ORIGINAL RESEARCH

To determine the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) and identify the risk factors associated with wound infection in patients

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ABSTRACT
Aim: To determine the prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) and identify the risk factors associated with wound infection in patients. **Material and methods:** Cross-sectional research was undertaken at the department of microbiology to evaluate the antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus that was isolated from wound infections, as well as the risk factors associated with it. This research included all patients who were suspected of having wound infections and had not received any antibiotics during the two weeks before the study period. A total of 100 participants were included in this trial. Data pertaining to sociodemographic characteristics and their corresponding risk factors were gathered via the use of a well-organized questionnaire. **Results:** Among a group of 100 patients who were suspected of developing wound infection, 50 of them (50%) were found to have S. aureus wound infections based on culture confirmation. Out of them, 15 (30%) were MRSA. The study population had an overall prevalence rate of 15% for MRSA, with 15 out of 100 individuals affected. Out of the 31 patients who were admitted to the hospital and the 69 patients who were treated as outpatients and suspected of having a wound infection, 38.71% (12 out of 31) and 33.33% (23 out of 69) were found to have a positive culture for S. aureus, respectively. The total incidence of Methicillin-resistant Staphylococcus aureus (MRSA) in hospitalized patients was 22.58% (7 out of 31), whereas in non-hospitalized patients it was 11.59% (8 out of 69). **Conclusion:** Among the 100 patients who were suspected to have wound infection, 50 of them, which is 50% of the total, were confirmed to have S. aureus by culture testing. Out of them, 15 (30%) were MRSA. Wound infection caused by MRSA had a significant correlation with profession, being diagnosed in the inpatient department, and body mass index. More than 50% of MRSA isolates exhibited resistance to gentamicin, ciprofloxacillin, cotrimoxazole, and erythromycin.
Keywords: MRSA, wound infection, S. aureus

INTRODUCTION
Staphylococcus aureus (S. aureus) is a prevalent bacterium that often leads to bacterial infections in humans. It is responsible for both community- and hospital-acquired infections, affecting many parts of the body such as the skin, urinary system, surgical sites, bones (osteomyelitis), bloodstream (septicaemia), and heart (endocarditis) [1]. Staphylococcus aureus have an exceptional capacity to acquire resistance to several antibiotics. The first discovery included the acquisition of β-lactamase on "penicillinase plasmids" and the subsequent adaptation of methicillin-resistant S. aureus (MRSA) by the acquisition of staphylococcal cassette chromosome (SCCmec) elements, which are resistant to β-lactamase. This information was initially reported in reference [2].
A wound is a disruption in the integrity of the skin that exposes the underlying tissue to the external environment. Wounding of the skin creates a favorable environment for microbial colonization, growth, and infection because to the presence of moisture, warmth, and nutrients [3]. Some examples of bacterial skin infections include Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes,

Proteus species, Streptococcus species, and Enterococcus species [4]. Staphylococcus aureus is a common source of nosocomial infections (NI) and surgical wound infections, making it one of the most prevalent types of wound infection [5]. In recent years, it has shown an increasing resistance to several antibiotics.

Methicillin-resistant S. aureus obtains its resistance via the methicillin resistance gene mecA, which produces a low-affinity penicillin-binding protein (PBP2a) that is not present in susceptible S. aureus strains. The penicillin-binding protein receptor, which is resistant, has poor binding affinity towards most β-lactams. As a result, MRSA is able to thrive in the presence of these antibiotics [8]. S. aureus strains that are resistant to methicillin have recently been categorized into two categories based on both epidemiological and molecular features. These groups are known as community-associated (CA) MRSA and healthcare-associated (HA) MRSA. Community-associated MRSA isolates often exhibit lower resistance levels compared to HA-MRSA isolates [6].

Methicillin-resistant Staphylococcus aureus (MRSA) is a significant global issue, responsible for hospital-acquired infections. MRSA infections in the hospital context alone were expected to impact about 150,000 people yearly in the European Union, resulting in an extra cost of 380 million euros [7]. The extensive and extended use of antibiotics results in the development of resistant bacterial pathogens during wound infections, which significantly contributes to increased rates of illness and death [8]. The range of infections caused by MRSA is diverse and is linked to negative outcomes, including longer hospital stays, greater treatment costs, and increased death [9].

MATERIAL AND METHODS

Cross-sectional research was undertaken at the department of microbiology to evaluate the antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus that was isolated from wound infections, as well as the risk factors associated with it. This research included all patients who were suspected of having wound infections and had not received any antibiotics during the two weeks before the study period. A total of 100 participants were included in this trial. Data pertaining to sociodemographic characteristics and their corresponding risk factors were gathered via the use of a well-organized questionnaire.

