Global Journal of Medical research: C
Microbiology and Pathology
Volume 14 Issue 2 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Staphylococcus aureus and its Antimicrobial Susceptibility Pattern in Patients, Nasalcarage of Health Personnel, and objects at Dessie referral hospital, Northern Ethiopia

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GJMR-C Classification : *NLMC Code: QW 161.5.S8*

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Result: Overall prevalence of *Staphylococcus aureus* was 40.5% and its occurrence in inpatients, health personnel and objects was 57.5%, 40% and 34.3% respectively. Penicillin G (90.4%), nalidixic acid (93.2%), and amoxicillin (82.9%) showed high level of resistance, whereas, gentamicin (84.3%)), tetracycline (62.9%) chloramphenicol (63.6%), ciprofloxacin (61.6%), and kanamycin (64.4%) were relatively effective against *Staphylococcus aureus* infection. Vancomycin exhibited 100% susceptible in all study subjects.

Conclusion: *Staphylococcus aureus* is still the most common cause of nosocomial infection and multi-resistant was very high and most of the isolates showed high levels of resistance to commonly used antimicrobials. In the absence of diagnostic bacteriologic services, vancomycin and gentamicin are the best therapeutic options to treat *S. aureus* infections.

Keywords: *Staphylococcus aureus*, antimicrobial susceptibility, ethiopia.

I. Background

Staphylococcus aureus (S. aureus) belongs to the genus Staphylococcus, which has more than 20 species. *S. aureus* is a Gram-positive coccus, catalase and coagulase positive and causes diseases through the production of toxins and enzymes and through direct invasion and destruction of tissues (1). It is one of the most common causes of healthcare- and community-acquired infections, such as localized cutaneous and life threatening systemic infections. Although most community infections are treated in the outpatient setting, some invasive infections, including bacteremia, septic arthritis, toxic shock syndrome, osteomyelitis, and endocarditis, have devastating complications and may require hospitalization (2, 3). Hospitalized patients are unusually at high risk of infection for various reasons, and tend to be more susceptible to infections. *S. aureus* causes more sever diseases in immunocompromised patients than in immune competent ones (4).

S. aureus is one of the most successful and adaptable human pathogens. Its remarkable ability to acquire antibiotic-resistance mechanisms and advent-ageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings. However, because of different selective pressures, several notable differences exist between nosocomial-and community-acquired strains (5). Healthcare workers are potential source of hospital-acquired infections. Pathogens are transmitted by carriage on hands from inanimate objects present in the hospital setting (6). However, the role of fomites and the inanimate hospital environment (e.g. surfaces and medical equipment) in the transmission of healthcare associated infections is controversial (7). Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections. It is a major risk for the development of infection in patients undergoing hemodialysis, continuous ambulatory peritoneal dialysis, surgical patients, and patients with intravascular devices (8).

Multidrug-resistant strains of *S. aureus*, particularly methicillin resistant *S. aureus* (MRSA), pose a major clinical and epidemiological problem in hospitals, as they are easily transferred among hospital staff and patients(9). Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries with severe consequences including increased medical

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costs, morbidity and mortality of patients (10). Since the first isolation of MRSA in the United Kingdom in 1961 (11), increasing rates of methicillin resistance among *S. aureus* strains have been a cause for concern, especially in developed countries. Until recently, vancomycin was believed to have retained activity against all strains of MRSA; therefore, the spread of MRSA has led to increased vancomycin usage and hence increased selective pressure for the development of resistance (12). The first report of MRSA in Ethiopia was made from 1987-1988 and the overall MRSA isolation rate was 31% while 71% out of the MRSA strains were multiple drug resistant (13). Nosocomial infection causes substantial morbidity and mortality, prolong hospital stay of patients, and increase direct patient-care costs. Widespread uses of antibiotics, together with length of time over which they have been available have led to major problems of resistant organisms. *S. aureus* as a cause of various nosocomial infections has not been recognized in Dessie Referral Hospital. Studying staphylococcal nosocomial infections in the area is essential for early prevention and control, correct diagnosis and treatment, and reducing morbidity and mortality of hospitalized patients owing to this infection. The aim of this study was therefore to assess prevalence of *S. aureus* and its susceptibility pattern to antimicrobials in inpatients isolates, nasal carriage of hospital personnel and hospital objects of Dessie Referral Hospital.

