Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN	Print	2231 – 3648
	Online	2231 – 3656

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIAL ISOLATES FROM PATIENTS WITH POSTOPERATIVE SURGICAL SITE INFECTION, HEALTH PROFESSIONALS AND ENVIRONMENTAL SAMPLES AT A TERTIARY LEVEL HOSPITAL, NORTHWEST ETHIOPIA

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Abstract

The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality. The emergence of multi-drug resistant organisms in hospitals results in difficulty to treat nosocomial infections. Therefore, the aim of this study was to assess the antimicrobial resistance patterns of bacterial isolates from patient's health professionals and the hospital environments. Cross sectional study was conducted at the University of Gondar Hospital from November 2010 to February 2011. Two hundred twenty specimens of pus, nasal, hand and surfaces swabs were collected using sterile cotton tipped applicator stick moistened with sterile saline. Biochemical tests were done to confirm the species of the organisms. Sensitivity tests were performed using agar disc diffusion technique. Two hundred sixty eight bacterial pathogens were recovered from all the specimens processed. Most of the isolates, 142(52.9%) were from the environments. The rest, 77(28.8%) and 49(18.3%) were from health professionals and patients, respectively. Gram-negative rods were resistant to ampicillin 72(90%), cotrimoxazole, 68 (85%), doxycycline, 66 (82.5%), tetracycline, 63(78.8%), chloramphenicol, 48 (60%), nalidixic acid, 46 (57.5%) and gentamicin, 38 (47.5%). *S. aureus* demonstrated high level of resistance to nalidixic acid and tetracycline while, ceftriaxone and ciprofloxacin were found to be relatively effective. Single as well as multiple antimicrobial resistances to the antimicrobials tested was alarmingly high. 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 antibiotic practice and good hygiene in hospitals.

Keywords: Bacterial pathogen, Antimicrobial Resistance, Hospital environments.

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Introduction

The rate of nosocomial infections is markedly higher in many developing countries[1]. Bacterial Pathogens that infect patients in the hospital can be part of the patient's normal flora or acquired from the hospital environment or other infected patients. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality[2]. Pathogens that can able to survive in the hospital environment for long period and resist disinfection are particularly more important for nosocomial infections[3]. Those bacterial pathogens isolated from hospital environments are known to be resistant to multiple antimicrobial agents[4].

Bacterial pathogens may be intrinsically resistant to more than one class of antimicrobial agents, or may acquire resistance by mutation or via the acquisition of resistance genes from other organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug; express efflux systems that prevent the drug from reaching its intracellular target, modify the drug's target site, or produce an alternative metabolic pathway that bypasses the action of the drug[5]. The major cause of community and hospital acquired infections, *s. aureus*, used to respond to β -lactam and related group of antibiotics. However, due to development of methicillin resistance amongst *S. aureus* isolates; treatment of these infections has become problematic[6]. Furthermore, antibiotic resistance often complicates the treatment of serious infections due to *Acinetobacter baumannii*, extended-spectrum β-lactamase producing Enterobacteriaceae, and *Pseudomonas aeruginosa* in clinical practice. The other problematic organisms were the Gram-positive pathogens, coagulase negative staphylococci and vancomycin-resistant *Enterococcus* species[4,7].

Information obtained from this antimicrobial resistance surveillance study is important for establishing trends in pathogen antimicrobial resistance and for identifying emerging pathogens at the national levels. This information enables the development of targeted approaches to help control antimicrobial resistance. Therefore, this study was undertaken to investigate the antimicrobial susceptibility pattern of potential bacterial pathogens isolated from patients, health professionals and environmental samples in the operating room, orthopedic and surgical wards at the University of Gondar Teaching Hospital.

Materials and methods

Study area and period

The 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. Gondar is 739 km far from Addis Ababa to the Northwest of Ethiopia. This University Hospital is one of the biggest tertiary level referral and teaching hospitals in the region. About 5 million people from the surrounding zones and nearby regions visit the hospital for both inpatient and outpatient treatment. The hospital consists of an operating room, intensive care unit (ICU) with 12 beds, 13 wards with 427 beds, and outpatient Departments.

Study design and population

Hospital based cross sectional study was conducted. Patients who had developed postoperative surgical site infection during the study period, health professionals and inanimate objects in operating room orthopedic and surgical ward were the study populations.

