ORIGINAL ARTICLE

THE PREVALENCE OF METHICILIN RESISTANT STAPHYLOCOCCUS AUREUS AND ASSOCIATED RISK FACTORS AMONG PATIENTS WITH WOUND INFECTION AT DESSIE REFERRAL HOSPITAL, NORTHEAST ETHIOPIA

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ABSTRACT

Back ground: *The emergence of Methicilin resistant Staphylococcus aureus (MRSA) has posed serious therapeutic challenges globally. The aim of this study was to determine the prevalence of MRSA infection and related risk factors among patients suffering from wound infection.*

Materials and Methods: *A hospital based cross-sectional study was conducted among patients suffering from wound infection. Socio-demographic characteristics and potential risk factors were assessed using a pre-tested and structured questionnaire. Wound swabs were collected following Levine's technique and deposited in a tube that had brain heart infusion. Each wound specimen was inoculated on Blood agar plate (BAP), MacConkey agar (MAC), and Mannitol salt agar (MSA) then incubated at 37^{O}C for 24 hrs. S. aureus suspected colonies were identified using standard laboratory procedures and MRSA was determined based on the resistance pattern of cefoxitin.*

Results*: A total of 266 wound swabs were investigated and 66.2% were found positive for bacterial pathogens. The overall prevalence of S. aureus was 34.6%. The prevalence of MRSA infection was 28.3% (26/92) and that of Methicilin sensitive Staphylococcus aureus (MSSA) was 71.7% (66/92). All MRSA isolates were resistant to penicillin. On the other hand, 61.5% of the MRSA isolates were resistant to erythromycin and ciprofloxacin and 53.8% for cotrimoxazole and gentamicin. However, MRSA isolates demonstrated lower resistance to clindamycin (7.7%). Data also showed that 69.8% of the MRSA isolates showed multidrug resistance, but MDR among MSSA isolates were only 3%. Hospital admission (P = 0.006), Low BMI (P<0.001) and farming occupation (P = 0.040) were the risk factors significantly associated with wound infection due to MRSA.*

Conclusion: *The prevalence of S. aureus infection was high, and a significant proportion of the isolates were Methicilin resistant. The occurrence of MRSA among patients with wound infection indicates a need for regular investigation of wound samples by culture and drug susceptibility testing.*

Key words: *Staphylococcus aureus, Methicilin resistant Staphylococcus aureus, wound infection.*

BACKGROUND

Wound is a break in the skin and exposure of the underlying tissue to the outside environment. Loss of skin integrity by wounding provides a moist, warm, and nutritious environment for microbial colonization, proliferation, and infection [1,2].Wound contaminating microorganisms may originate in the environment, the surrounding skin, and endogenous sources that primarily include the gastrointestinal, pharyngeal, and genitourinary mucosa [3]. Wound progression to an infected state may involve a complex interaction of multiple factors related to bacteria, the local environment and host defense mechanisms with host response playing a key role [4].

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Common bacteria that cause skin infections include *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus* species, and *Enterococcus* species [5, 6]. *Staphylococcus aureus* is the most frequently isolated bacteria from wound infections [7], the leading cause of nosocomial infections (NI), and surgical wound infections [8]. Methicillin Resistant *Staphylococcus aureus* (MRSA) is a bacterium resistant to conventional therapies against Gram -positive organisms, in particular beta (β) - Lactam antibiotics [9]. Methicillin resistant *S. aureus* acquires its resistance through the methicillin resistance gene *mecA*, which encodes a low-affinity penicillin-binding protein (PBP2a) that is absent in susceptible *S. aureus* strains [10, 11]. This foreign penicillin-binding protein does not bind well to most β-lactams, allowing MRSA to grow in their presence. The *mecA* gene is carried on a mobile genetic element called the *Staphylococcal* chromosomal cassette *mec* (SCC*mec*) [11].