II. Material and Method

a) Study area

The study was conducted in Dessie, capital of South Wollo Zone in Amhara Regional State Northern Ethiopia, located 401 km far from Addis Ababa, on the way to Asmara. This town has a latitude and longitude of 11°8N 39°38E/11.133°N 39.633°E with an elevation of between 2,470 and 2,550 meter above sea level. The town has an estimated total population of 151,094 of whom, 78,203 are women (**14**). Dessie has one referral hospital, three general hospitals (private), three health centers, five higher clinics (private) and one regional health research laboratory where culture and susceptibility tests are performed.

b) Study Design and period

A cross sectional study was conducted from February 01 to May 30, 2013.

III. Population

a) Source population

All patients visited Dessie referral hospital, all health personnel who were working in this hospital and Objects (blankets, floor and cupboards) which were being used by patients in the hospital.

b) Study population

All patients who were admitted to Dessie referral hospital and who had developed signs and symptoms of hospital acquired infection after 48hs of admission during the study period, all health personnel who were working in inpatient wards of the hospital and who were willing to participate in the study and the objects (blankets, cupboards and floor) which were being used by patients in the hospital.

c) Inclusion criteria

Patients who had signs and symptoms of hospital acquired infection after 48 hours of admission to hospital, and health personnel who had not antimicrobials within seven days of specimen collection during the study period.

IV. Variables

a) Dependent variable

Prevalence and antimicrobial susceptibility pattern of *S. aureus*

b) Independent variables

Sex, age, hospitalization, catheterization, surgery

c) Sample size determination and sampling technique

Convenient sampling technique was used. All the 40 patients who had developed signs and symptoms of hospital acquire infection during the study period were included in the study. Thirty five volunteer health personnel in five inpatient wards (medical, surgical, gynecology, pediatric and orthopedic) were also included. In addition, 105 specimens were taken from Objects (blanket, cupboards and floor) that could be touched with hands of health personnel and patients within the five wards.

V. Data Collection and Laboratory Methods

a) Socio-demographic data collection

Data on socio-demographic characteristics from each study participant was collected using pre-tested questionnaire guided interview.

b) Specimen collection

Specimens were collected from the study participants using the standard operational procedures. Thirty six swabs of wound secretions were aseptically obtained from patients after patients were diagnosed as wound sepsis by a physician. The specimens were collected with sterile cotton swabs before the wound was cleaned with an antiseptic solution and 10ml of four blood samples were aseptically collected from each patient, and mixed into blood culture bottle containing 90ml of nutrient broth. Nasal swabs were taken from 35 health personnel with sterile cotton swab. A separate sterile cotton swab was passed into the anterior nares of both the nostrils and rotated in both directions and then

placed into sterile test tube. One hundred five specimens were collected from Objects (blanket, cupboards and floor). Sterile cotton swabs moistened with normal saline was rotated against the surface of objects to obtain specimens. All collected specimens were labeled and transported to Dessie Regional Health Research Laboratory for culturing and antimicrobial susceptibility testing.

c) Bacterial isolation and identification

Swab specimens were cultured onto mannitol salt agar and incubated at 35-37°c for 24 hrs. Each culture was read and then sub-cultured onto blood agar and incubated at 35-37°c for 24 hrs. Blood samples were incubated at 35-37°c for 7-14 days (until growth was seen) and growth was sub-cultured on mannitol salt agar. Identification of growth was based on colony morphology, Gram staining and appropriate biochemical test. *S. aureus* is a gram positive, beta hemolytic, catalase, and coagulase positive.

d) Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (15). Single colony was selected and emulsified in 3ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculums. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 35-37°c for 24 hrs. An inhibition zone diameter of each antimicrobial was then measured and interpreted as 'Resistant', 'Intermediate' and 'Sensitive' by comparing with recorded diameters of a control organism, ATCC25923 (16). Antimicrobials used, include oxacillin (1μg), vancomycin (30 μg), penicillin G (10IU), tetracycline (30μg), sulphamethoxazole (25 μg), chloramphenicol (30μg), gentamicin (10μg), ciprofloxacin (5μg), nalidixic acid (30μg), amoxicillin (10μg), ceftriaxone (30μg) and kanamycin (30 μg). All media and antibiotics used were Oxoid, UK products.

e) Quality control

Pre-tested questionnaire guided interview was used for data collection on socio-demographic characteristics. Specimens were collected and processed according to the standard operating procedure. Sterility of culture media was checked by incubating 5% of the batch at 35-37°c overnight and observed for bacterial growth and the standard reference strains, *S aureus* ATCC25923 (16) was tested weekly as controls on the biochemical tests and agar plates including Mueller Hinton agar with antimicrobial discs to assure testing performance of the potency of antimicrobial discs.

f) Data processing and analysis

Data was checked for its completeness and entered and analyzed using SPSS statistical software version 16.0. Results were explained in words and tables. Proportions for categorical variables were compared using chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

g) Ethical consideration

The project was started after ethical clearance was obtained from the Ethical Clearance Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Written informed consent was obtained from the study participants. Permission was obtained from Dessie Referral Hospital. For each confirmed infection cases, the responsible clinician of the participant was informed and treatment was started as per the culture result and antimicrobial susceptibility pattern. Confidentiality of information of the participants was maintained.

VI. Results

a) Prevalence of S. aureus infection in inpatients, nasal carriage of health personnel and hospital objects

Of 180 specimens collected, 40(22.2%) were from inpatients, 35(19.4%) from health personnel and 105(58.3%) from hospital objects. From 40 inpatients, 36(90%) had undergone surgery and developed hospital acquired wound infections and the other 4 (10%) were blood samples. A total of 73 *S. aureus* isolates were identified and of which, 23(31.5%), 14(19.2%), and 36(49.3%) were from inpatients, health personnel and objects respectively(table1)..

Table1 : Prevalence of *S. aureus* infection in inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Study participants	*S. aureus* status		
	Positive (%)	Negative (%)	Total (%)
Inpatients	23(57.5)	17(42.5)	40(22.2)
Health personnel	14 (40)	21 (60)	35 (19.4)
Hospital objects	36 (34.3)	69 (65.7)	105(58.3)
Total	73 (40.5)	107 (59.5)	180 (100)

As it is shown in table2, among 36 *S. aureus* isolates of objects, 13 were from blankets, 10 from cupboards and 13 from floor. Based on this finding, there was no significant difference (p=0.684) among these objects on the prevalence of *S. aureus*.

Table 2 : *S. aureus* isolates from objects* at Dessie Referral Hospital, May 2013

Variable	*S. aureus* status			
	Positive	Negative	Total	P-value
Hospital objects				0.684
Blankets	13 (36%)	22 (64%)	35 (33.3%)	
Cupboards	10 (27.7%)	25 (72.3%)	35 (33.3)	
Floor	13 (36%)	22 (64%)	35 (33.3%)	
Total	36 (34.3%)	69 (65.7%)	105 (100%)	

**blankets, cupboards and floor*

b) S. aureus infection in relation to sex and age groups of inpatients and health personnel

As presented in table 3, a total of 75: inpatients (40) and health personnel (35) had participated in the study. Of these, 45(60%) were females. Among 40 inpatients, 22 were females. From males, 61.1% and from females, 54.5% had *S. aureus* infection. Among 35 health personnel, 23 were females.

