Journal of Environmental and Occupational Science
www.jenvos.com
DOI: 10.5455/jeos.20140512124135

Original Research

GESDAV

Isolation of bacterial pathogens from patients with postoperative surgical site infections and possible sources of infections at the University of Gondar Hospital, Northwest Ethiopia

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Received: March 31, 2014
Accepted: May 12, 2014
Published: May 21, 2014

ABSTRACT

Background: Hospital environment is a potential reservoir of bacterial pathogens since it houses both patients with diverse pathogenic microorganisms and a large number of susceptible individuals. The increased frequency of bacterial pathogens in hospital environment is associated with a background rise in various types of nosocomial infections. The rate of nosocomial infections is markedly higher in developing countries. Of these, surgical site infection (SSI) is one of the most frequent types of infections. **Aim:** The aim of this study was to isolate and correlate bacterial pathogens from hospital environments to that of patients with postoperative SSIs. **Methods:** A cross sectional study was conducted at the University of Gondar Teaching Hospital from November 2010 to February 2011. In the study, 220 specimens of pus, nasal, hand and surfaces swabs were collected using sterile cotton tipped applicator sticks moistened with normal saline. Colony characteristics and Gram's technique were used to differentiate the organisms. Biochemical tests were done to confirm the species of the organisms. **Results:** A total of 268 bacterial pathogens were recovered from all specimens processed in the study. Most of the isolates, 142 (52.9%) were from the hospital environments such as medical devices, inanimate objects and air. The rest, 77 (28.8%) and 49 (18.3%) were recovered from the health professionals and patients, respectively. Organisms associated with postoperative SSIs were *Staphylococcus aureus* 11 (22.4%) followed by Klebsiella species 10 (20.4%) and Proteus species 9 (18.4%), *Escherichia coli* 6 (12.2%), Enterobacter species and coagulase negative staphylococci each 4 (8.2%), *Pseudomonas aeruginosa* 3 (6.1%) and Citrobacter species 2 (4.1%). The predominant causes of postoperative SSIs were *S. aureus*, Klebsiella and proteus species. **Conclusion and Recommendation:** Medical equipment, inanimate objects, air and hands of health professionals were contaminated with various types of bacterial pathogens of nosocomial importance. It is imperative that all professionals should take an active role in infection control within their organization and more resources should be provided to encourage good hygienic practice in the hospital.

KEY WORDS: Bacterial pathogen, hospital environments, postoperative surgical site infection

INTRODUCTION

Hospital acquired infections are those infections that develop in patients during their stay in hospitals or other type of clinical facilities, which were not present at the time of admission [1]. The increased frequency of bacterial pathogens in the hospital environments is associated with a background rise in various types of nosocomial infections [2].

Surgical site infection (SSI) is one of the most frequent types of nosocomial infections in developing countries. The infection follows interference with the skin barrier, and is associated with the intensity of bacterial contamination of the wound at surgery or later in wards during wound care [3]. The rate of nosocomial infections is markedly higher in many developing countries, especially for infection that are largely preventable (e.g. those following surgical procedures). For instance, the prevalence of postoperative SSI was reported as 44.1% of the patients with nosocomial infection from Ethiopia [4]. Other studies from different parts of Ethiopia showed that *Staphylococcus aureus*, *Klebsiella* species, *Escherichia coli*, *proteus* species, *streptococcus* species, *Enterobacter* species, *pseudomonas* species and coagulase negative staphylococci were the most common pathogens isolated from wound [5,6].

The source of postoperative SSIs can be either endogenous or exogenous. Patients may be infected by their body flora following surgical manipulation which in most cases suppresses the natural body defence mechanisms [7] and exogenous sources of infections include hospital staff, other patients, and visitors, food, water, fomites [8,9].

Bacterial pathogens that able to survive in the hospital environment for long period and resist disinfection are particularly more important for nosocomial infections [10]. A systematic review of nosocomial pathogens indicated that most Gram-positive bacteria such as Enterococcus species including vancomycin resistant Enterococci, *S. aureus* including methicillin resistance *S. aureus* (MRSA) and *Streptococcus pyogenes* survive for months on dry surfaces. Many Gram-negative bacteria, such as *Acinetobacter* species, *E. coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, and *Serratia marcescens* can survive on inanimate surfaces even for months [10].

