Original Article

Methicillin-Resistant Staphylococcus Aureus Conundrum: Elucidating the Spectrum of Antibiotic Resistance

Awadh Alanazi

ABSTRACT

Objective: To assess MRSA's impact and the latest treatment strategies, seeking to improve management and containment efforts.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia, from March 2023 to February 2024.

Methods: S. aureus isolates from various clinical sources were identified using culture, biochemical, and phenotypic tests. MRSA isolates were identified using the cefoxitin (30 µg) disc as a surrogate marker. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method to report antimicrobial resistance.

Results: Forty-eight S. aureus isolates, comprising 32 MRSA and 16 methicillin-sensitive S. aureus (MSSA) infections. Nasal swabs exhibited 100% MRSA occurrence, with high prevalence in wound swabs and blood cultures. MRSA isolates demonstrated multidrug resistance to various classes of antibiotics. In contrast, these isolates exhibited no or less resistance to fewer antibiotics such as vancomycin (0%), teicoplanin (0%), rifampin (0%), tetracycline (0%), linezolid (3%), and quinupristin/dalfopristin (3%).

Conclusion: The spectrum of antibiotic resistance in MRSA, emphasizing the need for tailored antibiotic treatment strategies and ongoing efforts to combat the spread of multidrug-resistant pathogens in healthcare settings.

Key Words: Staphylococcus aureus, Gram-positive infections, MRSA, antibiotic resistance, multidrug resistance

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INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium belonging to the genus Staphylococcus. Several species and subspecies have been identified. S. aureus is part of the human microbiome, with 20% to 40% of humans carrying this organism as part of their normal flora.[1] However, S. aureus is the most commonly isolated organism in hospitals, community settings, and food sources, causing severe complications for patients and the hospital environment.[2,3] It causes many human diseases, including bacteremia, endocarditis, wound infections, and lung infections. The pathogenicity of S. aureus has been reported worldwide to be problematic in both community and hospital settings.[4]

Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia.

Correspondence: Dr. Awadh Alanazi, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia.
Contact No: +966501411613
Email: aaanazi@ju.edu.sa

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S. aureus infections rely on the evasion of the host's immune response, invasion of host tissues, and initiation of infection by producing enzymes and extracellular toxins that destroy host tissue and cells.[5] Clinically, the main issue with S. aureus is its ability to develop resistance to multiple drugs, leading to treatment complications. Traditionally, infections were treated with penicillin, which interferes with peptidoglycan assembly - the major component of the bacterial cell wall. This interference makes the bacterial cell wall fragile and deficient. Unfortunately, overexposure to penicillin led to the emergence of globally resistant strains, primarily through the deactivation and hydrolysis of the antibiotic's β-lactam ring by plasmid-encoded β-lactamase. A few years later, penicillin-resistant S. aureus rapidly became the predominant strain worldwide, causing severe infections. In 1959, methicillin was introduced as a treatment for β-lactamase-resistant penicillin. However, within a year of methicillin's introduction, the bacteria acquired the mecA gene, which encodes an alternative penicillin-binding protein. This gene conferred resistance to most β-lactam antibiotics.[6]

The rapid acquisition of resistance to penicillin and methicillin has been recognized as a global issue, making it increasingly challenging to treat S. aureus infections. In hospital settings, vancomycin, a late-stage

peptidoglycan synthesis inhibitor, is the preferred treatment for methicillin-resistant S. aureus (MRSA). However, vancomycin-resistant strains have been reported worldwide.[7] S. aureus can develop resistance to all classes of antibiotics available in clinical settings through various mechanisms, thereby limiting treatment options.[8]

Globally, MRSA is a life-threatening problem, leading to severe complications and a high risk of mortality, high therapeutic costs, and prolonged hospital stays.[9-11] In 2019, a study reported approximately 4.95 million deaths worldwide from multidrug-resistant infections, with around 100,000 associated with MRSA.[12] In Saudi Arabia, the number of MRSA cases has increased, with a prevalence rate of 35.6% derived from a pooled estimate of 22,793 infections with S. aureus.[13] Therefore, considering the significance of MRSA infections and the disease burden, the current study aimed to report the MRSA isolates and empirical therapeutic options for treating MRSA-associated infections.

