to non Critical Zone of ORs in the present study might be due to high number of people (staffs and students) were using Intermediate Zone where as few people were likely working in non Critical Zone.

The bacterial profile of indoor air sample showed that *S. aureus* was the most frequently isolated species among potential pathogenic bacteria isolated in both SW and OR units. The isolation rate of *S. aureus* was higher in Critical Zone (66.7 %) than Intermediate (25%) and non Critical (25%) Zone of OR. Moreover, the isolation rate of *S. aureus* in Female room-2 and Male room-2 was twice than Female room-1 and Male room-1 of SW units (Figure 3). This variation may be explained by differences in the presence and number of carrier as well as infected people and difference in cleaning procedures which are risk factors that affects the load of *S. aureus* in the air as it was suggested by Chikere BC *et al* (10) and Suzuki A *et al* (43).

Only 3 isolates of *S. pyogenes* and no Gram negative bacteria were identified from a total of 108 air samples examined. This might be due to the inability of Gram negative bacteria to survive for a long period in the aerosolized state as it was explained by Beggs BC (67) and to resist harsh conditions like drying (47, 96) when they are compared to their counter parts. This might be also true for low isolation rate of streptococci as these bacteria have relatively low survival time in the environment since they are fastidious and susceptible for environmental stress by their nature (97).

The mean ACC obtained for housekeeping surfaces; Tables with 34 CFU/cm^2 ($P<0.001$) and Floor with 19 CFU/cm^2 ($P<0.001$) in SWs as well as Floor with 10 CFU/cm^2 in ORs ($P=0.009$) in the present study is significantly higher than the set ACC standards (< 5 CFU/cm^2) for hand contact surfaces (32). Whereas the mean ACC 6 CFU/cm^2 obtained for Tables in ORs though higher compared with the set standard, it was not statistically significant ($P=0.164$) (Figure 4). The highest ACC from housekeeping surfaces was also seen in Sterilized Cloth Store (SCS) of Intermediate Zone (18 CFU/cm^2) and in Critical Zone (10 CFU/cm^2) of ORs. These results are in contrary to the study conducted by Hambraeus A *et al* (58) where ACC in dressing room of Intermediate Zone and Critical Zone of ORs was reported to be 7 and 0.72 CFU/cm^2 respectively. The highest ACC obtained in the present study could be increased movement of people in the rooms as it is a teaching hospital and cleaning of the entire SCS might not be appropriately done because the room (crowded with a lot of clothes) is relatively smaller in size than other rooms in the Intermediate Zone.

Unlike to indoor air samples, both Gram positive and Gram negative bacteria were isolated in samples taken from housekeeping surfaces. The distribution of potential pathogenic bacteria isolated from these surfaces was comparable with studies conducted on hospital environment by Oytayo VO *et al* (51), Wolde Tenssay Z (73) and Chikere BC *et al* (10). The most frequently isolated bacteria in the present study includes *S. aureus* (48 isolates) which is frequently responsible for SSI through air born transmission in SWs and ORs (67) followed by *Klebsiella* spp and *E. coli* (16 isolates each). One of the possible reason that *S. aureus, Klebsiella* spp and *E. coli* were frequently isolated than other bacteria may be due to their long survival time in the hospital environment (31).

Unexpectedly, high frequencies of potential pathogenic Gram negative bacteria were isolated in Critical Zone than in Intermediate and non Critical Zone of ORs and similarly in Female rooms than Male rooms of SWs. The high load of pathogenic bacteria in Critical Zone observed in the present study (Figure 6) may be due to uncontrolled (now and then) opening of OR doors and high movement of students in each surgical procedure during the study period.

In a study by Wolde Tenssay Z (from clinical and hospital environment) indicates *S. aureus* were 93% resistant to ampicillin whereas *E. coli*, *Klebsiella* species and *Enterobacter* species were 100% resistant for ampicillin (73). These is almost comparable with the present study (Table 2 and Table 3) where the sensitivity pattern of *Klebsiella* species, *E. coli*, *Enterobacter* species and *S. aureus* to ampicillin were 100%, 93.8%, 83.3% and 82.3% resistance respectively. Similarly, *S. aureus* also showed 100 % resistance for methicillin and 77.1% for penicillin whereas *Klebsiella* spp, *Citrobacter* spp and *Enterobacter* spp were resistant to ceftriaxone (third generation cephalosporins) with 68.8%, 30% and 33.3% respectively.