Methicillin -resistant *Staphylococcus aureus* (MRSA) is a major public health concern responsible for both hospital and community-associated infections worldwide [12]. It accounted for up to 40% of the nosocomial infections (NI) in large hospitals in the USA [13]. The prevalence of MRSA infection was 33% in tertiary-care hospitals in Jeddah, Saudi Arabia [14]. In Mozambique, the prevalence of hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) infection was reported as 15.1%, whereas the prevalence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infection was 1.0% [15]. The prevalence of MRSA infection was reported as 31.5% in Kampala, Uganda [16] and 20.23% in Nigeria [17]. A higher prevalence of MRSA infection (63.5%) was reported in Kinshasa (Democratic Republic of Congo) [18]. The prevalence of MRSA infection among pus specimens was reported as 9% in Eritrea [19]. In Ethiopia, the prevalence of MRSA infection among patients infected with wound at Debremarkos Hospital was 19.6% [20].

The most recognized risk factors for MRSA infections includes age, gender, chronic disease such as diabetics, liver disease and HIV infection. A prolonged hospital stay and irrational antibiotic use are also important risk factors. The link between hospitalization and MRSA was reported earlier [21]. In one study, the prevalence of nosocomial MRSA isolates was reported to be higher (84.2%) than the 15.8% isolates from a corresponding community [22]. A widespread and prolonged use of antibiotics leads to the emergence of resistant bacteria pathogens during wound infections [23]. Antibiotic resistant pathogens are acquired either from health care setting environments, health care personnel or inpatients [24]. Antimicrobial resistance can increase complications and costs of treatments [25, 26].

The burden of MRSA has been increasing at an alarming pace throughout the globe with a considerable variation in prevalence from region to region and even from institution to institution. Even though studies have been conducted on bacterial profile of wound infections in some health institutions in Ethiopia, studies on specific pathogen, such as MRSA infection are scarce. The aim of this study was to determine the prevalence of MRSA isolates in wound infections and associated risk factors at Dessie Referral Hospital, northeast Ethiopia.

METHOD

Study design, area, and period: The study was conducted at Dessie Referral Hospital (DRH), South Wollo zone of the Amhara Regional State, northeast Ethiopia. Dessie is located at a distance of 400 km from the capital, Addis Ababa, and 471 km from Bahir Dar, the capital of the Amhara Regional State. The town is at latitude and longitude of 11°8′N39°38′ with an elevation between 2,470 and 2,550 above sea level. According to the 2007 E.C population and housing census the town had a total population of 151,094 (72,891 males and 78,203 females). A Hospital based cross-sectional study was conducted to determine the prevalence of MRSA infection among patients suffering from wound infection between February and April 2016.

Study Population and Variables: All patients suffering from wound infection and visited Dessie Referral Hospital during the study period were used as source and study population. The prevalence of MRSA infection was used as the dependant variable, whereas age, sex, educational background, occupation, residence, patient setting, prolonged hospitalization, invasive or surgical procedures, prolonged or recurrent exposure to antibiotics, chronic diseases (diabetics, liver disease, HIV), polymicrobial infection and malnutrition were used as the independent variables.

Sample Size and Sampling Technique: The sample size was determined using a single population proportion formula as follows: $n = z^2 p (1-p)/ d^2$; where: n = the number of MRSA suspected cases; Z = Standard normal distribution value at 95% CI, which is 1.96; P = the prevalence of MRSA infection taken from a previous study report at Debremarkos Hospital which was 19.6% [20]; d = the margin of error taken as 5%. Thus, sample size 242 was determined, but considering a 10% nonresponse rate, the required sample size, 266 was determined.

Operational Definitions: Methicillin-resistant *Staphylococcus aureus* (MRSA): is defined as strains of *S. aureus* that are resistant to cefoxitin ((30 µg) using disk diffusion method on Mueller Hinton agar.

Multi-drug resistance: is antimicrobial resistance shown by a species of microorganism to multiple antimicrobial drugs.

Socio-demographic and clinical data collection: Data on socio-demographic characteristics and associated risk factors were collected from each participant by a trained nurse, using a pretested and structured questionnaire-guided interview. The socio-demographic characteristics included were age, sex, educational status, residence, and occupation of patients. The clinical characteristics and risk factors assessed were patient setting (admitted or outpatient), history of surgical procedures, history of antimicrobial therapy, history of chronic diseases, and nutritional status of patients.