METHODOLOGY

Wound samples were obtained via Levine's methodology. The wound surface was sterilized using sterile gauze soaked in 70% alcohol. Wounds that had been dressed were cleaned with sterile normal saline solution once the dressing was removed. Using a sterile cotton-tipped applicator, the end was spun over a 1 cm2 region for 5 seconds with enough pressure to release fluid and microorganisms from inside the wound tissue, following the approach described by Levine and Gardner [10]. Specimens were obtained from a closed wound after the application of 70% alcohol for skin cleansing. Two wound swabs were collected from each site simultaneously in order to maximize the likelihood of detecting bacterial infections. The obtained specimens were appropriately labeled and delivered to the Dessie Regional Health Research Laboratory utilizing brain-heart infusion transport medium. The purpose of this transportation was to facilitate culture and antibiotic susceptibility tests, which were conducted within a time frame of 1 hour. Every wound sample was introduced to blood (Oxoid, Ltd., Basingstoke, Hampshire, England) and then transferred to mannitol salt agar for further growth. The plates were placed in an environment with oxygen at a temperature of 35–37°C for a duration of 24 hours. Staphylococcus aureus was detected by seeing Gram-positive cocci arranged in clusters, the presence of β-hemolytic colonies on blood agar, the production of catalase and coagulase enzymes, and the presence of a yellow colony surrounded by a yellow zone on mannitol salt agar.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The disc diffusion technique on Muller Hinton agar (MHA) was used to conduct an antimicrobial susceptibility test on each bacterial isolate. Three to five distinct colonies of each bacteria were selected and transferred to a tube containing 5 ml of sterile nutritional broth. The mixture was vigorously mixed to achieve homogeneity in the suspension. The suspension was kept at a temperature of 37°C until the cloudiness of the suspension reached a turbidity standard of 0.5 McFarland (indicating a bacterial concentration of 1.5 × 108 colony-forming units per milliliter). Aseptic swab was immersed in the solution, and the whole surface of the MHA plates was evenly saturated with the suspensions and let to air dry for about 15–30 minutes.

The disks containing antimicrobial agents were carefully positioned on the medium using sterile forceps. Each disk was put at a minimum distance of 24 mm from each other to prevent the zones of inhibition from overlapping. Once the disk was put on the infected medium, the plates were left undisturbed for 30 minutes to enable the antibiotic to spread throughout the media. The plates were flipped over and placed in an incubator at a temperature of 35 ± 2°C for a duration of 24 hours. They were then examined for the presence of a zone of inhibition.

The antibiotic disks used in the study were penicillin (10IU), ciprofloxacillin (5 μg), cotrimoxazole (1.25/23.75 μg), doxycycline (30g), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), and gentamicin (10 μg) from Oxoid UK. The susceptibility pattern was determined by comparing the zone of inhibition according to the guidelines set by the Clinical and Laboratory Standards Institute

(CLSI, 2014). The results were reported as either sensitive, moderate, or resistant. Standard strains of S. aureus (ATCC25923) were used as controls in the biochemical tests and agar plates, which included MHA with antimicrobial discs. This was done to ensure the accurate assessment of the antimicrobial discs' effectiveness.

The data were inputted and analyzed using SPSS version 23.0 for the Windows operating system. A stepwise logistic regression model was used to identify the parameters that are linked with wound infection. The strength of the link was measured by calculating the adjusted odds ratio and 95% confidence interval. Statistically significant values were defined as those with a significance level of less than 0.05.

RESULTS

A total of 100 study participants were enrolled in this investigation. Out of the total, 68 individuals (68%) were male, while 32 individuals (32%) were female. The average ages of the individuals included in the research were 34.54 ± 5.64 years. 25% of the research participants had no formal education, whereas the majority resided in metropolitan areas (77%) (Table 1).

