e-ISSN 2795-3276
p-ISSN 2795-3270

10.5281/zenodo.12759828 Vol. 07 Issue 05 May - 2024 Manuscript ID: #01487

Epidemiological Assessment of the Burden and Determinants of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infections in Wounds

Mousa Muftah Khalil[1], Mahmoud F. Gaballa[2], Gomaa Sulaiman[3], Idress Hamad Attitalla[2*]

[1]Faculty of Medical Technology, Omar Al-Mukhtar University AL-Bayda, Libya
[2]Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University AL-Bayda, Libya
[3]Faculty of Medical Technology, The University of Tripoli, Libya

***Corresponding author E-mail:** idress.hamad@omu.edu.ly

Abstract:

Background and Objectives: One important pathogen linked to healthcare is *Methicillin-resistant Staphylococcus aureus* (MRSA), which is known to cause wound infections that have higher rates of morbidity and death.

The purpose of this study is to ascertain the frequency of *Methicillin-Resistant Staphylococcus aureus* (MRSA) and to identify the risk factors linked to patient wound infections .

Material and methods: *Methicillin-resistant Staphylococcus aureus* isolated from wound infections was studied using the cross-sectional study to assess its antibiotic resistance pattern and risk factors. All patients who were suspected of having wound infections and had not taken antibiotics in the two weeks before the study period was included in the present study. The research study had 100 participants in total. Utilizing a structured questionnaire, information about sociodemographic traits and the risk factors associated with them was acquired.

Results: Among a group of 100 patients who were suspected of developing wound infection, 45 of them (45%) were found to have *S. aureus* wound infections based on culture confirmation. Out of them, 9 (20%) were MRSA. The study population had an overall prevalence rate of 9% for MRSA, with 9 out of 100 individuals affected. Out of the 20 patients who were admitted to the hospital and the 80 patients who were treated as outpatients and suspected of having a wound infection, 55.0% (11 out of 20) and 31.3% (25 out of 80) were found to have a positive culture for *S. aureus*, respectively. The total incidence of *Methicillin-resistant Staphylococcus aureus* (MRSA) in hospitalized patients was 10.0% (2 out of 20), whereas in non-hospitalized patients it was 8.8% (7 out of 80).

Conclusion: Among the 100 patients who were suspected to have wound infection, 45 of them, which are45% of the total, were confirmed to have *S. aureus* by culture testing. Out of them, 9 (20%) were MRSA. Wound infection caused by MRSA had a significant correlation with profession and being diagnosed in the inpatient department. More than 50% of MRSA isolates exhibited resistance to gentamicin, ciprofloxacin, cotrimoxazole, and erythromycin.

Keywords: **Methicillin-resistant Staphylococcus aureus, MRSA, wound infection, prevalence, risk factors, antimicrobial susceptibility**

Introduction

One common bacterium that frequently causes bacterial infections in humans is *Staphylococcus aureus* or *S. aureus*. It is the cause of infections that are acquired in both community and hospital settings, affecting many body regions such as the skin, urinary system, surgical sites, bones (osteomyelitis), bloodstream (septicemia), and heart (endocarditis) [1]. The remarkable ability of *Staphylococcus aureus* to develop resistance to multiple antibiotics was first recognized. This involved the acquisition of β-lactamase on "penicillinase plasmids" and the subsequent adaptation of *methicillin-resistant S. aureus* (MRSA) through the acquisition of β-lactamase-resistant staphylococcal cassette chromosome (SCCmec) elements [2].

A wound is a break in the skin's barrier function that lets the outside world seep into the underlying tissue. Because of the wetness, warmth, and nutrition, skin wounds provide an ideal habitat for microbial colonization, development, and infection [3]. Bacterial skin infections can be caused by a variety of pathogens, including *Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus species,* and *Enterococcus species* [4]. One of the most frequent kinds of wound infection is *staphylococcus aureus*, which is a common cause of surgical wound infections and nosocomial infections (NI) [5]. It has demonstrated a growing resistance to many antibiotics in recent years. The methicillin resistance gene mecA, which results in the low-affinity penicillin-binding protein (PBP2a) that is absent from susceptible *S. aureus* strains, is the source of methicillin resistance in *methicillin-resistant S. aureus*. The majority of β-lactams bind poorly to the resistant penicillin-binding protein receptor. Because of this, MRSA can flourish when these medications are present [6]. Methicillin-resistant strains of *S. aureus* have lately been divided into two groups according to molecular and epidemiological characteristics. These subsets are referred to as healthcare-associated (HA) and community-associated (CA) MRSA. When compared to HA-MRSA isolates, community-