Sample collection: Wound samples were collected using Levine's technique [27]. Briefly, the immediate surface of the wound was cleaned with sterile gauze moistened with 70% ethyl alcohol. Dressed wounds were cleansed with sterile normal saline after removing the dressing. Aseptically, the end of a sterile cotton-tipped applicator swab was rotated over 1 cm^2 area of the wound for 5 seconds with sufficient pressure to express fluid and bacteria to surface from within the wound tissue as the technique stated by

Levine and Gardner [27, 28]. Double wound swabs were taken from each wound at a point in time to increase the chance of recovering bacterial pathogens. In case of closed wounds, the infected material was aspirated using a sterile syringe and needle and deposited into sterile test tubes that had brain heart infusion broth. All collected specimens were labeled and transported to Dessie Regional Health Research Laboratory within an hour to perform culture and antimicrobial susceptibility testing.

Wound Culture and identification: Wound samples were cultured, incubated, and examined using the standard operational procedures (SOPs) of the regional laboratory, following the standard microbiological techniques [29]. Briefly, each wound specimen was inoculated on Blood agar plate (BAP) (Oxoid, Ltd., Basingstoke, Hampshire, England), Mannitol salt agar (MSA) medium, and MacConkey agar plate (MCA). All plates were incubated in an aerobic atmosphere at 35-37^{O}C for 24 hrs. All positive cultures were identified by the grown bacterial colony characteristics and appearance on the respective media. Each grown bacterial colony was tested by Gram stain and confirmed by the pattern of biochemical reactions. Beta-hemolytic colony on blood agar plate, golden yellow colony on mannitol salt agar that appeared as Gram positive cocci in clusters, catalase and coagulase positive and also mannitol fermentation positive was identified as S. *aureus* [27].

Antimicrobial susceptibility test: Antimicrobial susceptibility test was carried out on each bacterial isolate using the disc diffusion method on Muller Hinton agar (MHA). Three to five pure colonies of each bacterium were picked and transferred to a tube containing 5 ml sterile nutrient broth (Oxoid). The preparation was vortex mixed thoroughly to make the suspension homogenous. The suspension was incubated at 37°C until the turbidity of the suspension adjusted to a 0.5 McFarland turbidity standard (Bacterial concentration of 1.5×10^8 colony forming unit/ml) in order to standardize the size of the inoculums [29]. A sterile swab was dipped into the suspension and the entire surface of the Muller Hinton agar plate was uniformly flooded with the suspensions. The antimicrobial impregnated disks were placed to the media using sterile forceps in such a way that each disk was placed at least 24 mm away from each other to avoid the over lapping zone of inhibition. After the disk was placed on the inoculated media, the plates were allowed to stand for 30 minutes so that the antibiotic would diffuse into the media. The plates were inverted and incubated at 37 ^{O}C for 24 hrs and observed for zone of inhibition. The zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline as susceptible (S), intermediate (I) or resistant (R) [30].

Quality Assurance: The validity and completeness of the data were checked daily by the principal investigator. The sterility of the culture media and biochemical tests were checked by overnight incubation of un-inoculated media from each batch of preparation. A standard strain of *S. aureus* (ATCC25923) was used as control during the biochemical and susceptibility testing. To standardize the inoculum density of bacterial suspension for the susceptibility test, 0.5 McFarland standard was used [29].

Data Processing and Analysis: Data was checked for completeness, coded, and first entered into EPI-info version 7, and then it was rechecked and transferred to Statistical Package for Social Sciences

(SPSS) version 20 for analysis. Logistic regression was used to determine the effect of independent variables on the dependent variable. Binary regression analysis was conducted and associations with a p-value < 0.2 were passed to backward logistic regression stepwise model multivariate analysis. Finally, the strength of association was interpreted based on the adjusted odds ratio (AOR). P-values < 0.05 were considered as statistically significant.