Table 3 : *S. aureus* infection in relation to sex and age groups of inpatients and health personnel at Dessie Referral Hospital, May 2013

Sex	Inpatients			Health personnel		
	Positive (%)	Negative (%)	Total (%)	Positive (%)	Negative (%)	Total (%)
Male	11 (61.1)	7 (38.9)	18 (45)	2 (16.7)	10 (28.6)	12 (34.3)
Female	12 (54.5)	10(45.5)	22 (55)	12(83.3)	11 (31.4)	23(65.7)
Total	23(57.5)	17(42.5)	40(100)	14 (40)	21(60)	35 (100)
Agegroup (year)						
0-10	0 (0)	2 (100)	2 (5)	0 (0)	0 (0)	0 (0)
11-20	2 (66.7)	1 (33.3)	3 (7.5)	0 (0)	0 (0)	0 (0)
21-30	10 (60)	7 (40)	17 (42.5)	5 (31.2)	11 (68.8)	16 (45.7)
31-40	4 (50)	4 (50)	8 (22.9)	4 (57.1)	3 (53.9)	7 (20)
≥41	7 (75)	3 (25)	10 (28.6)	5 (41.7)	7 (58.3)	12 (34.3)

c) Antimicrobial susceptibility pattern of S. aureus infection

Antimicrobial resistance patterns of *S. aureus* infection was 43.8%, 0%, 90.4%, 38.4%, 45.2%, 34.2%, 6.8%, 37%, 93.2%, 83.6%, 47.9%, and 35.6% for oxacillin, vancomycin, penicillin G, tetracycline, sulphamethoxazole, chloramphenicol, gentamicin, ciprofloxacin, nalidixic acid, amoxicillin, ceftriaxone, and kanamycin respectively. High level of resistance was demonstrated to penicillin G (90.4%), nalidixic acid (93.2%), and amoxicillin (82.9%), whereas, gentamicin (84.3%)), tetracycline (62.9%) chloramphenicol (63.6%), ciprofloxacin (61.6%), and kanamycin (64.4%) were relatively sensitive to *S. aureus* infection. Vancomycin exhibited 100% susceptible in all study participants (table4).

Table 4 : Antimicrobial susceptibility patterns of all *S. aureus* isolates (n=73) from inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Antimicrobial agents	Antimicrobial susceptibility patterns			
	Susceptible	Resistance	Intermediate	Total
Oxacillin	41(56.2%)	32 (43.8%)	0(0%)	73(100%)
Vancomycin	73(100%)	0 (0%)	0(0%)	73(100%)
penicillin G	6(8.6%)	66 (90%)	1(1.4%)	73(100%)
Tetracycline	45(62.9%)	28(37.1%)	0(0%)	73(100%)
Sulphamethoxazole	35(47.1%)	33(45.7%)	5(7.1%)	73(100%)
Chloramphenicol	47(62.9%)	25(35.7%)	1(1.4%)	73(100%)
Gentamicin	62(84.3%)	5(7.1%)	6(8.6%)	73(100%)
Ciprofloxacin	45(62.9%)	27(35.7%)	1(1.4%)	73(100%)
Nalidixic acid	1(1.4%)	68(92.9%)	4(5.7%)	73(100%)
Amoxicillin	10(14.3%)	61(82.9%)	2(2.9%)	73(100%)
Ceftriaxone	34(48.6)%	35(47.1%)	4(4.3%)	73(100%)
kanamycin	47(62.9%)	26(37.9%)	0(0%)	73(100%)

Different antimicrobials showed different antimicrobial susceptibility patterns in different study participants. Resistance pattern of isolates for nalidixic acid (91.3%), penicillin G(100%) and amoxicillin (100 %) were demonstrated in inpatient, whereas, in health personnel, the level of resistance were 85.7% for nalidixic acid, 92.9% penicillin G and 78.6% amoxicillin. In objects, the level of resistance for nalidixic acid, penicillin G and amoxicillin were 97.2% 83.3% and 75% respectively (table5).