METHODS

This cross-sectional study was conducted at the Department of Clinical Laboratory Sciences from March 2023 to February 2024. Leftover bacterial cultures from various clinical sources and wards of a tertiary care hospital were processed following the ethical considerations outlined in the WMA Declaration of Helsinki[14]. The study did not involve any animal or human participants.

The study specifically targeted S. aureus and excluded other bacterial isolates. S. aureus cultures were incubated aerobically on blood, chocolate, and mannitol salt agar overnight at 35–37°C. Identification of S. aureus was based on morphological characteristics, mannitol fermentation, catalase, coagulase, and DNase tests. Confirmed S. aureus isolates were screened to differentiate between MRSA and methicillin-sensitive S. aureus (MSSA) using a cefoxitin (30 µg) disc on Mueller-Hinton (MH) agar, following guidelines outlined in the CLSI manual.[15]

The antimicrobial sensitivity testing (AST) was performed according to CLSI guidelines using various antibiotic discs representing different classes of antibiotics, including amoxicillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, ciprofloxacin, clarithromycin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamicin, imipenem, levofloxacin, linezolid, moxifloxacin, mupirocin, penicillin, rifampin, quinupristin/dalfopristin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin. The inoculum size of S. aureus was adjusted in sterile saline using 0.5 McFarland standard and all MRSA and MSSA isolates were streaked on MH agar plates using sterile cotton swabs. Antibiotic discs were then placed on the MH agar using the Kirby-Bauer disc diffusion method, and cultures were incubated at 35–37°C overnight. The susceptibility results were interpreted as resistant, intermediate, or sensitive based on predefined criteria.[15]

Data analysis was conducted using SPSS-27.0. A Chi-square test was utilized to investigate the significant association between antimicrobial resistance (AMR) and MRSA, with the significance threshold set at p-values lower than 0.05.

RESULTS

This study examined 48 bacterial isolates of S. aureus, comprising samples from 32 MRSA and 16 with MSSA. The distribution of MRSA and MSSA across different clinical specimens was investigated. Nasal swabs exhibited a 100% occurrence of MRSA, with no MSSA detected. A high prevalence of MRSA was also observed in wound swabs and blood cultures (66.67%), as well as in urine samples (60%). Conversely, sputum samples predominantly yielded MSSA, accounting for 66.7% of the isolates compared to 33.3% for MRSA. Ear swabs showed an equal distribution, with MRSA and MSSA each constituting 50% of the isolates. However, no significant difference ($p = 0.84$) was observed in the isolation rate of MRSA from various clinical sources (Fig. 1).

The results revealed that, for most antibiotics tested, a significantly higher proportion of MRSA strains were resistant than MSSA. Notably, amoxicillin/clavulanic acid and imipenem both demonstrated 100% resistance in MRSA strains, with highly significant p-values ($p< 0.001$). Additionally, resistance to ampicillin was observed in 100% of MRSA strains, with a p-value of 0.05. Resistance to ciprofloxacin and levofloxacin was also significantly higher in MRSA, at 78% for both antibiotics, with p-values of 0.001 and 0.003, respectively. In contrast, MSSA strains exhibited lesser resistance to most antibiotics, with no resistance (0%) noted for cefoxitin, imipenem, penicillin, and vancomycin (Table 1).