Ethics approval: Ethical clearance was obtained from the University of Gondar, School of Biomedical and Laboratory Sciences Ethical Review Committee and a permission letter was submitted to Dessie Referral Hospital prior to data collection. A written informed consent was obtained from participants, parents, guardians or caretakers of children after explaining the purpose and objective of the study. Any patient who was not willing to participate in the study was not forced. All data obtained were kept confidential by using codes instead of any personal identifiers, and the samples were used only for the purpose of this study. Laboratory results were communicated to their attending physicians for appropriate treatment.

RESULT

Socio-demographic characteristics of participants: A total of 266 patients were included in the study. The proportion of male patients was 67.7% and that of females 32.3%. The mean (SD) age of the study participants was 33.2 (± 17.8) years (range 5 to 81). One -fourth of the participants had no formal education; the majority, (77.1%) lived in urban areas and 17.7% were civil servants by occupation (Table 1).

Patterns of microbial isolates from wound infections: Among the 266 wound samples subjected to culture, 66.2% (176/266) were positive for bacterial pathogens. A total of 228 bacterial pathogens were recovered and 29.5% (52/176) of the culture positive wound samples showed polymicrobial growth, while 124 (70.5%) had a single bacterial pathogen. In this study, 33.8% (90/266) of the wound swabs showed no bacterial growth. Eleven different types of bacterial species were isolated, and the proportion of Gram positive bacterial pathogen was 67.5% (154/228), while that of Gram negative was 32.4% (74/228). *S. aureus* was the predominant bacteria isolated (92/228; 40.35%), followed by coagulase negative *Staphylococci* (CoNS) (62/228; 27.2%), *P. aeruginosa* 31(13.6%), *K. pneumoniae* (4%) and *E. coli* (3.5%).

Table 1: Socio-demographic characteristics of patients with wound infections at Dessie Referral Hospital,north east Ethiopia, 2016

Characteristics		Frequency	Percentage
Age(years)	5-14	25	9.4
	15-24	83	31.2
	25-34	58	21.8
	35-44	31	11.7
	45-54	22	8.3
	55-64	28	10.5
	>64	19	7.1
Sex	Male	180	67.7
	Female	86	32.3
Educational status	No formal	67	25.2
	Primary	93	35
	Secondary	75	28.2
	College/University	31	11.7
Residence	Rural	61	22.9
	Urban	205	77.1
Occupation	Civil servant	47	17.7
	Farmer	30	11.3
	Merchant	29	10.3
	House wife	26	9.8
	Daily labor	42	15.8
	Other	92	34.6
Patient setting	In-patient	82	30.8
	Out-patient	184	69.2

Bacterial profile of wound swab cultures among inpatients and outpatients: Data of the current study showed that 30.8% (82/266) and 69.2% (184/266) were inpatients and outpatients, respectively. A total of 79 and 149 bacterial pathogens were recovered from the inpatients and outpatients, respectively. The predominant isolated bacteria in both cases were *S. aureus* (n = 92; 40.3%) followed by CoNS (n = 62; 27.2%) and *P. aeroginosa* (n=31; 13.6%) (Table 2).

Table 2: Frequency of occurrence of bacterial isolates among wound swab cultures along with inpatients and outpatients attending the Dessie Referral Hospital from Feb 08- May **08/2016**

Bacterial isolate	In-patient	Percentage(%)	Out-patient	Percentage(%)	Total	Percentage(%)
S. aures	34	39.9	58	63.1	92	40.3
CoNs	18	29	44	71	62	27.2
P. aeroginosa	18	58	13	42	31	13.6
K. pneumonia	3	33.3	6	66.7	9	4.0
K. ozanae	0	0	6	100	6	2.6
K. rhinose	0	0	2	100	2	0.87
Enterobacter spp	1	33.3	2	66.7	3	1.3
Citrobactor spp	2	28.6	5	71.4	7	3.1
E.coli	2	25	6	75	8	3.5
Providencia spp	0	0	4	100	4	1.8
Proteus spp	1	33.3	2	66.7	3	1.31
Acetinobacter spp	0	0	1	100	1	0.42
Total	79	34.6	149	65.4	228	100