Definition of terms

Surgical site infection: a type of healthcare-associated infection in which wound infection occurred at the site of surgery. The diagnosis of surgical site infection was based on the observation of pus, serous or non-purulent discharge from surgical site, signs of inflammation (oedema, redness, heat, fever, indurations and tenderness).
Environmental samples: swab specimens that were taken from inanimate objects and air in operating room, surgical and orthopedic wards of the Hospital.

Sample size

The sampling technique was convenient. All the 42 patients who had developed postoperative surgical site infection during the study period were included in the study. Thirty-six volunteer individuals out of fourty health professionals in operating room, surgical and orthopedic wards were also included.

In addition, 142 inanimate objects within the surgical units and that could be touched with hands of health professionals, patients or attendants were screened for bacterial contamination.

Specimen Collection

From all the 42 patients whose diagnosis was confirmed as wound sepsis by a surgeon, wound secretions were aseptically obtained from surgical sites using sterile cotton tipped applicator sticks. 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. One hundred forty two environmental specimens were collected from medical devices(such as suction machine, operating table, oxygen cylinder, blood pressure apparatus, light source, 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 swabs moistened with normal saline was rotated against the surface of inanimate objects to obtain specimens. For air samples, blood agar plates were distributed at various distance in the operating room and wards and left opened to the air for 1hour[8]. Following collection specimens were transported by placing each swab in a separate sterile test tube to the microbiology laboratory within 30 minutes.

Specimen Processing

The swabs were inoculated into MacCkonkey agar, blood agar plates (BAP) and manitol salt agar (Oxoid, LTD) and incubated at 35°C for 24-48 hours. Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. International Control bacteria strains**,** *Escherichia coli* (ATCC 25922) S. *aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used in controlling the performance of culture media and antimicrobial discs[9, 10].

Antimicrobial Susceptibility test (AST)

Susceptibility testing was performed based on the agar disc-diffusion technique[9]. The suspensions of the test organism were prepared by picking parts of similar test organisms with a sterile wire loop, suspended in sterile broth. The densities of suspension to be inoculated were determined by comparison with 0.5 McFarland standards. A sterile swab was dipped into the suspension of the isolate, squeezed free from excess fluid against the side of bottle. The test organism were uniformly seeded over the Mueller-Hinton agar surface (Oxoid, LTD) and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated discs into the agar medium. The medium was incubated at 35^{o}C for 18-24 hours. Grades of susceptibility pattern were recognized as sensitive and resistant by comparison of zone of inhibition as indicated in the manufacturer's guide. The drugs tested for both gram negative and gram positive bacteria were ampicillin (10µg), ciprofloxacin (5µg), gentamicin (10µg), tetracycline (µg), cotrimoxazole (25µg) chloramphenicol (30µg), doxycycline (30µg), nalidixic acid (15µg) and ceftriaxone (30µg). Methicillin (5µg), penicillin (10IU), erythromycin (15µg) and vancomycin (30µg) were used for only gram-positive bacterial isolates.

Data Analysis

The data was analyzed using SPSS statistical software version 17. Study findings were explained in words, 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.

Results

Profiles of study subjects

During the study period 510 operations were done. Of these, 42 patients developed surgical site infection and the rate of surgical site infection was 8.2%. Twenty-seven (64.3%) of the patients with surgical site infections were males and 15(35.7%) were females. 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 to 50 years (mean age 32.4 year). The duty of these health professionals were surgeons, nurses and anesthetics. 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.

Bacterial isolates

A total of 220 specimens were collected from patients, health professionals and hospital environments. Of these, 42(19.1%) were from patients, 142 (64.5%) from inanimate objects and 36(16.4%) from health professionals. A total of 268 bacterial pathogens were recovered. Of these, 70.1% (n =188) were gram-positive and 29.9% (n = 80) were gram-negative bacteria. Forty-three out of the 220 swabs (19.5 %) had mixed growth, while 144 (65.5 %) had pure growth. The rest, 33 (15 %) had no bacterial growth. Majority, 142(53%) were isolated from the environment. 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.* whereas, *Klebsiella* specie, *Escherichia coli*, *Proteus* species and *Pseudomonas aeruginosa* were the most common isolate of the gram-negative rods (Table 1).