Despite the advance in modern medicine nosocomial infection still poses a risk of increased morbidity and mortality to patients. It is thereby important to identify environmental surfaces that are rich in bacteria and have the potential to harbor pathogens. Therefore, this study was undertaken to investigate the distribution of potential bacterial pathogens isolated from patients with postoperative SSI, health professionals and environmental samples in operating rooms, orthopedic and surgical wards at Gondar University Teaching Hospital.

MATERIALS AND METHODS

Study Design, Area and Period

A hospital based cross sectional study was conducted at the University of Gondar Teaching Hospital from November 2010 to February 2011. The hospital is located in Gondar town in Amhara regional state. The University Hospital is one of the biggest tertiary level referral and teaching hospitals in the region. A large number of people from the surrounding zones and nearby regions visit the hospital for both inpatient and an outpatient treatment.

Sample Size and Sampling Technique

All the 42 patients who had developed postoperative SSI during the study period were included in the study. Thirty six volunteer health professionals in operating room, surgical and orthopedic wards were also included. In addition, 142 inanimate objects within the operating room, surgical and orthopedic wards that could be touched with hands of health professionals, patients and attendants were screened for bacterial contaminations. Patients who do not develop postoperative SSI on clinical examination during the study period were excluded from the study.

Definition of Terms

SSI: A type of healthcare associated infection in which wound infection occurs at the site of surgery. The diagnosis of SSI was based on the observation of pus, serous or nonpurulent discharge from surgical site, signs of inflammation (edema, redness, heat, fever, indurations and tenderness). Operations were classified as clean where no inflammation is encountered and the respiratory, alimentary or genitourinary tracts were not entered. Clean contaminated where the respiratory, alimentary or genitourinary tracts were entered but without significant spillage. Contaminated when acute inflammation is encountered, or there is visible contamination of the wound. Dirty in the presence of pus, where there is a previously perforated hollow viscous or compound/open injury more than four hours old [11].

Specimen Collection

After operation, patients were followed by a surgeon to assess the progress of wound healing until they become discharged as part of the routine activity. From all the 42 patients whose diagnosis was confirmed as wound sepsis by a surgeon, wound secretions were aseptically obtained before the wound was cleaned with an antiseptic solution and before antibiotic therapy was started. Specimens were collected on sterile cotton swabs without contaminating them with skin commensals. Wound secretions were taken from patients who had SSI the first episodes of infection. Nasal and hand swabs were taken from 36 health professionals. A sterile cotton swab moistened with normal saline was passed into the anterior nares of both the nostrils and rotated in both directions. A separate sterile cotton swab was rotated on the palms, fingers and fingernails of the dominant hand of health professionals. Specimens from health professionals were collected later in the shift (after 5 pm in the afternoon).

One hundred forty two environmental specimens were collected from medical devices (such as suction machine, operating table, oxygen cylinder, blood pressure apparatus, light sources, sterile materials), air, and inanimate objects (such as floor areas, walls, bed-frames, door handles, light switches, sinks, stands for infusion apparatus and disinfectants). Sterile cotton tipped applicator sticks moistened with normal saline was rotated against the surface of inanimate objects to obtain specimens. For air samples, blood agar plates (BAP) were distributed at various distances in the operating room and wards. The plates were left opened to the air for 1 h [12]. Following collection specimens were transported by placing each swab in a separate sterile test tube to the microbiology laboratory within 30 min.

Specimen Processing

Following collection, the swabs were inoculated into MacConkey agar, BAP and manitol salt agar (Oxoid Limited). The inoculated agar plates were incubated at 35°C for 24-48 h. Then the growth was inspected to identify the bacteria. Preliminary identification of bacteria were performed based on gram reaction, colony characteristics of the organisms like hemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms [13]. Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests (Oxoid Limited). Namely, triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, manitol and motility. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results [14]. International control bacteria strains, *E. coli* (American Type Culture Collection [ATCC] 25922) *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were used in controlling the tests carried out [14].

Data Analysis

Two different data clerks double entered data. Entry data verification was done in order to clean data in readiness for statistical analysis. The data was analyzed using SPSS statistical software version 17. Then, study findings were interpreted in words, number, percentage and tables.