Prevalence of MRSA: The overall prevalence of culture confirmed *S. aureus* infection among patients with wound infection was 34.58% [92/266]. The prevalence of MRSA infection determined based on the resistance pattern of cefozitine was 28.3% (26/92) and that of Methicilin sensitive *Staphylococcus aureus* (MSSA) 71.7% (66/92). Among the 82 inpatients and 184 outpatients, 41.5% (34/82) and 31.5 % (58/184) were culture positive for *S. aureus*, respectively. The prevalence of MRSA infection among inpatients and outpatients was 19.5% (16/82) and 5.4 % (10/184), respectively.

Anti-microbial susceptibility patterns of bacterial isolates: The proportion of penicillin resistant *S. aureus* was high (84.8%; 78/92) but that of clindamycin resistant *S. aureus* was low (4; 4.3%). On the other hand, all MRSA (n=26) bacterial isolates demonstrated resistance to penicillin (100%), followed by erythromycin and ciprofloxacin (n=16; 61.5%), cotrimoxazole and gentamicin (n=14; 53.8%) (Table 3). The proportion of clindamycin resistant MRSA isolates was 7.7%. In this study, 69.2% (18/26) of MRSA isolates showed multidrug resistance anti-microbial susceptibility pattern, while only 3% of the MSSA isolates did so. On the other hand, 15.2% (10/66) of the MSSA isolates were sensitive to all antibiotics tested (Table 4).

A total of 74 Gram negative rod shaped bacteria were identified. The proportions of *Pseudomonas* species and *Klebsiella* species were 41.9% and 23%, respectively. *E. coli* accounted for 10.8%, *Citrobacter* species for 9.5%, but *Providencia* species, *Enterobacter* species, *Proteus mirabilis* and *Acinetobacter* species collectively accounted for 14.8%. Data on drug susceptibility patterns of the Gram negative rod shaped bacterial isolates showed that 16 (51.6%) *Pseudomonas* species were resistant to ceftazidime (CAZ), 14 (45.2%) to ciprofloxacin (CIP), 13 (41.9%) to gentamycin (CN), and 10 (32.2%) to pipracillin-tazobactum (PEP-TAZ). Moreover, the majority of the *Klebsiella* species (12/17) showed resistance to ampicillin (AMP), while all the *E.coli* isolates were resistant to augmentin (AUG) (n=8). On the other hand, the majority of the *Citrobacter* isolates were resistant to AMP and AUG. A higher proportion of *Providencia* species, *Enterobacter* species and *P. mirablis* isolates were resistant to AMP and AUG. Multidrug resistance was observed in all Gram negative bacterial isolates.

Table 3: Antimicrobial resistance pattern of *S.aureus* and MRSA isolate among patients with wound infection at Dessie Referral Hospital, 2016

ANTIBIOTICS	RESISTANCE PATTERN (%)	
	S. AUREUS(N=92)	MRSA(N=26)
Penicillin	78(84.8)	26(100)
Gentamicin	14(15.2)	14(53.8)
Ciprofloxacilin	17(18.4)	16(61.5)
Clindamycin	4(4.3)	2(7.7)
Cefoxitin	26(28.3)	26(100)
Erythromycin	25(26.1)	16(61.5)
Cotrimoxazole	15(16.3)	14(53.8)
Doxycycline	9(9.7)	8(30.8)
Chloramphenicol	8(8.7)	7(26.9)

Association of risk factors with MRSA wound infection: The association of MRSA infection with the possible risk factors was determined by both bivariate and multivariate logistic regression analyses models. In this study, the bivariate regression analysis of wound infection due to MRSA showed a significantly high rate among farmer patients [COR = 4.7; 95% CI = 1.099-19.817]. Patients with history of recent admission and had history of recent surgery were 4 and 3 times at more risk of being infected by MRSA [COR = 4.1; 95% CI = 1.712-9.83], [COR=3.063; 95% CI=1.330-7.054), respectively. On the other hand, admitted patients suffering from wound infection were almost 4 times at risk [COR = 4.2; 95% CI = 1.833-9.765] to being infected by MRSA compared with corresponding outpatients. Malnourished patients (low BMI <18.5) were 15 times at more risk [COR, 15.2; 95% CI, 5.462-42.216] of being infected with MRSA compared to their counterparts. However, factors such as age, sex, education status, residence, history of previous antibiotic therapy and chronic illness had no statistically significant association with infection caused by MRSA.