Table No. 01: Bacterial pathogens isolated from environmental samples, patients and health professionals of surgical units at Gondar University Hospital, November to February 2011

Bacterial isolate	Environment No (%)	Patients No (%)	Health professionals No (%)	Total
Gram positive	**101(71.1)**	**15(30.6)**	**72(93.5)**	**188(70.1%)**
CN Staphylococcus	69 (68.3)	4(26.7)	44(61.1)	117(62.2)
Staphylococcus aureus	31(30.6)	11(73.3)	28(38.9)	70(37.3)
Enterococcus species	1(0.99)	0(0)	0(0)	1(0.5)
Gram negative	**41(28.9)**	**34(69.4)**	**5(6.5)**	**80(29.9%)**
Escherichia coli	10 (24.4)	6(17.6)	1(20)	17(21.3)
Klebsiella species	11(26.8)	10(66.7)	3(60)	24(30)
Pseudomonas aeruginosa	8(19.5)	3(20)	0(0)	11(13.8)
Enterobacter species	4(0.9)	4(26.7)	1(20)	9(11.2)
Citrobacter species	2(0.5)	2(13.3)	0(0)	4(5)
Proteus species	5(12.2)	9(60)	0(0)	14(17.5)
Serratia species	1(2.4)	0(0)	0(0)	1(1.2)
Total	142(53)	49(18.3)	77(28.7)	268(100)

Antimicrobial susceptibility test

Gram-negative rods isolated from different sample sources were deemed highly resistant to most of the antibiotics tested. Of the isolates, 72(90%), 68(85%), 66(82.5%), 63(78.8%), 48(60%), 46(57.5%) and 38(47.5%) were found to be resistant to ampicillin, cotrimoxazole, doxycycline, tetracycline, chloramphenicol, nalidixic acid and gentamicin in their respective order. Among other antibiotics ceftriaxone 48(40%) and ciprofloxacin 61(76.3%) were relatively effective against gram-negative bacterial isolates.

Among the gram negatives, *Klebsiella* specie demonstrated high level of resistance to ampicillin 22(91.7), cotrimoxazole, gentamicin, chloramphenicol and doxycycline each 20(83.3%), tetracycline 19(79.2%), ceftriaxone and nalidixic acid each 16(66.7%). Relatively ciprofloxacin were effective against 15(62.5%) of the *Klebsiella* species. More than 80% of *Escherichia coli* isolates were resistance to tetracycline, ampicillin, cotrimoxazole, and doxycycline. Ciprofloxacin 12(70.6%) and ceftriaxone 14(82%) were effective against *Escherichia coli*. 10 out of 11(90. 9%) isolates of *Pseudomonas aeruginosa* demonstrated high level of resistance to tetracycline, ampicillin, cotrimoxazole, nalidixic acid, doxycycline and chloramphenicol. Three (75%) of *Citrobacter* species were resistance to all the antibiotics tested with the exception of 2 (50%) susceptibility to ciprofloxacin. Twelve (85.7%) of *Proteus* species were found to be resistant to chloramphenicol, ampicillin and ciprofloxacin. Gentamicin and tetracycline each 12(85.7%), ceftriaxone, cotrimoxazole, doxycycline and nalidixic acid 8(57.1%) were effective against *Proteus* species. *Entrobacter* isolates were also highly resistant to most of the antibiotics tested: cotrimoxazole, ampicillin and chloramphenicol each 7(77.8%), doxycycline and nalidixic acid each 5(55.6%). Whereas gentamicin and ciprofloxacin each 7(77.8%) were effective. *Serratia* species were susceptible to all the antibiotics tested with the exception of ampicillin (Table 2).

Table No. 02: Antimicrobial susceptibility pattern of gram-negative bacteria isolated from patients, environment and health professionals at Gondar University Hospital, November 2010 to February 2011.