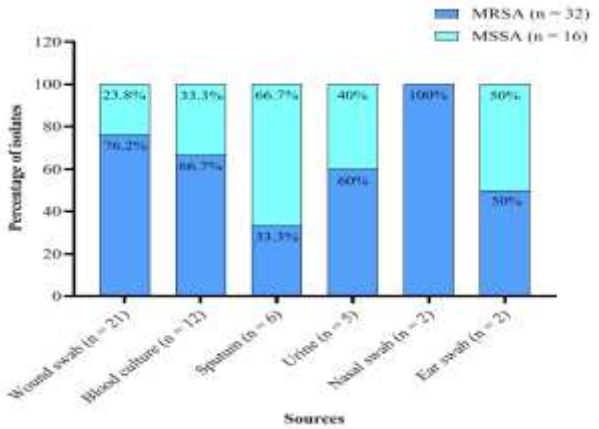

Figure No. 1: Comparative prevalence of MRSA and MSSA in clinical sources

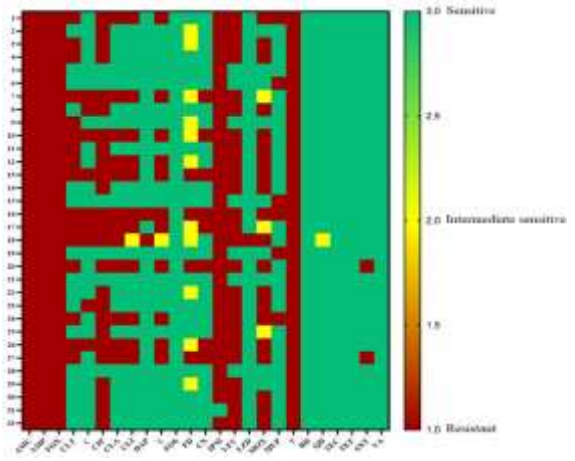

Figure No. 2: Antimicrobial spectrum in MRSA isolates

The variability in antibiotic response was evident among the MRSA isolates. A considerable number of these isolates were resistant to antibiotics such as amoxicillin/clavulanic acid, ampicillin, cefoxitin, penicillin, imipenem, and ciprofloxacin. Conversely, a few organisms exhibited resistance towards vancomycin, rifampin, teicoplanin, tetracycline, linezolid, and quinupristin/dalfopristin. These observed resistance patterns in the MRSA isolates underscore the evolving nature of bacterial resistance, highlighting the imperative for tailored antibiotic treatments in clinical practice (Fig. 2).

Antimicrobial response spectrum of MRSA isolates using a color-coded system: red indicates resistance (represented by a value of 1), yellow signifies intermediate sensitivity (value of 2), and green denotes sensitivity (value of 3). Amoxicillin/clavulanic acid (AMC), Ampicillin (AMP), Cefoxitin (FOX), Cephalothin (CLT), Chloramphenicol (C), Ciprofloxacin (CIP), Clarithromycin (CLA), Clindamycin (CLI), Daptomycin (DAP), Erythromycin (E), Fosfomycin (FOS), Fusidic Acid (FD), Gentamicin (CN), Imipenem (IPM), Levofloxacin (LEV), Linezolid (LZD), Moxifloxacin (MOX), Mupirocin (MUP), Penicillin (P), Rifampin RD, Quinupristin/dalfopristin (QD), Teicoplanin (TEC), Tetracycline (TET), Trimethoprim/sulfamethoxazole (SXT), Vancomycin (VA)

Table No. 1: Comparative analysis of antibacterial activity against MRSA (n = 16) and MSSA (n = 32).

Antibiotic	MRSA (n=32) Resistance		MSSA (n = 16) Resistance		p-value
Amoxicillin/clavulanic acid	32	100%	1	6%	<0.001*
Ampicillin	32	100%	13	81%	0.05*
Cefoxitin	32	100%	0	0%	-
Cephalothin	16	50%	6	38%	0.41
Chloramphenicol	9	28%	1	6%	0.07
Ciprofloxacin	25	78%	5	31%	0.001*
Clarithromycin	12	38%	1	6%	0.02*
Clindamycin	11	34%	2	13%	0.09
Daptomycin	2	6%	-	-	1
Erythromycin	10	31%	1	6%	0.04*
Fosfomycin	15	47%	-	-	0.004*
Fusidic Acid	4	13%	2	13%	0.71
Gentamicin	8	25%	1	6%	0.27
Imipenem	31	97%	-	-	<0.001*
Levofloxacin	25	78%	4	25%	0.003*
Linezolid	1	3%	-	-	0.6
Moxifloxacin	20	63%	5	31%	0.01*
Mupirocin	6	19%	1	6%	0.24
Penicillin	32	100%	-	-	-
Rifampin	-	-	-	-	-
Quinupristin/dalfopristin	1	3%	-	-	0.8
Teicoplanin	-	-	-	-	-
Tetracycline	-	-	-	-	-
Trimethoprim/sulfamethoxazole	2	6%	1	6%	0.9
Vancomycin	-	-	-	-	-