Table 5 : Antimicrobial susceptibility patterns of *S. aureus* isolates from inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Antimicrobial agents	Study participants and antimicrobial susceptibility patterns								
	Inpatients			Health personnel			Objects		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
Oxacillin	14(60.9)	9(39.1)	0(0)	11(78.6)	3(21.4)	0(0)	16(44.4)	20(55.6)	0(0)
Vancomycin	23(100)	0(0)	0(0)	14(100)	0(0)	0(0)	36(100)	0(0)	0(0)
penicillin G	0(0)	23(100)	0(0)	1(7.1)	13(92.9)	0(0)	5(13.9)	30(83.3)	1(1.4)
Tetracycline	16(69.6)	7(30.4)	0(0)	7(50)	7(50)	0(0)	22(61.1)	14(38.9)	0(0)
Sulphamethoxazole	12(52.2)	9(39.1)	2(8.7)	8(57.1)	5(35.7)	1(7.1)	15(41.7)	19(52)	2(5.6)
Chloramphenicol	16(69.6)	6(26.1)	1(4.3)	12(85.7)	2(14.3)	0(0)	19(52.8)	17(47.2)	0(0)
Gentamicin	22(95.7)	0(0)	1(4.3)	14(100)	0(0)	0(0)	26(72.2)	5(13.9)	5(13.9)
Ciprofloxacin	15(65.2)	8(34.8)	0(0)	10(71)	3(21.4)	1(7.1)	20(55.5)	16(44.5)	0(0)
Nalidixic acid	0(0)	21(91.3)	2(8.7)	0(0)	12(85.7)	2(14.3)	1(2.8)	35(97.2)	0(0)
Amoxicillin	0(0)	23(100)	0(0)	2(14.3)	11(78.6)	1(7.1)	8(22.2)	27(75)	1(2.8)
Ceftriaxone	10(43.5)	10(43.5)	3(13)	10(71.4)	3(21.4)	1(7.1)	14(38.9)	22(61.1)	0(0)
kanamycin	18(78.3)	5(21.7)	0(0)	10(71.4)	4(28.6)	0(0)	19(52.8)	17(47.2)	0(0)

S= susceptible R= resistance I= intermediate

d) Multi drug resistance pattern of S. aureus isolates from inpatients, heath personnel and objects

Multi-drug resistance (resistance to ≥2 drugs) was recorded in 79 (95.9 %) of *S. aureus* isolates. About half, 39(53.4%) of the isolates were demonstrated resistant to at least five antibacterials. Four (5.5%), 2 (2.7%), 17 (23.3%) and 11(15.1%) of the *S. aureus* were found to be resistant for one, two, three and four antibacterials respectively. None of the *S. aureus* isolates were susceptible for all tested antibacterials (table6).

Table 6 : Multi drug resistance pattern of *S. aureus* isolates from inpatients, health personnel and objects, at DRH*, May 2013

Study participants	Antibiogram pattern						Total
	R0	R1	R2	R3	R4	≥R5	
Patients	0(0%)	0(0%)	1(4.3%)	5(21.7%)	4(17.4%)	13(56.5%)	23(31.5%)
Health personnel	0(0%)	0(0%)	1(7.1)	5(35.7%)	3(21.4%)	5(35.7%)	14(19.2%)
Objects	0(0%)	4(11.1%)	0(0%)	7(19.4%)	4(11.1%)	21(58.3%)	36(49.3%)
Total	0(0%)	4(5.5%)	2(2.7%)	17(23.3%)	11(15.1%)	39(53.4%)	73(100%)

R0= resistant to zero antimicrobial
R1= resistant to one antimicrobial
R2= resistant to two antimicrobial
R3= resistant to three antimicrobial
R4= resistant to four antimicrobial
R≥5= resistant to greater or equals to five antimicrobial
*Dessie referral hospital

VII. Discussion

Results of previous studies which are also confirmed in this study had shown that *S. aureus* is the common cause of nosocomial infection. Overall prevalence of *S. aureus* infection in this study (table1) is comparable to other study done elsewhere in the world (37.3%) (17). The present study also showed that the frequency of *S. aureus* isolated from hospital objects of different wards (table2) is consistent with studies done in Gondar and Nigeria (17,18).

