Journal of Bacteriology and Parasitology

Gizachew et al., J Bacteriol Parasitol 2015, 6:3
DOI: 10.4172/2155-9597.1000228

Research Article Open Access

Antimicrobial Susceptibility Patterns of *Staphylococcus aureus* at the University of Gondar Tertiary Hospital, Northwest Ethiopia: A Retrospective Cross Sectional Study

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Received date: March 27, 2015; **Accepted date:** June 05, 2015; **Published date:** June 11, 2015

Abstract

Background: *Staphylococcus aureus* is the most common cause of nosocomial and community acquired infections. The infections caused by the drug resistant strains of *Staphylococcus aureus* are difficult to treat in hospital settings.

Objective: This study was aimed to determine antimicrobial susceptibility patterns of *Staphylococcus aureus* at the University of Gondar Teaching and Referral Hospital, Northwest Ethiopia.

Methods: A retrospective cross sectional study was conducted from September 2013 to February 2014 to investigate *S. aureus* prevalence and its antibiotic susceptibility patterns among different specimens. Antibacterial susceptibility testing was done by disk diffusion method on Mueller-Hinton agar. ens collected from various sections of the Hospital in the University. Completeness of the collected data was checked and entered into the computer. Chi-square test was used to check whether there is the association between the socio-demographic characteristics of the study participants and positivity to *S. aureus*. P-value less than 0.05 was considered as statistically significant.

Results: Of the 4321 different samples cultured, 309 of them were positive for *S. aureus*. The overall prevalence of *S. aureus* was 7.2% and the prevalence in the type of samples cultured were: abscess (22%), followed by body discharges (19.2%), wound secretions (17.9%), pus (17.5%), blood (9.1%), urine (4.4%) and body fluid (0.6%). The sensitivity rates of the isolates from the highest to the lowest were: vancomycin (99.6%), cefoxitin (92.6%), clindamycin (89.5%), Ceftriaxone (86.7%), ciprophloxacillin (81.2%), gentamycin (80%), chloramphelicol (78%), norfloxicillin (65%), erythromycin (53.2%), co-trimoxazole (39.7%), penicillin (37.7%), ampicillin (36.3%), amoxicillin (34.5%) & tetracycline (30.6). One hundred and sixty six (53.7%) of the isolates showed multi-resistance to antimicrobial agents.

Conclusion: *Staphylococcus aureus* isolates in this study showed higher multi-drug resistance patterns to several antimicrobials and thus further studies should be conducted in the hospital.

Keywords: Prevalence; *Staphylococcus aureus*; Antimicrobial susceptibility patterns

Introduction

Staphylococcus spp belongs to the family of Micrococcaceae [1]. *Staphylococcus* spp is characterized as gram positive, non-sporing, non-motile, non-capsulated and spherical cells, usually arranged in grape-like clusters. It has three medically important species, namely, *S. aureus*, *S. epidermidis* and *S. saprophyticus*. This organism can readily grow in ordinary media under aerobic and anaerobic conditions [2,3].

Staphylococcus aureus is a major pathogen to human with higher rates of nasal carriage in the health personnel and patients [4]. The bacterium is transmitted through aerosols, when an infected individual coughs and/or sneezes. It can also transmit via contact with inanimate objects that are contaminated with such a bacterium. Nasal carriage of *S. aureus* in health personnel provides the real sources for infections of the hospital patients, mainly, pediatrics, patients in the intensive care units and operating theater [1,3,4].

Staphylococcus aureus causes wider variety of infection in man that is associated with considerable morbidity and significant mortality. Staphylococcal infections range from skin and soft tissues to different systemic infections [4-6]. It is an important human pathogen causing nosocomial and community acquired diseases [7].

A recent study conducted in USA estimated that MRSA causes 9,400 infections and over 18,000 deaths in 2005 [8]. Another study in South Africa showed approximately 27% of MRSA infections were observed more frequently in females and children [9]. Penicillin's stable beta-lactams (i.e. methicilin, oxacillin and cephalosporin) developed as a first line anti- staphylococcus drugs ,but soon, methicilin resistant strains of *S. aureus* (MRSA),emerged invalidating almost all antibiotics including B-lactams [5,6].