Antimicrobials tested	Pattern	Organisms isolated						
		***Klebsiella* species**	***E. coli***	***P. aeruginosa***	***Proteus* species**	***Enterobacter* species**	***Citrobacter* species**	***Serratia* species**
Gentamicin	S	4(16.7)	8(47.1)	4(36.3)	12(85.7)	7(77.8)	1(25)	1(100)
	R	20(83.3)	9(52.9)	7(63.7)	2(24.3)	2(22.2)	3(75)	0(0)
Ciprofloxacin	S	15(62.5)	12(70.6)	9(81.8)	2(14.3)	7(77.8)	2(50)	1(100)
	R	9(37.5)	5(29.4)	2(18.2)	12(85.7)	2(22.2)	2(50)	0(0)
Tetracycline	S	5(20.8)	2(11.8)	2(18.2)	12(85.7)	6(66.7)	1(25)	1(100)
	R	19(79.2)	15(88.2)	9(81.8)	2(14.3)	3(33.3)	3(75)	0(0)
Ampicillin	S	2(8.3)	2(11.8)	1(9.1)	2(14.3)	2(22.2)	1(25	0(0)
	R	22(91.7)	15(88.2)	10(91)	12(85.7)	7(77.8)	3(75)	1(100)
Ceftriaxone	S	8(33.3	14(82.4)	5(45.6)	8(57.1)	5(53.6)	1(25	1(100)
	R	16(66.7)	3(17.6)	6(54.4)	6(42.9)	4(44.4)	3(75)	0(0)
cotrimoxazole	S	4(16.7)	3(17.6)	1(9.1)	8(57.1)	2(22.2)	1(25	1(100)
	R	20(83.3)	14(82.4)	10(91)	6(42.9)	7(77.8)	3(75)	0(0)
Nalidixic acid	S	8(33.3)	5(29.4)	1(9.1)	6(42.9)	4(44.4)	1(25	1(100)
	R	16(66.7)	12(70.6)	10(91)	8(57.1)	5(55.6)	3(75)	0(0)
Doxycycline	S	4(16.7	3(17.6)	1(9.1)	8(57.1)	4(44.4)	1(25	1(100)
	R	20(83.3)	14(82.4)	10(91)	6(42.9)	5(53.6)	3(75)	0(0)
Chloramphenicol	S	4(16.7)	8(47.1)	1(9.1)	4(28.5)	2(22.2)	1(25	1(100)
	R	20(83.3)	9(52.9)	10(91)	10(71.5)	7(77.8)	3(75)	0(0)

S= sensitive R= Resistance

On the other hand, gram-positive cocci isolated from patients, health professionals and environment were sensitive to most of the antibiotics tested. Coagulase negative staphylococci were resistant to ampicillin 23(20%), cotrimoxazole 27(23%), doxycycline 42 (35.9%), chloramphenicol 45(38.5%, tetracycline 57(48.7%), nalidixic acid 102(87%). Ciprofloxacin 106(95.6%), erythromycin 96(82.1%), ceftriaxone 98(83.8%), and penicillin 93(79.5%) were effective for coagulase negative staphylococci. Thirty-three (28%) of the coagulase negative staphylococcus isolates were resistance to methicillin. *S. aureus* demonstrated high level of resistance to nalidixic acid 58(82.9%) and tetracycline 40(57.1%). Whereas, ceftriaxone 63(90%), ciprofloxacin 60(85.7%), cotrimoxazole 56(80%), erythromycin and chloramphenicol each 55(78.6%), ampicillin 53(75.7%), and doxycycline 47(67.1%) were relatively effective against *S. aureus* isolates. A total of 70 *S. aureus* strains were isolated on samples taken from patient's surgical site infection, health professionals and various environmental samples. Of these, 17(24.0%) were methicillin resistant, while 53(76%) were methicillin sensitive (MSSA). None of the S. *aureus* strains was resistance to vancomycin (Table 3).

Table No. 03: Antimicrobial susceptibility pattern of gram-positive bacteria isolated from patients, environment and health professionals at Gondar University Hospital, November 2010 to February 2011.

Antibiotics tested	pattern	Organisms isolated		
		Coagulase negative staphylococcus	*S. aureus*	*Enterococcus species*
Gentamicin	S	92(78.6)	58(82.8)	1(100)
	R	25(21.4)	12(17.2)	0(0)
Erythromycin	S	96(82.1)	55(78.6)	1(100)
	R	21(17.9)	15(21.4)	0(0)
Ciprofloxacin	S	106(95.6)	60(85.7)	1(100)
	R	11(4.4)	10(84.3)	0(0)
Tetracycline	S	60(51.3)	30(42.9)	1(100)
	R	57(48.7)	40(57.1)	0(0)
Penicillin	S	93(79.5)	58(82.8)	0(0)
	R	24(20.5)	12(17.2)	1(100)
Ampicillin	S	94(80)	53(75.7)	1(100)
	R	23(20)	17(24.3)	0(0)
Ceftriaxone	S	98(83.8)	63(90)	1(100)
	R	19(16.2)	7(10)	0(0)
cotrimoxazole	S	90(77)	56(80)	1(100)
	R	27(23)	14(20)	0(0)
Methicillin	S	84(72)	53(76)	0(0)
	R	33(28)	17(24)	1(100)
Nalidixic acid	S	15(13)	12(17.1)	1(100)
	R	102(87)	58(82.9)	0(0)
Vancomycin	S	117(100	69(100)	1(100)
	R	0(0)	0(0)	0(0)
Doxycycline	S	75(64.1)	47(67.1)	1(100)
	R	42(35.9)	23(32.9)	0(0)
Chloramphenicol	S	72(61.5)	55(78.6)	1(100)
	R	45(38.5)	45(21.4)	0(0)