Ethical Considerations

The study was approved by the Department Ethical Review Committee of Microbiology Immunology and Parasitology, School of Medicine, Addis Ababa University. Permission was also obtained from the Gondar University Hospital administrator. Subjects were recruited after they become informed about the objectives and use of the study. In addition, written consent was obtained from study subjects (patients and health professionals).

RESULTS

A total of 220 swab specimens were collected from patients, health professionals and hospital environments. Of these, 42 (19.1%) were from patients with postoperative SSIs, 142 (64.5%) from inanimate objects in the hospital and 36 (16.4%) from health professionals. A total of 268 bacterial pathogens were recovered from all specimens processed. Of these, 70.1% ($n = 188$) were Gram-positive and 29.9% ($n = 80$) were Gram-negative bacteria. Majority of the bacteria, 142 (53%) were isolated from the environmental samples. The rest, 77 (28.7%) and 49 (18.3%) were recovered from health professionals and patients, respectively. Among the Gram-positive isolates, coagulase negative staphylococci were predominant from the health professionals and the environments followed by *S. aureus*, *Klebsiella* species, *E. coli*, *Proteus* species and *P. aeruginosa* were the most common isolates of the Gram-negative rods.

Postoperative SSIs

During the study period 510 operations were done. Of these, 42 patients developed SSI and the rate SSI was 8.2%. Twenty-seven (64.3%) of the patients with SSIs were males and 15 (35.7%) were females. The age of study groups ranged from 4-77 year. The majority of wound swabs, 37 (88.1%) had bacterial growth within 18-24 h of incubation. Twelve out of 37 (32.4%) had mixed growth, while 25 (67.6%) had pure bacterial growth. The rest, 5 (11.9%) had no bacterial growth even after 48 h of incubation. The rate of bacterial isolation among those patients who had clinically septic wound infections was 88.1%. The number of bacterial isolates from males 25 (59.5%) were higher than females 12 (28.5%). The difference was not statistically significant ($P = 0.227$). Thirty-four (69.4%) of the isolates of postoperative SSIs were Gram-negative bacteria. Fifteen (30.6%) of the isolates were Gram-positive cocci. Most of the isolate, 22 (44.9%) were recovered from patients who had Laparatomy. Of these, Gram-negative bacteria accounted for 19 (86.4%) of the isolates. The predominant isolate from Laparatomy was *Proteus* species 6 (27.2%) followed by *Klebsiella* species 4 (18.2%), *Enterobacter* species and *P. aeruginosa* each 3 (13.6%) and C*itrobacter* species 1 (4.5%). The rest, 3 (13.6%) of the isolates were *S. aureus* from Gram-positives. Eight (88.9%) of the isolates from patients who had prostatectomy was Gram-negatives. *Klebsiella* species 4 (44.4%), *E. coli* 2 (22.2%) and *Proteus* species 1 (11.1%) were the common isolates. Only coagulase negative staphylococcus 1 (11.1%) was isolated among the gram positives. The predominant isolate in amputation and thyroidectomy were *S. aureus* [Tables 1 and 2].

Environmental Survey of Bacterial Pathogens

In this study, 142 specimens were also collected from various environmental sources 56 located in the operating room, 42 in surgical and 44 in orthopedic wards [Table 3]. From these, 118 (83.1%) of the inanimate objects; 48 (33.8%) in operating room, 36 (25.4%) in surgical ward and 34 (23.9%) in orthopedic

Table 1: List of surgical procedures and corresponding bacterial isolates from patients who had postoperative surgical site infection, November 2010-February 2011

Site of surgical procedures	Patients (no (%))	Bacterial isolates (no (%))		Total (%)
		Gram- positive	Gram- negative	
Laparatomy	13 (30.9)	3 (13.6)	19 (86.4)	22 (100)
Prosteoctomy	7 (16.7)	1 (11.1)	8 (89.1)	9 (100)
Cesarean section	7 (16.7)	2 (33.3)	4 (66.7)	6 (100)
Amputation	5 (11.9)	3 (60)	2 (40)	5 (100)
Thyroidectomy	3 (7.1)	2 (66.7)	1 (33.3)	3 (100)
Appendectomy	3 (7.1)	1 (50)	1 (50)	2 (100)
Excision	1 (2.4)	0 (0)	0 (0)	0 (0)
Fixation	1 (2.4)	0 (0)	1 (100)	1 (100)
Debridement	1 (2.4)	1 (100)	0 (0)	1 (100)
Incision	1 (2.4)	0 (0)	0 (0)	0 (0)
Total	42 (100)	15 (30.6)	34 (69.4)	49 (100)