Generally most of Enterobacteriaceae isolated in the present study showed higher rate of resistance for most of antibiotic tested. This is to say that multi drug resistant Enterobacteriaceae were seen in more than 90% of isolates. On average, each Enterobacteriaceae isolate showed resistance for 4.4 antibiotics. This may be due to β-lactamases and extended spectrum β-lactamases (ESBLs) producing Enterobacteriaceae are now relatively common in healthcare settings and often exhibit multidrug resistance including for recent antibiotics (newer generation cephalosporin groups and quinolones) and also responsible for different outbreaks and HAIs as it was explained by Hobson RP *et al* (66) Paterson LD (71) and Jones NR (72). Fortunately, ciprofloxacin was identified as the most effective antibiotics even with 100% sensitivity for *P. aeruginosa*, *Enterobacter*, *Citrobacter* and *Acinetobacter* species isolated in the present study. Hence, this drug might be used for the treatment of HAI in the health care settings taking it as a best option.

6.1.1. Limitation of the study

1. During sample collection by settle plate method, because the plate is new in the environment for patients, health personnel and visitors; they were giving attentions especially at the first round of sample collection. This may, to some extent, hinder the natural movement of air in the area that will affect the result.

6.2. Conclusion

Almost all indoor air samples except OR-1 when active and non-Critical Zone of ORs showed higher mean aerobic bacterial load compared to the standard (48). Thus, the bacterial load of indoor air was high which is considered as a risk factor for SSI since there is a linear relationship between bacteria count in air (operating rooms and surgical ward) and surgical site infection rate (39). Similarly, housekeeping surfaces also showed high mean aerobic bacterial load ranging from 5 to 41 CFU/cm^2. As indicated in the standard (32), the presence of ACC in $1cm^2$ of housekeeping surfaces higher than 5 CFU (as observed in the present study) is an indication of increased risk of infection for the patient in that environment.

Over all, the possible reason for the high bacterial load obtained in OR and SW units in the present study could be due to high number of people (including patients, visitors and health professionals) in the room, inappropriate cleaning procedures, high amount of materials brought in from outside, environmental disturbances, delayed maintenance and inappropriate ventilation as indicated by different researchers (43, 45, 46, 76).

In the present study, *S. aureus* which is the most important cause of SSI (47) was the most frequently isolated bacteria with isolation rate of 38.9 and 47.2% in indoor air of ORs and SWs respectively. Moreover, the isolation rate of this bacterium in housekeeping surface was 22.2% for ORs and 44.4% for SWs. Surprisingly, most of pathogenic bacteria isolated from housekeeping samples in OR were from Critical Zone (the zone where the actual surgical procedure is undergoing). In general, Enterobacteriaceae were most frequently isolated (53 isolates) from housekeeping samples which were also resistant for most of antibiotics used in the study area as a current treatment option. These poses a high risk for SSI since the bacteria isolated can persist for a long period (31, 49, 95) acting as a potential source of HAI (15) since they are frequently involved in different HAI as well as outbreaks (13, 60, 64, 65, 66). Thus, the present study results indicated that ORs and SWs of JUSH have high risk of SSI for the patient and high risk of infection for the health personnel as well. So the hospital (mainly the general surgical department) should implement appropriate infection prevention measures to reduce the environmental bacterial contamination level of the OR and SW units.

6.3. Recommendation

All the bacteria isolated in the present study were implicated in different HAIs and have the potential to cause outbreaks due to their presence in hospital environment (63, 65, 66, 74, 98). Though the exact reason why the total aerobic colony count and potential pathogenic bacteria was high in the present study needs further research, there are different measures which are known to reduce the total bacterial load and potential pathogenic bacteria to the optimal level. Thus, measures like reducing foot trafficking in and out of the OR, improving the ventilation system, appropriate educational intervention on routine cleaning which is under implementation, programmatic intervention and enhanced cleaning (50, 57, 76, 77) are known to reduce the total bacterial load.