associated MRSA isolates frequently show lower levels of resistance [7]. Resistant to methicillin MRSA, and s*taphylococcus aureus*, is a major worldwide health concern that causes infections acquired in hospitals. In the European Union, MRSA infections in hospitals alone were predicted to affect around 150,000 individuals annually and incur additional costs of 380 million euros [8]. The prolonged and widespread use of antibiotics causes resistant bacterial pathogens to emerge during wound infections, which greatly raises the incidence of disease and mortality [9]. MRSA infections can range widely and are associated with several unfavourableoutcomes, such as increased hospital admissions, higher treatment costs, and a higher mortality rate [10].

Material and Methods

Study Design:

Methicillin-resistant Staphylococcus aureus was identified in wound infections from patients who were treated at Al-Bayda Medical Centre. To assess the antibiotic resistance pattern of the bacteria and the risk factors associated with it, a cross-sectional study was carried out in the laboratory of the Faculty of Medical Technology at Omar Al-Mukhtar University in Al-Baydacity. All patients with suspected wound infections who had not taken antibiotics in the two weeks before the research period were included in this investigation. This experiment had 100 individuals in total. A well-structured questionnaire was used to gather information on sociodemographic traits and the associated risk factors.

Sample Collection and Laboratory Procedures:

Levine's approach was utilized to gather samples of wounds. Sterile gauze dipped in 70% alcohol was used to sterilize the wound area. After the dressing was taken off, a sterile normal saline solution was used to clean the wounds. Following the method outlined by Levine and Gardner, the end of a sterile cotton-tipped applicator was spun over a 1 cm2 area for 5 seconds at a pressure

high enough to release fluid and bacteria from inside the wound tissue [11]. Specimens were collected from a closed wound following the use of 70% alcohol to clean the skin. To increase the chance of finding bacterial infections, two wound swabs were taken concurrently from each location. The acquired specimens were transported to the laboratory using brain-heart infusion transport media after being suitably labelled. This transportation was needed to make it easier for the one-hour culture and antibiotic susceptibility testing to be completed. Each wound sample was placed in blood (Oxoid, Ltd., Basingstoke, Hampshire, England) and allowed to develop further before being moved to mannitol salt agar. For a whole day, the plates were kept in an oxygen-rich atmosphere between 35 and 37 degrees Celsius. The presence of β-hemolytic colonies on blood agar, the formation of catalase and coagulase enzymes, clusters of Gram-positive cocci, and a yellow colony encircled by a yellow zone on mannitol salt agar were all indicators that Staphylococcus aureus was present.

Antimicrobial susceptibility test

Every bacterial isolate underwent an antibiotic susceptibility test using the disc diffusion method on Muller Hinton agar (MHA). Each bacterium was divided into three to five separate colonies, which were then placed in a tube with 5 ml of sterile nutritive soup. To ensure that the suspension was homogeneous, the mixture was thoroughly stirred. The suspension was maintained at 37°C until it attained a turbidity standard of 0.5 McFarland, which denotes a concentration of 1.5 x 108 colony-forming units per milliliter of bacteria. After dipping an aseptic brush into the solution, the suspensions were equally applied to the entire surface of the MHA plates, which were then allowed to air dry for 15 to 30 minutes.

Using sterile forceps, the antimicrobial agent-containing discs were carefully placed on the medium. To avoid the zones of inhibition overlapping, each disc

was placed at least 24 mm apart from the others. The plates were kept undisturbed for 30 minutes after the disc was placed on the contaminated medium to allow the antibiotic to disperse throughout the media. After being turned over, the plates were kept in an incubator set at 35 ± 2°C for a whole day. The existence of a zone of inhibition was then investigated. Penicillin (10IU), ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), erythromycin (15µg), chloramphenicol (30µg), and gentamicin (10µg) from Oxoid UK were the antibiotic discs used in the investigation. By comparing the zone of inhibition to the recommendations established by the Clinical and Laboratory Standards Institute (CLSI, 2014), the susceptibility pattern was ascertained. Sensitive, moderate, or resistant outcomes were reported. Standard strains of *S. aureus* (ATCC25923) were employed in agar plates containing MHA and antimicrobial discs as controls for the biochemical experiments. This was carried out to guarantee an accurate evaluation of the antimicrobial discs' efficacy.