Table No. 04: Multi drug resistance pattern of bacterial isolates from patients, heath professionals and hospital environmental sources, at Gondar university hospital, November 2010 - February 2011

Bacterial isolate	Total	Antibiogram pattern					
		R0	R1	R2	R3	R4	≥R5
Gram negative	**80(29.9)**	**3(3.6)**	**7(8.8)**	**4(5)**	**4(5)**	**11(13.8)**	**43(53.6)**
Klebsiella species	24(30)	0(0)	1(4.2)	0(0)	0(0)	1(4.2)	22(91.7)
Escherichia coli	17(21.3)	0(0)	2(11.8)	1(5.9)	0(0)	3(17.6)	11(64.7)
P. aeruginosa	11(13.6)	0(0)	2(18.2)	0(0)	1(9.1)	1(9.1)	7(63.6)
Proteus species	14(17.5)	2(14.3)	1(7.1)	1(7.1)	0(0)	5(35.7)	5(35.7)
Enterobacter species	9(11.3)	1(11.1)	0(0)	2(22.2)	0(0)	1(11.1)	5(55.6)
Citrobacter species	4(5)	0(0)	1(25)	0(0)	0(0)	0(0)	3(75)
Serratia species	1(1.3)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
Gram positive	**188(70.1)**	**12(6.4)**	**22(11.7)**	**50(26.6)**	**31(16.5)**	**22(11.7)**	**50(26.6)**
S. aureus	70(37.2)	5(7.1)	11(15.7	21(30)	6(8.6)	9(12.9)	18(25.7)
CN staphylococcus	117(62.2)	7(6)	11(9.4)	29(24.8	25(21.4)	13(11.1)	32(27,4)
Enterococcus species	1(0.5)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)
Total	**268(100)**	**15(5.6)**	**29(10.8)**	**54(20.1)**	**35(13.1)**	**33(12.3)**	**93(34.7)**

R0- No antibiotic resistance, R1- Resistance to one, R2-Resistance to two , R3-Resistance to three, R4-Resistance to four, ≥ R5-resistance to five and more antibiotics

More than half of the Gram negative isolates demonstrated evidences of multiple antibiotics resistance. For example, 22(91.7%) of *Klebsiella* species were resistant to at least five of the antibiotics tested. Eleven (64.7%) of *Escherichia coli and* 7(63.6%) of *Pseudomonas aeruginosa* were resistant to more than five of the antibiotics tested. Ten (71.4%) of *Proteus* species were resistance to at least for four of the antibiotics tested. Among the gram negative isolates 2(14.3%) *Proteus* species and 1(11.1%) *Enterobacter* species were susceptible to all of the antibiotics tested. Pan-antibiotic resistance was noted among 4(23.5%) *Escherichia coli*, 3(27.3%) *Pseudomonas aeruginosa* and 5 (20.8%) *Klebsiella* species isolates. On the other hand, more than 25% of gram positive isolates were resistant to at least five of the antibiotics. Six (8.6%), 9(12.9%) and 18(25.7) of the *S. aureus* isolates were found to be resistant for three, four and at least five of the antibiotics tested, respectively. five out of seventy isolates were susceptible to all of the antibiotics. None of the *S. aureus* isolates were pan- resistant. Coagulase negative staphylococci species had similar pattern of multiple antibiotic resistance with that of *S. aureus* isolates (Table 4).

Discussion

The successful management of patients suffering from hospital-acquired infections depends upon the identification of the types of organisms that cause the diseases and the selection of an effective antibiotic against the organism in question[11]. Antibiotics are the pillars of modern medical care and play a major role in prophylaxis and treatment of infectious diseases. Cognizant of this, the issue of their availability, selection and proper use are of critically important to the global community[11]. The data presented in this study could provide information of immediate public health importance to clinicians in Northwest Ethiopia on the selection of antimicrobial agents for prophylaxis and treatment of patients suffering from nosocomial infections.