Table 1Basic parameter of patients with wound infections

Age	Frequency	Percent
Below 15	9	9
15–25	31	31
25–35	22	22
35–45	12	12
45–55	8	8
55–65	10	10
Above 65	8	8
Gender		
Male	68	68
Female	32	32
Residence		
Rural	23	23
Urban	77	77
Patient setting		
Inpatient	31	31
Outpatient	69	69

Among a group of 100 patients who were suspected of developing wound infection, 50 of them (50%) were found to have S. aureus wound infections based on culture confirmation. Out of them, 15 (30%) were MRSA. The study population had an overall prevalence rate of 15% for MRSA, with 15 out of 100 individuals affected. Out of the 31 patients who were admitted to the hospital and the 69 patients who were treated as outpatients and suspected of having a wound infection, 38.71% (12 out of 31) and 33.33% (23 out of 69) were found to have a positive culture for S. aureus, respectively. The total incidence of Methicillin-resistant Staphylococcus aureus (MRSA) in hospitalized patients was 22.58% (7 out of 31), whereas in non-hospitalized patients it was 11.59% (8 out of 69).Among the 50 S. aureus samples isolated from wound swabs, including MRSA, 42 (84%) exhibited a significant resistance to penicillin. Additionally, 2 (4%) samples showed a mild resistance to clindamycin. MRSA demonstrated a complete resistance (100%) to penicillin, as well as high resistance rates to erythromycin and ciprofloxacin (66.67%), and moderate resistance rates to cotrimoxazole and gentamicin (53.33%) (Table 2).

Table 2Antibiotic resistance pattern of S. aureus and MRSA from wound infection

Antibiotics	Resistance pattern (%)			
	S. aureus (N = 50)		MRSA (N = 15)	
	Number	Percentage	Number	Percentage
Penicillin	42	84	15	100
Gentamicin	8	16	8	53.33
Ciprofloxacillin	9	18	10	66.67
Clindamycin	2	4	1	6.67
Cefoxitin	14	28	15	100
Erythromycin	13	26	10	66.67
Cotrimoxazole	8	16	8	53.33
Doxycycline	5	10	5	33.33
Chloramphenicol	4	8	4	26.67

The research found a much higher occurrence of multidrug resistance (MDR) in methicillin-resistant Staphylococcus aureus (MRSA) compared to methicillin-sensitive Staphylococcus aureus (MSSA), with MDR accounting for 66.67% (10 strains) and MSSA accounting for just 4% (2 strains). None of the strains tested were resistant to all drugs. However, out of the total of 10 MSSA strains, which accounts for 15.2% of the sample, all of them exhibited sensitivity to all antibiotics that were tested, as seen in Table 3.

Table 3MDR pattern of bacteria isolated from wound infection

S.aureus (n = 50)	**Resistance pattern, n (%)**							
	R0	**R1**	**R2**	**R3**	**R4**	**R5**	**R ≥ 6**	**MDR (≥3)**
MSSA (n = 35)	5(14.29)	27(77.14)	2 (5.71)	1(2.86)	0	1(2.86)	0	2 (5.71)
MRSA (n = 15)	0	0	5(33.33)	1 (6.67)	1 (6.67)	1 (6.67)	8(53.33)	11(73.33)

Note. R0, R1, R2, R3, R4, R5, R6, sensitive to all, resistance to one, two, three, four, five, and greater than six antibiotics tested, respectively; MDR (≥3): multidrug resistance (for greater than or equal to three antibiotics). MSSA: methicillin-sensitive Staphylococcus aureus; MRSA: methicillin-resistant Staphylococcus aureus.

Occupation, recent admission, recent surgery, inpatient diagnosis, and low body mass index (BMI) (<18.5) were shown to be significantly associated with wound infection caused by MRSA in a bivariate logistic regression study. Nevertheless, variables such as age, gender, level of education, place of residence, prior antibiotic use, and chronic medical conditions did not exhibit a statistically significant correlation.

In a multivariate logistic regression analysis, all the characteristics stated before were shown to be linked with wound infection caused by MRSA, except for recent history of hospitalization. The likelihood of farmers developing MRSA wound infection was 6 times higher (AOR = 5.99; 95% CI (1.32–29.65)) compared to housewives. those with a low BMI had a 12.43 times higher likelihood of developing MRSA wound infection compared to those with higher BMI (AOR = 11.43; 95% CI (5.54–33.85)). Furthermore, the likelihood of MRSA infection was 3.5 times higher among inpatients (AOR = 3.44; 95% CI (1.65–9.64)) compared to those identified in the outpatient department (OPD).

