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IDENTIFICATION OF VANCOMYCIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM SURGICAL WOUNDS

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Abstract

The rise of antimicrobial resistance, particularly vancomycin-resistant *Staphylococcus aureus* (VRSA), is a *significant public health concern worldwide. The high prevalence of surgical site infections and unregulated antibiotic use in developing countries like Pakistan creates a favorable environment for multidrug-resistant strains. There is a notable lack of local data on VRSA prevalence in regions of Pakistan, including the Kohat district. The primary objective of this study was to determine the prevalence of vancomycin-resistant S. aureus (VRSA) among isolates from surgical patients in the Kohat district and analyze their antimicrobial susceptibility profiles. This was a cross-sectional study conducted at the District Hospital (CMH), Kohat, from July to December 2022. A total of 155 samples, including pus, tissue, and wound swabs, were collected from surgical patients with postoperative infections. S. aureus was identified using microscopic, macroscopic, and biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Vancomycin resistance was confirmed by the Minimum Inhibitory Concentration (MIC) test, with isolates having an MIC of ≥4μg/ml classified as resistant. Out of 155 samples collected, 80 isolates of S. aureus were identified. The prevalence of S. aureus was found to be 53.94% (n=82/152). Notably, 39% (n=32) of the isolates were Methicillin-Resistant S. aureus (MRSA)[12]. The study's main finding was that 11% (9 out of 80) of the S. aureus isolates showed resistance to vancomycin. The MIC values for these 9 isolates ranged from 4μg/ml to 8μg/ml. The findings indicate that VRSA is present in surgical patients in the Kohat district, a significant and alarming discovery given its previously rare reporting in Pakistan. This underscores a critical public health issue that necessitates immediate action to curb the spread of these pathogens. The study recommends implementing strict antibiotic stewardship, enhancing surveillance, and improving infection control measures in local healthcare facilities.*

INTRODUCTION

Staphylococcus aureus is a major Gram-positive bacterium responsible for a wide spectrum of human infections, ranging from mild skin and soft tissue infections to severe conditions like bacteremia and surgical site infections. Its ability to acquire resistance to antimicrobial agents has made it a significant public health concern globally. The emergence of methicillin-resistant *S. aureus* (MRSA) in the mid-20th century transformed clinical practice, leading to vancomycin becoming a critical last-resort antibiotic for serious *S. aureus* infections.

However, the effectiveness of vancomycin has been threatened by the emergence of vancomycin-intermediate *S. aureus* (VISA) and, more alarmingly, vancomycin-resistant *S. aureus* (VRSA). The spread of VRSA represents a major global challenge, as it severely limits treatment options for patients with life-threatening infections. In many developing countries, including Pakistan, the high prevalence of surgical site infections combined with the widespread and often unregulated use of antibiotics has created an environment conducive to the development of such multidrug-resistant strains.

Despite this pressing issue, there is a notable lack of local data on the prevalence of VRSA in many regions of Pakistan. Specifically, the prevalence of vancomycin resistance among *S. aureus* isolates from surgical patients in the Kohat district has not been systematically investigated. This knowledge gap is critical, as local data are essential for informing public health policies and for guiding appropriate antimicrobial prescribing. The lack of surveillance data in this region makes it difficult to assess the true burden of vancomycin resistance and to implement effective infection control measures. Understanding the local epidemiology is a fundamental step toward developing targeted strategies to combat the spread of these highly resistant pathogens, thereby protecting both individual patients and the broader community from the threat of untreatable infections.

Therefore, the objective of this study was to determine the prevalence of VRSA among *S. aureus* isolates from surgical patients in the District of Kohat and to analyze their antimicrobial susceptibility profiles.

Methodology

This cross-sectional study was conducted at the Pathology Department of Combined Military Hospital (CMH), Kohat, from July to December 2022. The study included surgical patients who developed postoperative infections. A total of 152 samples were collected, comprising pus, tissue, and wound swabs. The samples were processed and cultured on blood and nutrient agar at 37°C for 24 hours. The identification of *Staphylococcus aureus* was performed using a combination of microscopic and macroscopic examinations, including Gram staining and colony morphology. Confirmatory biochemical tests included catalase, coagulase, and DNase tests.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included oxacillin, erythromycin, clindamycin, vancomycin, linezolid, and others as per CLSI recommendations. The vancomycin susceptibility was specifically confirmed by the Minimum Inhibitory Concentration (MIC) test, with a cutoff of ≤2µg/ml. Isolates with an MIC of ≥4µg/ml were classified as vancomycin-resistant. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0.