So based on the findings of the present study, the following actions should be taken to improve the bacteriological quality of the ORs and SWs environment in JUSH for the sake of patient, health personnel and visitor safety by minimizing the rate of HAI. Thus, the management of General Surgical Department should reduce the overall bacterial burden of air and housekeeping surfaces of OR and SW units with especially emphasis on units which are found to have high bacterial burden by implementing an appropriate infection prevention measures. Some of the measures which are effective in infection prevention and control and can be implemented with a minimal cost by the department include;

- Educational intervention targeted mainly on hospital hygienic staffs and other health professionals to improve the existing cleaning system in the department,
- Programmatic intervention (arrangement) on the existing cleaning system in the department
- Enhancing the existing cleaning system by;
    - ✓ Increasing the number of cleaner
    - ✓ Increasing the cleaning time per week
    - ✓ Improving the ventilation system
- Reducing foot trafficking in and out of the ORs specially related to out proportion of students

The overall infection control measures should be with appropriate educational and programmatic intervention along with (if possible) appropriate culture based surveillance to evaluate the system.

Generally, the appropriate infection control measure is implemented when and only when the appropriate source of bacterial contamination is identified which needs further research and well designed study. Adapting and implementing recommendations and infection prevention strategies found in "INFECTION PREVENTION GUIDELINES for Healthcare Facilities in Ethiopia" (81) prepared by the Federal Ministry of Health-Ethiopia is surely helpful.

Additionally, health professionals who are involved in antibiotic prescription (in addition to appropriate infection prevention system and culture based surveillance (if possible)) should consider the current antibiotic susceptibility pattern of the bacteria to reduce the occurrence and spread of resistant pathogens since most of the bacteria are developing resistance for the antibiotics used currently in the study area as it is shown in the present study. Since susceptibility pattern of bacterial isolate showed a great variation, the hospital needs to seek proper antibiotic susceptibility testing services to have the best possible treatment choice for the patient.

Finally, it will be better if the hospital encourages the microbiology unit of Medical Laboratory Sciences and Pathology department to perform the culture based hospital-wide surveillance (which is important in the characterization and control of healthcare-associated infections and antimicrobial resistance (15) part of infection control system periodically.

6.3.1 Further research questions

1. Exact causes of high bacterial load from indoor air in OR and SW of JUSH
2. Is the distribution of bacteria and antibiotic susceptibility pattern from patient samples comparable with the present findings?
3. Evaluation of infection prevention system in OR and SW of JUSH
4. Molecular characterization of environmental and clinical isolates of Enterobacteriaceae (including other Gram negative bacteria) and *S. aureus* in OR and SW of JUSH.

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ANNEX-I

A survey format used for data collection in determining the degree of bacterial contamination of ORs and SWs environment and antibiotic susceptibility pattern of isolates at JUSH from October to January, 2009/2010 G.C

PART-I: preliminary data

1. Code number _____

2. Collection day _____/_____/_____E.C. at _____ (time)

3. Types of specimen collected

A. Surface samples (Floor or Table Top) B. Air samples

4. The unit in which the sample is collected

A. Critical Zone (OR-1, OR-2 or ER) B. Intermediate Zone (SCS, FCR or MCR)

C. Non-Critical Zone (SR, PR or SMS) D. SW-1 (Male room-1 or Female room-1)

E. SW-2 (Male room-1 or Female room-1)

5. Site of sample collection for SW units (for air samples)

A. Corner of the room B. Center of the room

6. Site of sample collection for Table Top surfaces in SWs

A. Bed side chair in SW room-1 (Female room-1 or male room-1)

B. Bed side chair SW room-2 (Female room-2 or Male room-2)

7. Site of sample collection for floor surfaces (in SW units)

A. Under bed in SW-1 (Male room-1 or Female room-1)

B. Under bed in SW-2 (Male room-2 or Female room-2)

C. Free in SW-1 (Male room-1 or Female room-2)

D. Free in SW-2 (Male room-2 or Female room-2)

8. The area in ORs in which floor surface sample is taken

A. Critical Zone B. Intermediate Zone C. Non-Critical Zone

PART-II: laboratory result

9. Culture result

9.1. Number of colonies (in CFU/dm^2/h for air samples and in CFU /cm^2 for housekeeping surface)

_____

9.2. Bacterial species identified

_____

_____

10. Antibiotic susceptibility result

Table 4: A format used to collect data of antibiotic susceptibility result of bacterial isolates from hospital environment of JUSH from October to January, 2009/2010 G.C

No ♠	**Susceptibility test results for different antibiotics**																																			
	Aml			Amp			Cro			KF			C			Cf			E			CN			Ox			P			T			SXT			Va		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1																																						
2																																						
3																																						
4																																						