Data Collection and Analysis:

SPSS software for Windows, version 23.0, was used to enter and analyze the data. To determine the factors associated with wound infection, a stepwise logistic regression model was employed. The adjusted odds ratio and 95% confidence interval were computed to determine the strength of the relationship. A significance level of less than 0.05 was used to identify values as statistically significant.

RESULTS

The investigation had 100 study participants in total 42 people (42%) and 58 people (58%) of the total were male and female. The mean age of the participants in the study was 29.8 years. Participants made up 80% of outpatients and 20 % of inpatients, andonly 3% of research participants did not have a formal education (Table 1).

Table 1: the fundamental characteristics of wound-infected patients

Age	Frequency	Percent
5-15	18	18
15–25	27	27
25–35	21	21
35–45	15	15
45–55	11	11
55–65	8	8
Gender		
Male	42	42
Female	58	58
Patientsetting		
Inpatient	20	20
Outpatient	80	80

Among a group of 100 patients who were suspected of developing wound infection, 45 of them (45%) were found to have S.aureus wound infections based on culture confirmation. Out of them, 9 (20%) were MRSA. The study population had an overall prevalence rate of 9% for MRSA, with 9 out of 100 individuals affected. Out of the 20 patients who were admitted to the hospital and the 80 patients who were treated as outpatients and suspected of having a wound infection, 55.0% (11 out of 20) and 31.3% (25 out of 80) were found to have a positive culture for S. aureus, respectively. The total incidence of Methicillin-resistant Staphylococcus aureus (MRSA) in hospitalized patients was 10.0% (2 out of 20), whereas in non-hospitalized patients it was 8.8% (7 out of 80). Among the 45 S. aureus samples isolated from wound swabs, including MRSA, 38 (84.4%) exhibited a significant resistance to penicillin. Additionally, 3 (6.7%) samples showed a mild resistance to Chloramphenicol. MRSA demonstrated a complete resistance (100%) to penicillin, as well as high resistance rates to Ciprofloxacin, Gentamicin, and Cefoxitin (66.7%,77.8%,88.9%), respectively, and moderate resistance rates to Erythromycin (55.6%) (Table 2).

Table 2:*S. aureus* and MRSA's Pattern of Antibiotic Resistance in Wound Infections

Antibiotics	Resistancepattern (%)			
	S.aureus(N = 45)		MRSA(N = 9)	
	Number	Percentage	Number	Percentage
Penicillin	38	84.4	9	100
Gentamicin	9	20.0	7	77.8
Ciprofloxacillin	8	17.8	6	66.7
Cefoxitin	12	26.7	8	88.9
Erythromycin	14	31.1	5	55.6
Cotrimoxazole	9	20.0	4	44.4
Chloramphenicol	3	6.7	4	44.4

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 77.8% (7 strains) and MSSA accounting for just 2.6% (1 strain). None of the strains tested were resistant to all drugs However, out of the total of 10 MSSA strains, which accounts for 26.3% of the sample, all of them exhibited sensitivity to all antibiotics that were tested, as seen in Table 3.

Table3:Bacteria isolated from wound infections with an MDR pattern

S.aureus (n = 45)	Resistance pattern, n (%)							
	R0	R1	R2	R3	R4	R5	R ≥ 6	MDR(≥3)
MSSA(n = 38)	10 (26.3)	24(63.2)	2(5.3)	1(2.6)	1(2.6)	0	0	1(2.6)
MRSA (n = 9)	0	0	3(33.3)	1(11.1)	1 (11.1)	1 (11.1)	3(33.3)	7 (77.8)

R0 is sensitive to all antibiotics, while R1 is resistant to one antibiotic, R2 to two, R3 to three, R4 to four, R5 to five, and R6 to more than six antibiotics that were tested. MDR (≥3): multidrug resistance which indicates resistance to 3 or more antibiotics.MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*.

Occupation, recent admission, recent surgery, and inpatient diagnosis 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. Furthermore, the likelihood of MRSA infection the crude odds ratio (OR) for the incidence of MRSA in hospitalized versus non-hospitalized patients is approximately 1.16. This

suggests that hospitalized patients have slightly higher odds of developing MRSA compared to non-hospitalized patients (OPD).