Staphylococcus aureus is a major pathogen implicated in skin infections such as impetigo, furuncles, boils, styes, pustules, burns and wounds and invasive (deep tissue) infections including osteomyelitis, necrotizing, pneumonia, infective endocarditic, mastitis, septicemia, meningitis, plural empyema, abscess and pneumonia. Invasive infections are frequently associated with life threatening bacteraemia, also, causes toxin mediated diseases such as food poisoning, toxic shock syndrome and toxic skin exfoliation [3,4,10].

Global, national and local studies showed that *S. aureus* is among the primary bacterial species showing a high prevalence rate of multidrug resistance patterns, because of its intrinsic ability to develop resistance to many antimicrobial agents. We are in the era when few antibiotics are available for the treatment of *S. aureus* infection [11]. The treatment of infections caused by *S. aureus* is a challenge for clinicians. The discovery of antimicrobial agents has been a critical element of the therapeutic armamentarium [12,13].

Antibiotic resistant strains of *S. aureus* are the major cause of infections especially in hospital setting [14,15]. Strains of *S. aureus* that were fully sensitive to penicillin and other antibiotics became penicillinase producers, which dominated the entire *S. aureus* population in the hospital environment and the community [16].

S. aureus is an important cause of nosocomial and community infections. Infections caused by MERSA are especially severe in hospitals of the developing world. Prolonged hospitalization exposes patients to unnecessary costs and possibly to new infections. The widespread uses of antibiotic in the hospital environment have increased the emergence of drug resistant Staphylococcal infections in the hospital personnel, patients and in the community. It is therefore, the goal of this study was to determine the prevalence of *S. aureus* and its antibiotic susceptibility patterns from different specimens of the patients attending the University of Gondar Teaching and Referral Hospital, Northwest Ethiopia from September 2013 to February 2014.

Materials and Methods

Study area

The study was conducted at the University of Gondar Teaching and Referral Hospital, Northwest Ethiopia. More than 5,000,000 people from the surrounding zones and nearby regions visit the hospital not only for indoor treatment but also outdoor services.

Study design and period

Retrospective cross sectional study was conducted from September 2013 to February 2014.

Population

Source population: The source populations were patients who were sent by the physician to the main lab of bacteriology laboratory for culture and sensitivity patterns during the study period.

Study population: Study population was patients whose specimens were cultured and drug susceptibility testing of isolates determined during study period.

Inclusion criteria

Patients who had adequate information from log book on culture and drug susceptibility pattern during study period.

Variables

Dependent variable

- Prevalence of *Staphylococcus aureus*.
- Drug susceptibility patterns of *Staphylococcus aureus*.

Independent variables

- Age
- Sex
- Sample
- Department

Sampling technique

This study was done on secondary data that were obtained from bacteriology registration log book available at the bacteriology laboratory of Gondar University Teaching Hospital, according to registration books having the necessary patient information since September 2013 to February 2014. Accordingly; all the necessary variables that have been selected for analysis in this study were taken from these registration books. The selected data from these registration books were transferred into the developed result record sheet, in order to increase the quality of data collection. Then, study findings were explained in words and tables. Finally appropriate data were compiled, analyzed and reported.

Antimicrobial susceptibility pattern

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on MullerHinton agar plates as per the National Committee for Clinical Laboratory standards [17]. 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 hours. 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 [18-20]. Antimicrobials used, include ampicillin (10 µg), amoxicillin (10 µg), clindamycin (2 µg), vancomycin (30 µg), penicillin G (10I U), tetracycline (30 µg), sulphamethoxazole (25 µg), norfloxacin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), erythromycin (15 µg), ciprofloxacin (5 µg), cefoxitin (30 µg) and ceftriaxone (30 µg). All media and antibiotics used were Oxoid, UK products.

Quality control

In order to control the quality of data collection, two individuals have collected the necessary data blindly to minimize the probability of individual error during data collection process; then, the collections were compared.

Data analysis and interpretation

Data were checked for completeness, and cleaned manually. The data were summarized using frequency tables, words and other statistical summary techniques. In all cases, P-value of less than 0.05 was considered as statistical significant.