Aml: Amoxicillin KF: Cephalothin E: Erythromycin P: Penicillin

Amp: Ampicillin C: Chloramphenicol CN: Gentamicin T: Tetracycline Va: Vancomycin

Cro: Ceftriaxone Cf: Ciprofloxacin Ox: Oxacillin SXT: Trimethoprim-sulphamethoxazole

♠: Bacterial isolates tested: 1. _____

2. _____

3. _____

4. _____

ANNEX-II

Air specimen collection procedure used using settle plate method[49, 79, 80]

1. The plates were examined for contamination prior to use.
2. The code number was written on the base of the plate (the part containing the culture media) with marker. The code number ranges from 001-108.
3. The plates were transported from microbiology laboratory to site of sample collection using clean box.
4. The plates were placed one meter above the floor (using alcohol cleaned chairs which are 1 meter long) and one meter away from the wall with the lids still on.
5. During transporting and placing the plates in their appropriate site of sample collection, sterile surgical gloves, surgical clothing (when collecting in ORs) and face masks were used.
6. Lids were raised to expose the surface of the medium and were rested on the very edge of the plate so that the entire agar surface is completely exposed. The plates were supervised for the whole collection time (1 hour) to avoid passing anything over the top of plates being exposed.
7. After 1 hour of exposure, the lids of the plates were closed and transported in to microbiology laboratory using clean box for incubation.

ANNEX-III

Specimen collection procedure used for housekeeping surfaces using swabs[79, 80]

1. The swab and the test tube were assembled with code number written on each tube. The code number ranges from 109-252.
2. The surface of sample collection site was checked to make sure that they are dry and free of any liquids.
3. Clean aluminum plate with a hallow area of 25cm^2 was placed on the surface to be sampled.
4. The sample collection cotton swab was moistened with sterile nutrient broth.
5. The swab was rubbed by rotating it in the predetermined surface area within the aluminum plate holding the swab at a 30° angle to the contact surface.
6. The swab was inserted into a test tube containing 5ml nutrient broth.
7. Then the swab within the test tube was transported in to microbiology laboratory.

ANNEX-IV

Table 8.2. Panels of antibiotic discs that were used in the study for susceptibility testing

Type of antibiotics	Abbreviation	Disc content	Product of
Ampicillin	AML	10 µg	Hi-media Ltd♠
Ceftriaxone	CRO	30 µg	Oxoid Ltd♣
Cephalothin	KF	30 µg	Oxoid Ltd
Chloramphenicol	C	30 µg	Oxoid Ltd
Ciprofloxacin	Cf	5 µg	Hi-media Ltd
Erythromycin	E	15 µg	Oxoid Ltd
Gentamicin	CN	10 µg	Oxoid Ltd
Methicillin	Met	5 µg	Hi-media Ltd
Penicillin G	P	10 IU	Oxoid Ltd
Tetracycline	T	30 µg	Hi-media Ltd
Trimethoprim-sulphamethoxazole	SXT	1.25/23.75 µg	Oxoid Ltd
Oxacillin	Ox	1 µg	Hi-media Ltd
Vancomycin	VA	30 µg	Oxoid Ltd

♠: Hi Media Company Limited, India

♣: Oxoid Ltd Basingstoke, Hampshire-UK

ANNEX-V

Table 8.3. Air total bacterial count according to Fisher in different hospital environments (CFU on Petri dishes 9 cm in diameter with blood-agar left open to air according to the scheme 1/1/1)[48,49]

Place	Total microbial count (cfu//h)		
	Optimal	Acceptable	Unacceptable
Surgical ward units	0-250	251-450	> 451
ORs (when at rest)	0-4	5-8	> 9
ORs (when active)	0-60	61-90	> 91

Annex VI

Pour Plate Technique procedure used in the study

1. The 5ml elution solution was mixed using vortex mixer to ensure homogenous distribution.
2. One ml suspension from the 5 ml elution solution was transferred into the sterile Petri dishes.
3. The plate count agar which were already prepared and placed at 50c^{o} water bath was poured in to a plat having a 1 ml specimen
4. The Petri dish was covered and swirled clockwise and anti clockwise to mix the inoculated sample well
5. The Petri dish was inverted and incubated at 37 c^{o} for 24 hour when the agar is set
6. The colonies in the plates were then counted and recorded

DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been dully acknowledged.

Name: __Chalachew Genet_____

Signature: _____

Date of submission: _____

Jimma, Ethiopia

This thesis has been submitted for examination with my approval as University advisor

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