*Significant p-value**.**

DISCUSSION

Despite improvements in healthcare facilities, S. aureus remains the primary cause of hospital infections worldwide, with a broad spectrum of clinical diseases ranging from superficial infections (such as folliculitis) to life-threatening.[16] This study focused on the characterization of S. aureus antibiotic resistance, mainly methicillin resistance. It has been reported that MRSA cases are frequently distributed in surgical

areas.[17] A study found that the prevalence of MRSA is more likely in males than in females.[18] This elucidates the variation in gender distribution based on geographic area and host factors such as age and general health status. The prevalence of MRSA fluctuated over various periods. A study from Pakistan in 2011 revealed a 35.8% incidence of MRSA in clinical environments.[19] There has been a notable variation in the prevalence of MRSA in Saudi Arabia, with a significant increasing trend reaching 14.5% in 2019 compared to 5.2% in 2009.[20]

According to the Centers for Disease Control and Prevention (CDC), in 2019, about 33% of healthy individuals carried MRSA in their noses without developing any severe MRSA infections. Although many people are considered carriers of MRSA, several studies have reported that these carriers are considered a source of infection in hemodialysis and surgical wards. In this study, despite the limited number of nasal samples, 2 out of 2 (100%) were found to be MRSA carriers, consistent with the findings reported by the CDC. In this study, bloodstream infections and skin infections were the most common sites of MRSA infection, in line with findings from other studies.[21]

Treating isolates resistant to multiple drugs, whether gram-negative or gram-positive, from clinical and non-clinical origins presents enduring challenges, with MRSA distinguishing itself as a particularly significant concern.[11,22,23] In 2014, the World Health Organization (WHO) Global Report on Surveillance reported that the treatment of verified or suspected cases of MRSA worldwide must rely on second-line treatments, which are costlier and have more side effects, since first-line treatments for severe MRSA have limited efficacy. Treatment of S. aureus infections is available based on different antimicrobial therapies; however, several reports have confirmed the limitations of treating the infection since the organism has become resistant to first-line drugs[24]. In this study, vancomycin, teicoplanin, and linezolid showed no resistance to MRSA and are considered excellent therapeutic options for MRSA infections.[25] Another study presented similar findings regarding vancomycin sensitivity, teicoplanin, and linezolid as treatment options for MRSA infections.[25,26] All MRSA strains showed resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, and penicillin as therapeutic options against MRSA infection. These findings are consistent with various reports from around the world.[27,28]

In this study, ciprofloxacin showed poor therapeutic activity against the MRSA strain, exhibiting resistance in 78% of cases. A variable degree of ciprofloxacin resistance to MRSA infection has been reported in different Gulf Cooperation Council countries.[29] Fusidic acid has been used clinically to treat MRSA infections in various parts of the world and is considered a drug of choice for treating MRSA.[30] In this study, fusidic acid showed meager resistance (13%) to the MRSA strain, which is lower than what was reported in Riyadh in 2006[31] and similar to findings in the UK.[32] The risk factors for acquiring MRSA, continuous reporting of new cases, and monitoring of colonized individuals should be considered to control and reduce the spread of this disease. The current study has limitations due to its inability to explore the clinical data and the molecular basis of mecA genes involved in the emergence of MRSA.

CONCLUSION

The emergence of MRSA presents a significant challenge in healthcare settings due to its diverse antibiotic resistance patterns. MRSA exhibited high resistance to commonly used antibiotics, whereas MSSA strains demonstrated greater sensitivity. Although therapeutic options for MRSA infections are limited by resistance to first-line antibiotics, vancomycin, teicoplanin, and linezolid continue to be effective treatments. Continuous surveillance and identification of risk factors are essential for controlling the spread of MRSA despite limitations in investigating the molecular mechanisms underlying its emergence. This resistance extends the duration of illness and escalates the financial burden of treatment.

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Author's Contribution:

Concept & Design of Study:	Awadh Alanazi
Drafting:	Awadh Alanazi
Data Analysis:	Awadh Alanazi
Revisiting Critically:	Awadh Alanazi
Final Approval of version:	Awadh Alanazi

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