Ethical consideration

Ethical clearance was secured prior to the commencement of data collection from School of Biomedical and Laboratory Sciences research and ethical review committee, University of Gondar. Permission was sought from the hospital laboratory director and bacteriology laboratory section.

Results

Socio demographic characteristics of the study participants

Over one and half year's period, a total 4321 different samples were cultured from patents attending the hospital, and 309 of them were positive for *S. aureus* which caused for infections to which the attending physician prescribed anti-*Staphylococcus aureus* agents. The overall prevalence of *S. auerus* was found to be 7.2%. Of the total number of cultures, 2265 (52.4%) were from males and 2056 (47.6%) were from females. The prevalence of *S. aureus* according to the patient settings: 235 (6.2%) and 74 (13.3%) isolates were from wards and outpatients respectively (p-value<0.001) (Table 1).

Variables	Total culture	*S. aureus* Positive N (%)	X^2	p-value
Sex				
Male	2265(52.4)	152(6.7)	1.39	0.24
Female	2056(47.6)	157(7.6)		
Age group				
<5	1158(26.8)	99(8.5)	47.5	<0.001
5-14	445(10.3)	56(12.6)		
15-24	735(17.1)	62(8.4)		
25-34	931(21.7)	54(5.8		
35-44	378(8.7)	12(3.2)		
>45	673(15.6)	26(3.9)		
Patient settings				
Wards	3766(87.2)	235(6.2)	36.6	<0.001
Out-patients	555(12.8)	74(13.3)		
Total	4321(100)	309(7.2)		

Table 1: Prevalence of *S. auerus* by patients' setting and their socio-demographic characteristics attending University of Gondar Tertiary Hospital, September 2013 to February 2014.

As shown in Table 2, the prevalence of *S. aureus* based on types of human specimens cultured, the highest isolation was found to be from abscesses (22%), followed by body discharge (19.2%), wound (17.9%), pus (17.5%), blood (9.1%), urine (4.4%) and body fluid (0.6%).

Sample	Total culture	
Discharge	501(11.6)	96(19.2)
Blood	747(17.3)	68(9.1)
Abscess	182(4.2)	40(22)
Pus	200(4.6)	35(17.5)
Wound	190(4.4)	34(17.9)
Urine	568(13.2)	25(4.4)
Body-fluid	1933(44.7)	11(0.6)
Total	4321(100)	309(7.2)

Table 2: Prevalence of *S. aureus* by types of specimens cultured from patients who attended University of Gondar Tertiary Hospital, September2013 to February 2014.

The highest isolation of *S. aureus* in proportion were from Medical wards (26.5%) followed by Opd (23.9%), Ped (17.65), Surgery (15.2), Neonatology (10.7%), Gyn-obs (3.2%) and kalazar (2.9%) (Table 3).

		OPD	Wards					
Type of sample Cultured	Frequency N (%)	N (%)	MW N (%)	PEDI N (%)	Surgery N (%)	Neo N (%)	Gyn-obs N (%)	Kalazar N (%)
Discharge	96(31.1)	31(10.1)	26(8.4	14(4.5)	7(2.3)	6(1.9)	3(0.97)	9(2.9)
Blood	68(22)	5(1.6)	24(7.8	16(5.2)	0(0)	22(7.2)	1(0.3)	0(0)
Abscess	40 (13)	7(2.3)	8(2.6)	8(2.6)	16(5.2)	0(0)	1(0.3)	0(0)
Pus	35 (11.3)	6(1.9)	9(2.9)	3(0.97)	16(5.2)	1(0.3)	0(0)	0(0)
Wound	34(11.1)	11(3.5)	6(1.9)	8(2.6)	5(1.6)	3(0.97)	1(0.3)	0(0)
Urine	25(8)	10(3.2)	7(2.3)	3(0.97)	2(0.6)	0(0)	3(1)	0(0)
Body fluid	11(3.5)	4(1.3)	2(0.6)	2(0.6)	1(0.3)	1(0.3)	1(0.3)	0(0)

Total	309(100)	74(23.9)	82(26.5)	54(17.6)	47(15.2)	33(10.7)	10(3.2)	9(2.9)

Table 3: Proportion of *S. aureus* isolated from patients attending in different wards of the University of Gondar Tertiary Hospital, September 2013 to February 2014.