4. Discussion

MRSA-caused wound infection is a serious problem in low-resource countries since there are insufficient methods for effectively avoiding and treating infections. According to the study, *S. aureus*-related wound infections affected 45% of the subjects. This outcome is in line with studies conducted in Cameroon (28.9%) and Debre Markos (39.7%) [12,13].This finding, however, is higher than the percentages reported in earlier studies conducted in Tanzania (26.7%), Jimma (23.6%), Nigeria (26.6%), and Brazil (20%) [14,15,16,17]. However, the current study's prevalence is lower than that of a study done in Addis Ababa (57.8%) [18]. changes in the research participants' characteristics, the date of the study, and the *S. aureus* identification technique could be the cause of the changes in prevalence. The study discovered that 9% of cases of MRSA were overall. The results of research undertaken in Addis Ababa (13.2%), Eretria (9%), and Cameroon (13.16%) agree with this finding[19,20,12]. It is less than the findings of earlier research, though, from Ethiopian studies like Debre Markos (19.6%) [13] and Jimma (17.4%), as well as from research conducted in other African nations like Uganda (41%) and Libya (31%) [13,15,21,22]. The results of this study, however, are higher than those of studies that were reported from Tanzania (4.3%), Brazil (5.6%), and Nigeria (5.8%) [14,17,16].

Potential risk factors for MRSA wound infections were employment (particularly in rural areas) and the proportion of the population that is hospitalized right now (inpatients) as opposed to the general population. This could be explained by farmers' ignorance of the importance of using healthcare. Moreover, their work exposes them to wound infections, which encourages the misuse of antibiotics without supervision. One possible explanation for the

increased prevalence of MRSA among hospitalised patients is the spread of drug-resistant bacterial strains within healthcare settings. While healthy people may harbour MRSA for extended periods without exhibiting any signs, immune system-compromised people are considerably more likely to experience symptoms [23, 24]. The profile of the isolates' antimicrobial resistance was examined in this study. Penicillin (84.4%), Gentamicin (20.0%), Ciprofloxacillin (17.8%), Cefoxitin (26.7%), Erythromycin (31.1%), Cotrimoxazole (20.0%), and Chloramphenicol (6.7%), among other antibiotics, were all resistant in the *S. aureus* isolates.Our investigation's *S. aureus* resistance profile to penicillin substantially resembles the results published in DRH (82.2%) [13].Penicillin resistance was shown to be somewhat higher in earlier studies carried out in Jimma, Ethiopia and Tanzania, with reported rates of (97%) and (100%) [25,14]. The results of the study showed that the MRSA isolates were resistant to 100% of penicillin, (77.8%) gentamicin, (66.7%) ciprofloxacillin, (88.9%) cefoxitin, (55.6%) erythromycin, (44.4%) of cotrimoxazole, and (44.4%) of chloramphenicol.Studies conducted in different areas have consistently demonstrated that MRSA isolates have a 100% resistance rate to penicillin and exhibit total resistance [26].

In contrast to a prior study carried out at Yekatit 12 Hospital in Addis Ababa, where the resistance rate was 38.2%, this study revealed that the MRSA isolates exhibited a higher level of gentamycin resistance [19]. In a similar vein, the percentage of ciprofloxacin resistance is a little higher than the results reported in Tanzania (54%) [14].The main reason for the disparity in drug resistance trends throughout studies could be the freely available and extensive use of some antibiotics in a particular area. The variation in antibiotic resistance rates between different locations indicates that the pattern of antibiotic resistance varies depending on geographical and regional factors as well as changes throughout time.Additionally, the study area demonstrated a noteworthy frequency of 77.8% for multidrug-resistant MRSA. This finding is consistent

with studies done in northern India, where 73% of MRSA strains had antibiotic resistance [27]. In a similar vein, a study conducted in Debre Markos found that every MRSA strain tested was resistant to at least three drugs [13]. Since patients are more likely to contract hard-to-treat strains of infection due to the high prevalence of multidrug resistance, it is imperative to improve infection control protocols and develop guidelines for the appropriate use of antibiotics in this setting.

Conclusion

45 of the 100 patients—or 45% of the total—who had been suspected of having a wound infection had S. aureus identified by culture testing. Nine (9%) of them contained MRSA. MRSA-caused wound infections were diagnosed in the inpatient department and correlated significantly with the occupation. Moreover, over 50% of MRSA isolates showed resistance to erythromycin, gentamicin, ciprofloxacin, and cotrimoxazole.

Declarations Source of Funding

This study did not receive any grant from funding agencies in the public or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

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