The profiles of bacterial isolates from patients with postoperative surgical site infection in this study are consistent with previous reports in Bahir Dar[12] and Gondar[13]. The organisms associated with the infections were *S. aureus* 11(22.4%), *Klebsiella* species 10(20.4 %), *Proteus* species 9(18.4%), *Escherichia 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[14-16]. 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 *Staphylococcus aureus*, coagulase negative staphylococci and methicillin resistant *Staphylococcus aureus* among health care workers were 48%, 46% and 14% respectively[18].

Among the environmental sources 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. 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[17]. The habit of leaving this equipment for long periods without cleaning and proper disinfection after use is possibly responsible for this contamination. Although the direct involvement of the inanimate objects in case of disease transmission is not investigated in this work, the isolation of *S. aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus* species*, Klebsiella* species and *Enterobacter* species is a concern for possible nosocomial transmission.

The results of antimicrobial susceptibility testing showed various percentage of resistance among the bacterial isolates from the environment, patients and health professionals. In general , more than 80% of gram negative rods were resistant to ampicillin , cotrimoxazole , doxycycline , and tetracycline. It was reported that, ciprofloxacin were effective for more than 90% of gram negative isolates in Gondar[19]. However, in the present study ciprofloxacin was found to be effective to 75% of the isolates. This decrease in effectiveness may be due to frequent use of it as empiric treatment option for most of the patient. Among gram-negative isolates *Klebsiella* species, *Escherichia coli, Pseudomonas aeruginosa* and *Proteus* species demonstrated high level of resistance to most o the antibiotics tested. Some of the strains of these gram-negative bacteria were pan-resistance. All of the *Klebsiella* species, 88% of *Escherichia coli*, 82% of *Pseudomonas aeruginosa* and 78.5% of *Proteus* species were multiple antibiotic resistances. Although they are not dependable for empiric treatment, ciprofloxacin and ceftriaxone were relatively effective to most of the gram-negative isolates. This finding was also in agreement with the findings of other studies[19-21].

Majority of the *staphylococci* 153(83.4%) were multiple antibiotic resistant and these multi-drug resistance patterns had been documented already[19, 23, 24]. In the present study, gentamicin, erythromycin, penicillin, ampicillin, ciprofloxacin and ceftriaxone were found to be active against more than 75% of *S. aureus* and coagulase negative staphylococci isolates (Table 3). Overall more than 85% resistance against tetracycline and nalidixic acid was observed in *S. aureus* and coagulase negative staphylococci. Methicillin, the first semi-synthetic penicillinase-resistant antibiotic, was introduced in 1961 to target strains of penicillinase producing *S. aureus*[25]. In present study 24% of the *S. aureus* and 28% of coagulase negative staphylococci isolates were found to be resistant to methicillin. Similar study in Pakistan demonstrated resistance for 29% of the *S. aureus* and 22% of the coagulase negative staphylococcus[22]. In the present study, none of the staphylococci isolates was resistance to vancomycin. This finding is in agreement with other study in Pakistan[23].

Multiple antibiotics resistance was seen in 83.4% of gram positive and 87.5% of the gram-negative isolates. This is high when compared to previous studies[19,20,21]. The high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics. According to a report by Abula on the pattern of antibiotic usage, ampicillin, tetracycline, cotrimoxazole, chloramphenicol and gentamicin were commonly used in the study area[22]. Multiple antibiotics resistance to these commonly used antibiotics was found to be extremely high is frustrating. Most of the isolates were resistant to these antibiotics. This finding is relatively higher as compared to other studies in Gondar[19, 21] and Bahirdar[20]. This may be explained by the fact that, irrational use of antibiotics for conditions that may not clinically indicate their use, over-the-counter sell of antibiotics , some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them hence antimicrobial resistance strains grow around.

Conclusion

In this study, single as well as multiple antibiotic resistance to most of the antibiotics tested were alarmingly high. This might be a reflection of inappropriate use of antibiotics, or unavailability of a guideline regarding the selection of antibiotics for prophylaxis or empiric treatment. Although not dependable ciprofloxacin and ceftriaxone can be used for empiric treatment of severe cases before culture and sensitivity test results become available. Our findings demonstrated the widespread problem of antibiotic resistance among nosocomial pathogens. 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 antibiotic practice and good hygiene in hospitals.

Acknowledgments

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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