Key-discharge (vaginal, eye, ear, nasal and skin),-body fluid (csf, sputum, synovial, pleural, and peritoneal), MW: Medical wards; OPD: Outpatient department; NEO: Neonatology; PED: Pediatrics.

Sensitivity rates from highest to low were recorded for vancomycin (99.6%), cefoxitin (92.6%), clindamycin (89.5%), Ceftriaxone (86.7%), ciprophloxacillin (81.2%), gentamycin (80%), chloramphelicol (78%), norfloxicillin (65%), erythromycin (53%), cotrimoxazole (39.7%), penicillin G (37.7%), ampicillin (36.3%), amoxicillin (34.5%) and tetracycline (30.6) (Table 4).

Antimicrobial agent %															
WARDS N (%)		AMPI N (%)	AMOX N (%)	CAF N (%)	CIP N (%)	CLN N (%)	CRO N (%)	CN N (%)	CXT N (%)	E N (%)	NOR N (%)	PG N (%)	SXT N (%)	TE N (%)	VANC N (%)
MW 82(26.5)	S R	12(8.2) 24(16.4)	5(6) 13(15.5)	10(16.9) 5(8.5)	37(21.1) 6(3.5)	16(28.1 4(7)	24(16) 10(6.6)	19(17 5(4.5)	41(23.2) 3(1.7)	45(19.5) 24(10.9)	25(17.6) 11(7.7)	11(7.2 32(20.7)	4(6.3 14(22.3)	10(6.1) 33(26.9)	66(26.9) 0(0)
OPD 74(23.9)	S R	16(11) 15(10.3)	11(13.1) 13(15.5)	13(22) 4(6.8)	37(21.2) 6(3.4)	11(19.3 2(3.5)	38(25.3) 1(0.6)	22(20 7(6.4)	39(22) 3(1.7)	23(10) 22(9.5)	20(14.1 10(7)	15(9.7) 14(9.1)	7(11.1) 12(19.1)	13(8.2) 25(15.6)	50(20) 0(0)
PEDI 54(17.6)	S R	7(4.8) 17(11.7)	6(7.2) 11(13.1)	9(15.3) 1(1.7)	25(14.3) 10(5.7)	10(17.5 0(0)	13(8.6) 1(0.6)	10(9) 5(4.5)	31(16.9) 1(0.6)	17(7.4) 24(10.4)	15(10.6 6(4.2)	10(6.5) 17(11	6(9.5) 3(4.8)	13(7.5) 17(10.6)	46(18.8) 0(0)
SURGERY 47(15.2)	S R	2(1.4) 17(11.6)	1(1.2) 9(11.7)	5(8.5) 1(1.7)	23(13.1) 5(2.9)	10(17.5 0(0)	24(1.6) 2(1.3)	11(10 2(1.8)	23(13) 3(1.7)	21(9.1) 14(6.1)	15(10.6 6(4.2)	9(5.8) 18(11.7	5(7.9) 4(6.3)	7(4.4) 14(8.8)	38(15.5 1(0.4)
NEO 33(10.7)	S R	9(6.2) 14(9.6)	4(4.8) 8(9.5)	5(8.5) 2(3.4)	12(6.9) 5(2.8)	2(3.5) 0(0)	17(11.3) 4(2.6)	10(9) 11(10	22(12.4) 2(1.2)	11(5.2) 17(7.3)	13(9.2) 11(7.7)	8(5.2) 12(7.8)	2(3.2) 4(6.3)	4(2.5) 10(6.2)	31(12.7) 0(0)
GYN-OBS 10(3.2)	S R	3(2.1) 3(2.1)	2(2.4) 2(2.4)	1(1.7) 0(0)	3(1.7) 1(0.6)	2(3.5) 0(0)	6(4) 2(1.3)	3(2.7) 0(0)	2(1.1) 0(0)	1(0.4) 4(1.7)	2(1.4) 3(2.10	4(2.6) 1(0.6)	1(1.6) 1(1.6)	1(0.6) 1(0.6)	5(2.04) 0(0)
KALAZAR 9(2.9)	S R	4(2.7) 3(2.1)	0(0) 0(0)	3(5.1) 0(0)	5(2.9) 0(0)	0(0) 0(0)	8(1.6) 0(0)	5(4.5) 0(0)	6(3.4) 1(0.6)	5(2.2) 3(1.3)	3(2.1) 2(1.4)	1(0.6) 2(1.3)	0(0) 0(0)	2(1.3 1(0.6	8(3.3) 0(0)
Total	S R T	53(36.3) 93(63.7) 146(100)	29(34.5 55(65.5) 84(100)	46(78 13(22) 59(100)	142(81.2 33(18.8) 171(100)	51(89.5 6(10.5) 57(100)	130(86.7 20(13.3 150(100)	80(80 20(20) 100	164(92.6 13(7.4) 177(100)	123(53.2 108(46.8) 231(100)	93(65.5 49(34.5) 142(100	58(37.8 96(62.2) 154	25(39.7 38(60.3) 63(100	49(30.6 111(69.4) 160(100)	244(99. 1(0.4) 245(10