In the multivariate logistic regression analysis model, the association of wound infection due to MRSA remains significant except in the case of recent history of admission. Farmers were 6 times more likely to develop MRSA wound infection [AOR = 6.1; 95% CI = 1.086-33.724) than housewives. Patients with low BMI had a high chance of developing MRSA wound infection than their counterparts. In addition, inpatients were 3.6 times more likely to be infected with MRSA compared to outpatients (Table 5).

Table 4: MDR pattern of bacteria isolated from wound infection among patients attending Dessie Referral Hospital, 2016.

S. aureus (n=92)	Resistance pattern, n (%)							
	R0	R1	R2	R3	R4	R5	R6	MDR
MSSA (n=66)	10(15.2)	50(75.8)	4(6.1)	1(1.5)	0	1(1.5)	0	2(3%)
MRSA (n=26)	0	0	8(30.8)	2(7.7)	1(3.8)	2(7.7)	13(50)	18(69.2)

R0: sensitive to all drugs; R1: resistance to one drug; 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; MDR: resistance to ≥3 drugs; MSSA: methicilin sensitive staphylococcus aureus; MRSA: methicilin resistant staphylococcus aureus

Table 5: Multivariate regression analysis of risk factors and MRSA infection among patients with wound infection at Dessie Referral Hospital, 2016

Variable		MRSA		COR (95% CI)	AOR(95% CI)	P-value
		Yes	No			
Age(years)	5-14	1	24	1	1	
	15-24	7	76	2.21(0.259-18.9)	0.74 (0.066-8.329)	0.808
	25-34	3	55	1.309(0.130-13.233)	0.881(0.056-13.759)	0.926
	35-44	1	30	0.800 (0.048-13.466)	0.26 (0.008-8.371)	0.448
	45-54	3	19	3.79(0.363-39413)	2.4 (0.121-47.993)	0.564
	55-64	6	22	6.545(0.729-58.756)	1.179 (0.58-23.863)	0.915
	>65	5	14	8.571(0.907-80.993)	2.54(0.158-40.847)	0.511
Sex	Male	16	164	0.741 (0.322-1.710)		
	Female	10	76	1		
Occupation	House wife	2	24	1	1	
	CS	3	44	1.63(0.181-2.835)	0.69 (0.110-4.429)	0.702
	Farmer	3	27	4.7(1.099-19.817)*	6.1(1.086-33.724)	0.040
	Merchant	7	22	3.341(1.093-10.216)*	1.47 (0.187-11.625)	0.713
	DL	3	39	0.875 (0.174-4.379)	1.11 (0.175-7.001)	0.913
	Others	8	84	0.808 (0.203-3.211)	2.1(0.429-9.939)	0.366
Admission	Yes	18	85	4.103 (1.712-9.830)*	1.21 (0.117-12.604)	0.872
	No	8	155	1	1	
Surgery	Yes	16	82	3.063 (1.330-7.054)*	1.94 (0.540-6.950)	0.311
	No	10	158	1	1	
Patient setting	In patient	16	66	4.218 (1.833-9.765)*	3.56 (1.429-8.857)	0.006
	Out patient	10	174	1	1	
Educational status	Formal	5	62	1.169(0.214-6.389)		
	Primary	14	79	2.570(0.550-12.004)		
	Secondary	5	70	1.036 (0.190-5.647)		
	College/ university	2	29	1	1	
Residence	Rural	7	54	1		
	Urban	19	186	1.269 (0.507-3.178)		
Previous antibiotic therapy	Yes	15	164	0.632 (0.277-1.44)		
	No	11	76	1		
Chronic infection	Yes	6	29	2.2(0.810-5.883) *	2.1(0.577-7.521)	0.263
	No	20	211	1		
BMI	<18.5	21	52	15.2(5.462 - 42.216)**	13.89(4.919-39.192)	<0.001
	>18.5	5	188	1	1	