DISCUSSION

Wound infection caused by MRSA was a significant issue in nations with little resources, namely in India, where there is a lack of effective techniques for preventing and controlling infections. The research found that 50% of the participants had a wound infection caused by S. aureus. This result is consistent with the research carried out in Debre Markos (39.7%) [11] and Cameroon (28.9%) [12]. However, this discovery surpasses the percentages seen in previous research undertaken in Jimma (23.6%) [13], Nigeria (26.6%) [14], Tanzania (26.7%) [15], and Brazil (20%) [16]. Nevertheless, the prevalence shown in the present research is lower compared to a study conducted in Addis Ababa (57.8%) [17] and Uganda (41%) [18]. The differences in prevalence may be attributed to variations in the characteristics of the research participants, the timing of the investigation, and the methodology used to identify S. aureus.The research found that the total incidence of MRSA was 15%. This finding is consistent with the results reported in studies conducted in Addis Ababa (13.2%) [19], Eretria (9%) [20], and Cameroon (13.16%) [12]. However, it is lower than the results reported in previous studies conducted in Ethiopia, such as Debre Markos (19.6%) [11] and Jimma (17.4%) [13], as well as in other African countries, including Uganda (41%) [18] and Libya (31%) [21]. However, this study's findings are greater than the reported findings from studies conducted in Nigeria (5.8%) [14], Brazil (5.6%) [16], and Tanzania (4.3%) [15]. The significant frequency of MRSA identified in our research may be attributed to the elevated use of certain antibiotics, either owing to their widespread availability or their cost-effectiveness.Concerning potential risk factors, MRSA wound infections were strongly linked to employment (specifically farmers), patients with low BMI, and those who are presently hospitalized (inpatients) compared to others. This might be attributed to the lack of awareness among farmers on healthcare use. Furthermore, their employment predisposes them to wound infections, leading to the unsupervised use of antibiotics. The high incidence of MRSA in hospitalized patients may be ascribed to the transmission of drug-resistant bacterial strains inside healthcare facilities. Patients with a low body mass index (BMI) had a greater likelihood of acquiring a wound infection caused by methicillin-resistant Staphylococcus aureus (MRSA). While healthy individuals may harbor MRSA without showing any symptoms for extended durations, those with weakened immune systems are at a far higher risk of developing symptomatic infections [22, 23].In this investigation, the antimicrobial resistance profile of the isolates was examined. The S. aureus isolates shown resistance to penicillin (84%), gentamicin (16%), ciprofloxacillin (18%), clindamycin (4%), erythromycin (26%), cotrimoxazole (16%), doxycycline (10%), and chloramphenicol (8%). The resistance profile of S. aureus to penicillin in our investigation closely resembles the findings reported in DRH (82.2%) [11]. In previous research conducted in Tanzania [15] and Jimma, Ethiopia [24], the resistance to penicillin was found to be somewhat greater, with reported rates of 97% and 100% respectively. In the present investigation, the level of resistance to clindamycin is comparable to other studies conducted in Ethiopia, where the resistance rate was shown to be below 50% [19, 17]. The investigation revealed that the MRSA isolates exhibited resistance to penicillin (100%), gentamicin (53.33%), ciprofloxacillin (66.67%), clindamycin (6.67%), erythromycin (66.67%), cotrimoxazole (53.33%), doxycycline (33.33%), and chloramphenicol (26.67%). Studies undertaken in various regions have consistently shown that MRSA isolates exhibit complete resistance to penicillin, with a resistance rate of 100% [25]. The research found that the MRSA isolates had a greater resistance to gentamycin compared to a previous study conducted at Yekatit 12 Hospital in Addis Ababa, where the resistance rate was 38.2% [19]. Similarly, the level of resistance to ciprofloxacin is somewhat elevated when compared to the findings published in Tanzania (54%) [15]. The primary discrepancy in drug resistance trends across various research may be attributed to the widespread and unrestricted use and accessibility of certain antibiotics within a specific region. The disparity in resistance rates across various places suggests that the pattern of antibiotic resistance differs based on regional and geographical factors, and also evolves with time.

Moreover, the research region exhibited a significant occurrence of multidrug-resistant MRSA, with a prevalence rate of 70%. This result aligns with the research carried out in northern India, where 73% of MRSA strains shown resistance to several drugs [26]. Similarly, a research done in Debre Markos revealed that all MRSA strains that were examined had resistance to at least three medications [11]. The high incidence of multidrug resistance increases the likelihood of patients becoming infected with difficult-to-treat strains, highlighting the need of enhancing infection control measures and establishing recommendations for antibiotic use in this context.

CONCLUSION

Among the 100 patients who were suspected to have wound infection, 50 of them, which is 50% of the total, were confirmed to have S. aureus by culture testing. Out of them, 15 (30%) were MRSA. Wound infection caused by MRSA had a significant correlation with profession, being diagnosed in the inpatient department, and body mass index. More than 50% of MRSA isolates exhibited resistance to gentamicin, ciprofloxacillin, cotrimoxazole, and erythromycin.

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