Table 4: Antimicrobial susceptibility patterns of *S. aureus* isolated from patients attending University of Gondar Tertiary Hospital, September 2013 to February 2014.

KEY: AMPI-ampicillin, AMOX-amoxicillin; CAF-Chloramphelicol; CIP-Ciprofloxicillin; CLN-Clindamycin, CN-Gentamycin; CXT-Cefoxitin; CRO-Ceftriaxone; E-Erythromycin, NOR-Norfloxicillin, PG-Penicillin G; SXT-Cotrimoxazole; TE-tetracycline; VANC-Vancomycin.

Table 5 briefly indicated that 166 (53.7%) isolates showed multi-drug resistance to two or more antimicrobial agents. Higher multi-drug resistance was observed in medical wards and least multi-drug resistance was observed in Gyn-obs and kalazar wards. Additionally, the highest multi-drug resistance was observed in R2 which was 59 (19.1%) followed by 55(17.8%) from R3, 23 (7.4%) from R4, 18 (5.8%) from R5, 8 (2.6%) from R6 and 3 (1%) from R7.

Discussions

Staphylococcus aureus is an important human pathogen causing nosocomial and community acquired infections [7]. Global, national and local studies showed that *S. aureus* is among the primary bacterial species showing a high prevalence rate of multidrug resistance

patterns, because of its intrinsic ability to develop resistance to many antimicrobial [11].

Our study showed a prevalence rate of 7.2% *S. aureus* which is lower than a study done in Eretria (17%) [10]. In this study, the prevalence of *S .aureus* observed across sex group (52.4%) were males and (47.6%) were females. Our finding is slightly lower than a report from Kano, Northwestern Nigeria (62%) for males and (38%) for females [21-24]. This difference might be due to difference in study population, and study design methods.

Wards N (%)	Frequency N (%)	R2 N (%)	R3 N (%)	R4 N (%)	R5 N (%)	R6 N (%)	R7
MED.W	82 (26.5)	20 (24.4)	18 (22)	9 (11)	3 (3.7)	0 (0)	1 (1.2)
OPD	74 (23.9)	12 (16.2)	6 (8.1)	6 (8.1)	7 (9.5)	1 (1.4)	0 (0)
PEDI	54 (17.6)	11 (20.4)	8 (14.8)	2 (3.7)	3 (5.5)	4 (7.4)	0 (0)
SURGERY	47 (15.2)	9 (19.1)	11 (23.4)	3 (6.4)	2 (4.3)	1 (2.1)	0 (0)
NEO	33 (10.7)	6 (18.2)	8 (24.2)	2 (6.1)	1 (3)	2 (6.1)	2 (6.1)
KALAZAR	9 (2.9)	1 (11.1)	3 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)
G-OBS	10 (3.2)	0 (0)	1 (10)	1 (10)	2 (20)	0 (0)	0 (0)
Total	309 (100)	59 (19.1)	55 (17.8)	23 (7.4)	18 (5.8)	8 (2.6)	3 (1)

Table 5: Multi-drug resistance patterns of *S. aureus* isolate from patients attending University of Gondar teaching hospital bacteriology laboratory from, September 2013 to February 2014.