Note: Gram-negative isolates were *Escherichia coli*, Klebsiella species, *Enterobacter* species, *Citrobacter* species *Pseudomonas aeruginosa* and *Proteus* species. Whereas Gram-positive isolates were *Staphylococcus aureus* and coagulase negative staphylococci

Table 2: Profiles of bacterial isolates identified in postoperative wound infection Gondar University Hospital, November 2010-February 2011

Bacterial isolate	Total	Percentage
Gram-positive	15	30.6
Staphylococcus aureus	11	22.4
Coagulase negative staphylococcus	4	8.2
Gram-negative	34	69.4
Klebsiella species	10	20.4
Proteus species	9	18.4
Escherichia coli	6	12.2
Enterobacter species	4	8.2
Pseudomonas aeruginosa	3	6.1
Citrobacter species	2	4.1
Total	49	100

Table 3: The distribution pattern of bacterial isolates in different surgical units of Gondar University Hospital, November 2010-February 2011

Bacterial isolate	Surgical units			Total
	Orthopedic ward	Surgical ward	Operating room	
Gram-negative	13 (30.9)	15 (31.3)	13 (24.1)	41 (28.9)
Klebsiella species	3 (23.1)	5 (33.2)	3 (23.1)	11 (26.8)
Escherichia coli	2 (15.3)	3 (20)	5 (38.4)	10 (24.3)
P. aeruginosa	3 (23.1)	4 (26.7)	1 (7.7)	8 (19.5)
Proteus species	1 (7.7)	1 (6.7)	3 (23.1)	5 (12.2)
Enterobacter species	3 (23.1)	1 (6.7)	0 (0)	4 (9.8)
Citrobacter species	1 (7.7)	0 (0)	1 (7.7)	2 (4.9)
Serratia species	0 (0)	1 (6.7)	0 (0)	1 (2.4)
Gram-positive	29 (69)	33 (68.7)	39 (75)	101 (71.1)
Coagulase negative staphylococcus	21 (72.4)	21 (63.6)	27 (69.2)	69 (68.3)
Staphylococcus aureus	7 (24.1)	12 (36.3)	12 (70.8)	31 (30.7)
Enterococcus species	1 (3.4)	0 (0)	0 (0)	1 (1)
Total	42 (29.6)	48 (33.8)	52 (38)	142 (100)

wards had demonstrated evidences of bacterial contaminations. Of 26, 118 (22.0%) inanimate objects had mixed bacterial growth, while 92 (77.9%) had pure growth. Twenty-four (16.9%) of the inanimate objects; 8 (5.7%) in operating room, 6 (4.2%) in surgical ward and 10 (7.0%) in orthopedic ward did not show any bacteria growth even after 24 h of incubation. Out of 142 bacterial pathogens isolated from inanimate objects 101 (71.1%) and 41 (28.9%) were Gram-positive and Gram-negative, respectively.

The hospital environment that had the highest number of bacterial isolates was the operating room. This room was accounted for 52 out of the 142 (38%) isolates from all the surgical units. Surgical ward followed closely with 48/142 (33.8%) isolate, while orthopedic ward had 42/142 (29.6%). The most commonly isolated Gram-positive bacteria from the surgical units were coagulase negative staphylococci 69 (68.3%) followed by *S. aureus* 31 (30.7%) and *Enterococcus* species, 1 (1%). Similarly, *Klebsiella* species 11 (26.8%), *E. coli* 10 (24.3%), *P. aeruginosa* 8 (19.5%), *Proteus* species 5 (12.2%) and *Enterobacter* 4 (9.8%) were common among Gram-negative isolates. Citrobacter 2 (4.9%), *Serratia* and *Enterococcus* species each 1 (2.4%) were the least isolated [Table 3]. Gram-positive bacteria in the genus *staphylococci* were found contaminating most dry surfaces. Whereas, Gram-negative rods especially in the family of Enterobacteriaceae were most frequently isolated on moisten objects such as sink. A total of 23 bacterial pathogens were isolated from sinks. Of these, 22 (95.6%) and 1 (4.4%) were Gram-negative and Gram-positive, respectively. From the total isolates of sinks *E. coli* 6 (27.3%), *Klebsiella* species 5 (27.7%), *P. aeruginosa* 4 (18.2%), *Enterobacter*, *Citrobacter* and *proteus* species each 2 (9%) and *Serratia* species 1 (4.5%) were the common isolates. Other inanimate objects such as floor, tables, door handles, light switch, infusion sands, bed-frames and medical equipment were found to be contaminated with Gram-positive bacteria of the genus staphylococcus. Fortunately, bacterial pathogens were not identified from antiseptic solutions and sterile materials such as forceps and scissors. A total of 22 bacterial pathogens were recovered from air samples of surgical units of the hospital. Eighteen (81.8%) and four (18.2%) were Gram-positive and Gram-negative, respectively. The predominant isolates in the air sample were coagulase negative staphylococci 12 (53.6%) followed by *S. aureus* 6 (27.3%), *P. aeruginosa* 2 (9.1%), *Proteus* species and *Enterobacter* species each 1 (4.5%).