Results

A total of 155 samples were collected, from which 80 isolates of *Staphylococcus aureus* were identified. The prevalence of *S. aureus* was found to be 53.94% (n=82/152). Among the isolated strains, a significant proportion were resistant to multiple antibiotics. Notably, 39% (n=32) of the isolates were identified as Methicillin-Resistant *S. aureus* (MRSA).

The study's primary objective was to assess vancomycin resistance. Of the 80 *S. aureus* isolates, 9 (11%) showed resistance to vancomycin. The Minimum Inhibitory Concentration (MIC) for these 9 isolates ranged from 4µg/ml to 8µg/ml. These findings indicate that Vancomycin-Resistant *S. aureus* (VRSA) is present in surgical patients in the Kohat district.

Figure. 4.1 Show the bacterial growth on nutrient agar

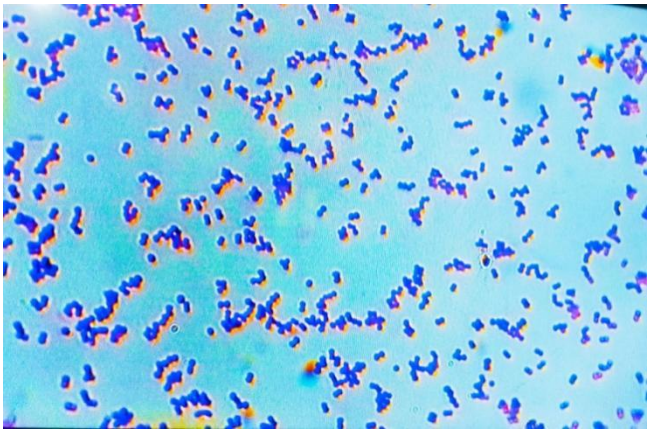
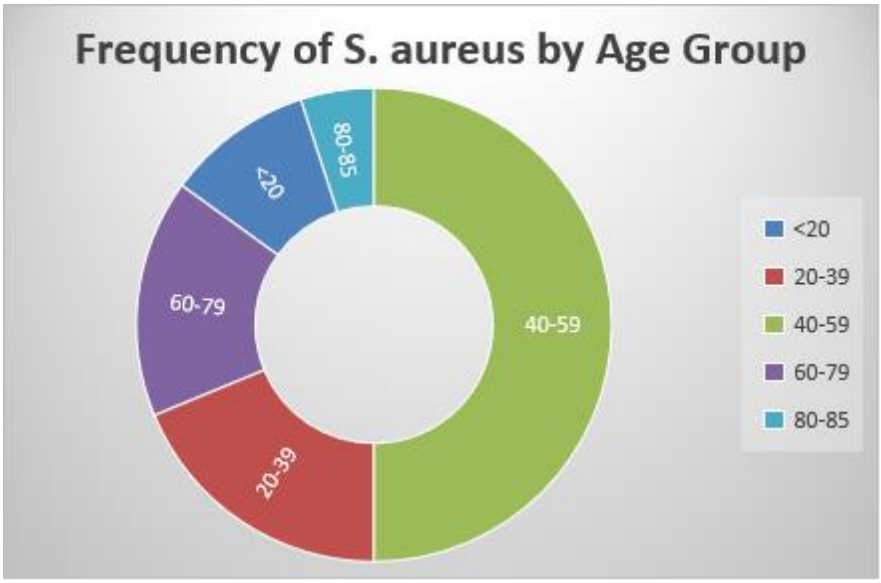


Figure. 3.2 Gram staining of S. *aureus*: Gram-positive *cocci* in grapes-like arrangement