*MRSA: Methicilin resistant Staphylococcus aureus; COR: crude odds ratio; AOR: adjusted odds ratio; Daily laborer; CS: Civil servant; BMI: body mass index; *: p-value<0.05; **: p-value<0.001*

DISCUSSION

Staphylococcus aureus is a frequent cause of bacterial infections in both developed and developing countries [31, 32, 33]. It is a highly versatile and adaptable pathogen, causing a range of infections of varying severity. *S. aureus* represents a prototype for drug resistance, especially to β-lactam antibiotics. It frequently gains resistance by gene mutations and horizontal gene transfer and has been implicated in episodes of epidemic and pandemic proportions [34].

The dominant pathogen identified from wound samples in the present study was Gram positive bacteria (67.5%). In Nigeria, Muhammed et al [35] reported that Gram positive cocci were more dominant (71.5%) pathogens isolated in hospitals than Gram negative bacilli (28.5%). This could be due to the fact that Gram positive bacteria are the most common organisms found on the skin, causing wound infections if they get access to the inner layer of the skin through cuts or abrasions. Previously, Cutting and White [36] reported that pathogens that affect the wound are primarily Gram positive bacteria such as *Staphylococcus aureus.*

In the current study the dominant Gram positive bacteria was *S. aureus* (34.5%) and the proportion of MRSA infection was 28.3%. Methicillin resistant *Staphylococcus aureus* (MRSA) infection was determined based on the resistance pattern of cefozitine. The gold standard for the identification of MRSA is to detect the *mecA* gene [37] or its product, Penicillin -Binding Protein 2a (PBP2a) by latex agglutination [38, 39]. However, these tests are not within the scope of many clinical laboratories as they are relatively expensive. Cefoxitin and moxalactam have been reported as surrogate markers for the detection of methicillin resistance [40, 41].

The current data showed a comparatively higher prevalence of MRSA (28.3%) infection than a previous report from Debremarkos Hospital (19.6%) [20]. However, a higher prevalence of MRSA infection (76.7%) was reported earlier from Jimma Hospital, south-west *Ethiopia* [42]. Different reports from different countries in Africa showed a significantly diverse prevalence of MRSA infection. The prevalence of MRSA infection was reported as 9% in Eretria [43] and 16% in Tanzania [44]. Comparatively higher prevalence of MRSA was reported in Libya (31%) [45] and Nigeria (20.23%) [17]. Genetic analysis of MRSA strains from around the world revealed that the transfer of SEC*mec* gene to a MSSA strain occurred only a few times, so the emergence of MRSA has resulted from dissemination rather than the development of new MRSA clones [46]. Thus virtually all patients with MRSA infection or colonization acquire their strain from external sources [47; 48].

The prevalence of MRSA infection among inpatients (19.5%) was by far higher than among outpatients (5.4 %). Methicillin-resistant *S. aureus* (MRSA) isolates have been recognized as health care-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). In the year 2000, CDC created a case definition for a CA-MRSA infection as any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks health care-associated MRSA risk factors [49]. Community-associated MRSA strains have been distinguished from their health care-associated MRSA (HA-MRSA) counterparts by molecular means. HA-MRSA strains carry a relatively large S*taphylococcal* chromosomal cassette *mec* (SCC*mec*) belonging to type I, II, or III. In contrast, CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V [50].

The other important finding of the current study is a high (84.8%) proportion of penicillin resistant *S. aureus* comparable to the report from Debremarkos (82.2%) and Jimma (100%) hospitals [20, 51]. In Nigeria, Uwaezuoke and Aririatu reported high resistance (95.8%) of *S. aureus* strains to penicillin isolated from clinical sources [52]. The current study

also demonstrated a low proportion of clindamycin resistant *S. aureus* (4.3%). Clindamycin is a frequently used alternative drug to treat MRSA and MSSA infections because of its patient tolerability, excellent tissue penetration, and capacity to accumulate in wound abscess [53]. In this study, 69.2% of the MRSA isolates showed multidrug resistance antimicrobial susceptibility pattern, while only 3% of the MSSA isolates did so. This finding is concordant with the finding in northern India, whereby 73% of MRSA strains were multidrug resistant [53].