Hands and Nasal Carriage of Health Professionals

Out of 40 health professionals approached, 36 agreed to participate. These comprise 24 (66.7%) males and 12 (33.3%) females with ages ranging from 22-50 years (mean age 32.4 year). Seventy-two swab specimens were collected from the dominant hand and nostrils of health professionals. A total of 77 bacterial pathogens of nosocomial importance were isolated. Of these, 36 (46.8%) and 41 (53.2%) were from nostril and hands, respectively. Seventy-two of the isolates were Gram-positive, while 5 (6.5%) were Gram-negatives. Coagulase negative staphylococcus 44 (57.1%) was the predominant isolate followed by *S. aureus* 28 (36.4%). Thirty-two (88.9%) of the health professionals hands were found to be contaminated with one or more bacterial pathogens of nosocomial importance. Coagulase negative staphylococci 23 (56.1%), *Klebsiella* species 3 (7.3%), *E. coli* and *Enterobacter* species 1 (2.4%) each were isolated from the dominant hands of health professionals [Table 4].

DISCUSSION

In countries where resources are limited, postoperative SSIs remain as one of the major types of nosocomial infections [15]. The successful management of patients suffering from bacterial illnesses depends upon the identification of the types of organisms that cause the diseases [16]. The profiles of bacterial isolates from swabs of postoperative SSI in this study are consistent with previous reports in Bahir Dar [6] and Gondar [7]. The organisms associated with the infections were *S. aureus* 11 (22.4%), *Klebsiella* species 10 (20.4%), *Proteus* species 9 (18.4%), *E. coli* 6 (12.2%), *Enterobacter* species and coagulase negative staphylococci each 4 (8.2%), *P. aeruginosa* 3 (6.1%) and *Citrobacter* species 2 (4.1%). This finding is again in agreement with similar studies in Addis Ababa, Uganda and logos-Nigeria [17-19].

Table 4: Common bacterial isolates from the dominant hand and nostrils of health professionals at Gondar University Hospital, November 2010-February 2011

Bacterial isolates	Hand swabs No (%)	Nasal swabs No (%)	Total No (%)
Gram-positive	36 (87.8)	36 (100)	72 (93.5)
Coagulase negative staphylococcus	23 (88.4)	21 (58.3)	44 (57.1)
Staphylococcus aureus	13 (11.6)	15 (41.7)	28 (36.4)
Gram-negative	5 (12.2)	-	4 (5.2)
Klebsiella species	3 (7.3)	-	3 (3.9)
Escherichia coli	1 (2.4)	-	1 (1.3)
Enterobacter species	1 (2.4)	-	1 (1.3)
Total	41 (53.2)	36 (46.8)	77 (100)

This result showed that *S. aureus*, *Klebsiella* species and *Proteus* species were the major bacterial pathogens associated with surgical wound infections. This result is consistent with data in Eastern Nigeria [20]. According to work done by Endalafer *et al*., 2011 [17] it was reported that out of 44 surgical wound patients examined microbiologically for surgical wound infection, 15.9% had Staphylococcal and Klebsiella surgical site wound infection while, 13.6% had Proteus infection. It was found that most of the inanimate objects (83.1%) in the hospital wards and operating room were variously contaminated by bacterial agents many of which are recognized pathogens. Coagulase negative staphylococci 69 (68.3%) were the most frequently isolated from all the samples collected from the wards and operating room followed by *S. aureus* 31 (30.7%). This finding is consistent with a study in Nigeria [21]. Other studies in Taiwan and Nigeria also demonstrate similar finding on patient's medical chart and X-ray machine contamination with coagulase negative staphylococci [22,23]. The reason of their high prevalence may because of the fact that staphylococci are members of the body flora of health and sick individuals that can be spread by their hands, expelled from respiratory tract to the immediate environment [24].