One of the important sources of *S. aureus* for nosocomial infection is nasal carriage among hospital personnel (19). In this study, prevalence of *S. aureus* isolates from nasal carriage of health personnel and hospital objects (table1) are comparable with other studies done in Gondar, Pakistan and Cameron (17, 20, 21). The occurrence of *S. aureus* in hospital objects (table2) may indicate poor infection control in the hospital environment, which could serve as a reservoir of the organism and it may be the potential source of cross contamination (infection) between objects and

patients. This may also account for the high incidence of the organism observed in health personnel. Out of 50 isolates of *S. aureus* from health personnel and objects, 19 had identical antibiogram pattern with the isolates of patients. This specifies that the objects and/or the health personnel may be the source of *S. aureus* infection in this study.

Antimicrobial resistance patterns of *S. aureus* infection in the present study (table4) is comparable with the previous study done in Dessie (22), but the susceptibility of ciprofloxacin and ceftriaxone are fall from the previous study which had such antimicrobial susceptibility patterns as 95.4% and 80% respectively. It may be due to overuse of it as empirical treatment.

S. aureus isolated in this study showed the highest vancomycin sensitivity pattern (table4) which is similar with the previous studies in Kontagora and Suleja Area of Niger State, in Gondar and Nigeria (17, 23, 24) The highest susceptibility of *S. aureus* to vancomycin in our study may be due to its uncommon use or being a new medication. In this study; however, *S aureus* was highly resistant to penicillin G, amoxicillin and nalidixic acid (table 4). This result is in line with previous studies in Gondar, Cameron, Dessie and Jimma (24, 25, 28, 25), respectively, but in the case of amoxicillin, our result is completely showed disparity to the study in Bahar-dar (26), which reported *S. aureus* as 100% susceptible. This difference may be due to inappropriate and incorrect administration of antibacterials as empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community in the study area. Forty four percent of *S. aureus* isolates were resistant to oxacillin which is similar to the previous studies in Kontagora and Suleja Area of Niger State and Jimma (23, 25).

Multi drug resistance patterns (table 6) of isolates of *S. aureus* in the current study is higher than the previous studies in Gondar (17) and Dessie (22) but in line with the previous study in Gondar (27). This is probably due to empirical use of broad-spectrum antibacterials, lack of culture and antimicrobial susceptibility tests and non adherence to hospital antimicrobial policy. About 24%, 16%, 6%, and 3% of *S. aureus* isolates were found to be resistant to three, four, two and one of the tested antibacterials respectively. No one of the isolates was susceptible to all of the tested antibacterials and also none of the *S. aureus* isolates were pan- resistant (resistant to all the tested antibacterialsl).

VIII. Limitation of the Study

In the present study, the antimicrobial susceptibility pattern was used in an attempt to identify possible cross infection from health personnel and/or hospital objects has a limitation. Since unrelated colony of a single species can undergo evolutionary convergence to the same resistance phenotype under antibacterial selective pressure through mutation and genetic exchange (28), unless confirmed by genomic analysis, no definite conclusions can be drawn with regard to the role of the possible sources of infection.

IX. Conclusion

The present study indicated that *S. aureus* is still the most common cause of nosocomial infection and hospital objects which were being used by inpatients may be a source of nosocomial *S. aureus* infections in this hospital. It also demonstrated that health personnel are at risk of the infection and can be a potential source of nosocomial *S. aureus* infections. In this study MDR was very high and most of the isolates showed high levels of resistance to commonly used antibacterials. However, gentamicin (84%) had high activity against most of the isolates *in vitro* when compare to other tested antibacterials. Susceptibility rate of *S. aureus* to vacomycin in this study was 100%. In the absence of culture and antibacterial susceptibility testing, vancomycin and gentamicin are the best therapeutic options to treat *S. aureus* infections. In order to confirm *S. aureus* cross infections among patients, health personnel and objects, further study with the aid of phage typing and other molecular studies should be done.

X. Acknowledgment

We would like to thank University of Gondar which covered the financial aspect of this work. Our deepest thank also go to Dessie Regional Health Research Laboratory for supporting the necessary material and equipments required for this research work and to the study participants.

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