All the MRSA bacterial isolates demonstrated 100% resistance to penicillin, and the drug resistance pattern to erythromycin and ciprofloxacin was still high (61.5%). Moreover, greater than 50% of the isolated MRSA strains encountered resistance to gentamicin, ciprofloxacilin, cotrimoxazole and erythromycin. A similar result was noted for ciprofloxacilin among MRSA strains. A high percentage of resistance was also reported to gentamicin, erythromycin, and cotrimoxazole, elsewhere [54].

The proportion of Gram negative bacterial pathogens among wound samples was 32.4% (74/228), and the most frequently isolated bacterial pathogens were *Pseudomonas aeroginosa* and *Klebesilla* species. The antimicrobial susceptibility pattern of *Pseudomonas aeroginosa* showed a relatively high resistance to CAZ, CIP and CN. Moreover, a high proportion of AMP resistance was observed in *Klebsiella* species, but all the *E. coli* isolates were resistant to augmentin. The current result that *Pseudomonas aeruginosa, E. coli* and *Klebsiella pneumoniae* were highly resistant to commonly used antibiotics is similar to the report from India [55]. In Jimma, southwest Ethiopia, Mama et al reported a 20% prevalence of *Escherichia coli*, 16% *Proteus* species, 10% *Klebsiella pneumoniae*, and 8% *Pseudomonas aeruginosa* with an overall multiple drug resistance patterns of 85% [56].

According to our work, 29.5% of the culture positive wound samples showed polymicrobial infection. Wound infection represents a very common health problem in the entire world. Microbes rarely exist as single-species planktonic forms; the majority are found thriving in complex polymicrobial biofilm communities attached to biotic and abiotic sites [57]. Polymicrobial synergy can occur during infection, in which the combined effect of two or more microbes on disease is worse than what is seen in any of the individuals alone [58]. In the current study, 33.8% of the patients demonstrated no bacterial growth which may indicate sterile pus or abscess. Sterile abscess can be defined as a localized swelling filled with fluid, where no organism is obtained by culture.

In this study, farmers were 6 times more likely to be infected with MRSA than housewives. Many of the farmers in Ethiopia lack knowledge and financial resources to utilize health care services. Moreover, the occupation may expose them to wound infection which may remain for a longtime without medication; besides, incomplete medication is also common.

The data also demonstrated that patients admitted to hospital wards (in-patients) and those who had low BMI had higher odds of developing wound infection due to MRSA. Methicilin resistant *Staphylococcus aureus* is a major nosocomial pathogen which has a high possibility of cross infection among patients and hospital staff, and the risk of transmission may be high among malnourished patients. Moreover, previous reports showed a significant association of wound infection due to a resistant strain of *S. aureus*

with age, sex, type of surgery, ward and hospital stay [20].

Limitation: The limitation of this study was that due to resource constraints, Vancomycin resistance was not determined among MRSA positive patients.

CONCLUSION

The prevalence of MRSA among patients with wound infection was 28.3%, and a high proportion of MRSA isolates was found among inpatients. Significant proportions of MRSA isolates showed multidrug resistance but lower drug resistance to clindamycin. Conducting culture as a routine laboratory procedure to diagnose wound infection and establishing antimicrobial stewardship strategies in the hospital could be helpful.

Authors' contribution: YT: Designed the study, collect and analyze the data and participated in draft and final write up of the manuscript; BG: Participated in conception and design of the study, data analysis and interpretations and preparation of the draft and final write up of the manuscript; MA: participated in proposal writing, data analysis and interpretation of results; AA: participated in data analysis and interpretation of results and also commented the final manuscript prior submission for publication. All authors reviewed and approved the final manuscript.

Competing interests: We declare that we have no competing interests.

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