Gram-negative organisms that comprises of 41 (28.9%) of the environmental isolate were found contaminating surfaces of sink drains, door handle, bed-frames, and floor areas of the wards and operating rooms. In this study, it was observed that, sinks harbor more than half of the Gram-negative bacterial isolates consistently than do other sites (dry surface areas, e.g. walls, floors, medical equipments and tables) in patient care areas. The common bacterial isolates of sink were *E. coli* 6 (27.3%), *Klebsiella* species 5 (27.7%), *P. aeruginosa* 4 (18.2%), *Enterobacter*, *Citrobacter* and *proteus* species each 2 (9%) and *Serratia* species 1 (4.5%) were the common isolates. Operating table, Suction machines, blood pressure apparatus, oxygen cylinder, light sources that are frequently used in the operating room during operation were found to be contaminated mainly with *S. aureus* and other coagulase negative staphylococci. This finding is in agreement with similar report in Nablus [25]. The habit of leaving this equipment for long periods without cleaning and proper disinfection after use is possibly responsible for this contamination. Similarly other immovable objects such as floor, wall, light switch, door handles, bed-frames and infusion stands were heavily contaminated with Gram-positive bacteria of the genes staphylococci. The detection of members of Enterobacteriaceae in some of the inanimate objects in the hospital environments may be due to poor hygienic practice in the wards and/or visitors who attend their sick relative at the hospital may be the source of transfer of these enteric pathogens to health care facilities.

The present study also showed that the percentage of bacterial pathogen isolated from the air of surgical units of the hospital was 18 (81.8%). The pattern of the isolates in this study is consistent with a study in Sudan [12]. Although the direct involvement of the inanimate objects in case of disease transmission is not investigated in this work, the isolation of *S. aureus*, *P. aeruginosa*, *E. coli*, *Proteus* species, *Klebsiella* species and *Enterobacter* species is a concern for possible nosocomial transmission.

Results of the nasal swabs culture of this study indicated that health professionals carried coagulase negative staphylococcus 21 (58.3), *S. aureus*, 15 (41.7%) and MRSA 3 (8.3%) in their anterior nares. This finding is inline with a study in Pakistan where the prevalence of *S. aureus*, coagulase negative staphylococci and methicillin resistant *S. aureus* among health care workers were 48%, 46% and 14% respectively [26]. However, this is lower when compared with the carrier rate of (76%) among burns unit staff of Indian tertiary care hospital [27]. This difference could be due to the fact that the Indian subjects were from special unit. This study also showed that nearly 90% health professional's dominant hands had bacterial contaminations mostly with coagulase negative staphylococcus. The rate of bacterial contamination of hands of health professionals in this study is higher as compared to a study in Iran, which was 39.3% [28]. This high rate of contamination may be due to habit of infrequent hand washing and lack of proper disinfection practice of the health professionals in our hospital.

CONCLUSION

The predominant causes of postoperative SSIs were *S. aureus*, *Klebsiella* species and *Proteus* species. Different medical equipment, environmental surfaces, air and hands of health personnel were contaminated with various types of bacterial pathogens. Table surfaces, infusion stands and other movable objects used by health professionals in daily practice may be a source of nosocomial infections in this hospital. Gram-positive staphylococci were more frequently isolated from the operating room and wards especially from the environmental samples and health professionals than from patients with postoperative SSI. It is imperative that all professionals should take an active role in infection control within their organization and more resources should be provided to encourage good hygienic practice in the hospital.

ACKNOWLEDGEMENTS

We would like to express our heartfelt gratitude to all staff of the Department of Microbiology Immunology and Parasitology, University of Gondar and particularly, the bacteriology unit of Hospital Laboratory for providing the necessary materials and reagents. We are also indebted to health professionals and patients who were participants of the study.

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Source of Support: Nil, Conflict of Interest: None declared.