Key: R2-resistance to two drugs, R3-resistance to three drugs, R4-resistance to four drugs, R5-resistance to five drugs, R6-resistance to six drugs, R7-resistance to seven drugs.

The highest frequency of *S. aureus* observed in this study was at the age group of under- five 8.5% and 12.6% for 5-14years, while the lowest isolation was (3.2%) for 34-44 years (p=0.001). This was similar to the study conducted in Kano; Northwestern Nigeria which indicates the highest isolation of *S. aureus* occurred in (0-10) age groups; while the least isolation occurred in (51-60) years [25].

In our study, the highest isolation of *S. aureus* were from pus (17.5%), blood (9.1%) and urine (4.4%). This is higher than the study conducted in Chitwan, Nepal where *S. aureus* isolation accounted from pus (17.0%), blood (1.7%) and urine (0.9%) [22]. However, our report is lower than the study conducted in Sikkim, India in which the isolates from blood accounted 50%, urine (45.83%), and pus (27.05%) [21].This difference may be due to difference in methods of study population and study design.

The sensitivity patterns of vancomycin and erythromycin in this study were 96.6% and 53.2% respectively. It is consistent to reports from Pakistan 100%, 55% and Northern Nigeria 100%, 52.4% respectively [19,24].

The sensitivity patterns of penicillin and ampicillin in our study were 37.8% and 36.3% respectively. These were higher than reports in Pakistan 3% and 11% respectively [19]. The sensitivity patterns of gentamycin and ciprofloxicillin in our report were 80% and 81.2%. These were higher than reports in Northern Nigeria 99.4% and76-6% [24-26]. This difference might be due to indiscriminate use of antimicrobial agents in the area.

The resistance patterns of amoxicillin in this study were 65.5%, which is consistent to a report in Northern Nigeria, 63% [24]. However, it was lower than report conducted in Jimma Town, Ethiopia in 2010, (46%) [27,28]. Resistance patterns of ampicillin and co-trimoxazole in this study were 63.7% and 60.3% correspondingly. This result is higher than reports from St-Marys Hospital, Lacor, 50% [20] and Gondar University Hospital, Ethiopia, 49.7% [29]. This difference might be due to using over dose and faired to use accordingly.

The resistance patterns of Penicillin G and tetracycline in this study were 62.3% and 69.4% in that order. A resistant pattern of tetracycline is consistent with the reports in Addis Ababa, Ethiopia (66.7%) [26], and in Gondar University Hospital, Ethiopia (68.0%) [29]. However, it is lower than a finding from research conducted in Nigeria during 2009, (76.6%). Penicillin G is higher than a result shown in a research conducted in Gondar University Hospital, Ethiopia in 2005, which was (54%) [24,29].This difference may be due to miss- consumption of drugs, time period of the research conducted, and/or advancement of the laboratory performance.

Conclusion

- In this study, higher prevalence of *S. aureus* was observed in the study participants who are under fifteen year of age.
- Higher multi-drug resistance of *S. aureus*, except vancomycin (100% sensitive) was recorded.
- High proportion of *S. aureus* was seen in body discharges, blood, abscess, pus and wounds of the specimens tested.
- Staphylococcal infections were high in medical, pediatrics and surgical wards.

It is therefore, on the bases of the findings of this research, we recommend that there should be:

- Further studies on *S. aureus* at the molecular level including the communities in Gondar University Hospital and around the study areas
- High concern on prevention and control of Hospital infection since these two things are very crucial.

- Drug susceptibility tests instead of trying to treat the patients empirically, and finally, we additionally want to recommend that Vancomycin, cefoxitin and clindamycin are the powerful antibiotics in the management of *S. aureus* infections.

Limitation

Incompleteness of the Bacteriological registration book or record may lead to under estimation of the overall and individual prevalence rates of *S. aureus* among patients visiting University of Gondar Teaching and Referral Hospital.

Acknowledgments

First and for most, we would like to express our acknowledgement in advance to the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, for providing us the opportunity to do this manuscript. We also like to express our deepest gratitude and heartfelt thanks to our staff members for their constructive comments and ideas throughout the development of this manuscript. In addition, we would like to thank the staff of bacteriology laboratory of University of Gondar Teaching and Referral Hospital for giving us the relevant information during data collection.

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