4.3.1 Age Wise Distribution Of *S.aureus*

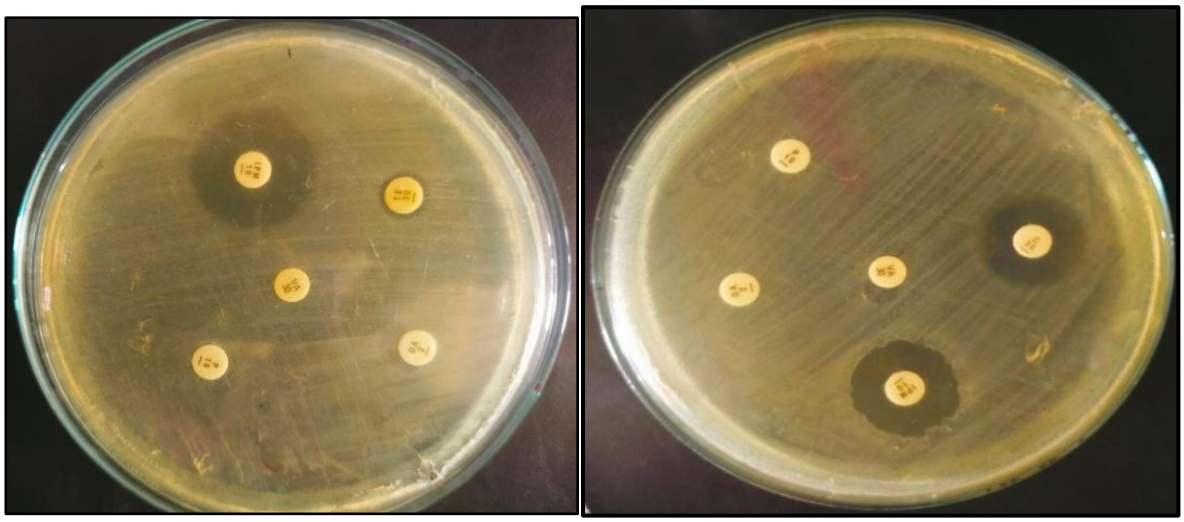


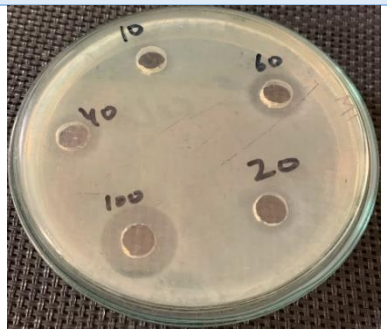
Figure 4.5. Show antimicrobial susceptibility testing of *S.aureus*

Show Drug resistance pattern of *S. aureus* isolates

Table I: Antibiotic Susceptibility Patterns of *S. Aureus*

Name of antibiotics	Sensitive	Intermediate	Resistant
Penicillin	42(38.8%)	08(7.4%)	58(53.7%)
Imipenem	100(92.5%)	08(7.4%)	00
Clindamycin	52(48.1%)	20(18.5%)	36(33.3%)
Tetracyclin	88(81.4%)	04(3.7%)	16(14.8%)
Chloramphenicol	48(44.4%)	04(3.7%)	56(51.8%)
Vancomycin	68(62.9%)	00	40(37%)

Table No 4.2. MIC Table of Vancomycin

MIC Values (ug/ml) For *S. aureus*					
MIC Values (µg/ml) For S. aureus					
Vancomycin ZOI & Concentration	10%	20%	40%	60%	100%
		≤16mm	17mm	≥20mm	≥22mm

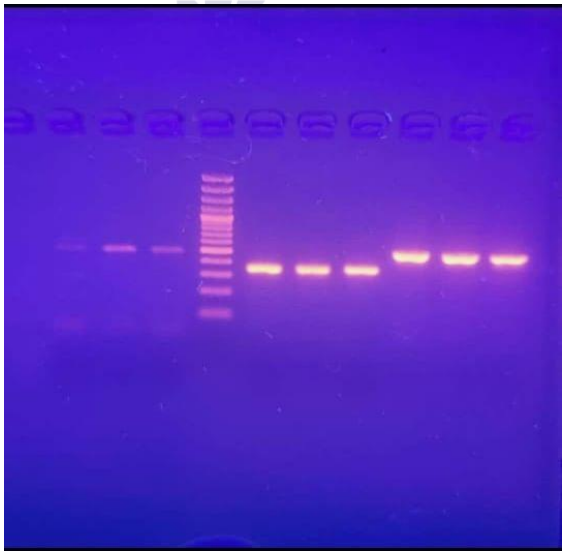


Figure. 4.6 Show Gel electrophoresis result

Discussion

The high prevalence of *Staphylococcus aureus* (53.94%) in surgical site infections observed in this study is consistent with global trends, where this pathogen remains a leading cause of nosocomial infections. A key finding of this research is the concerning rate of antibiotic resistance, particularly the 39% prevalence of Methicillin-Resistant *S. aureus* (MRSA), which aligns with recent studies from Pakistan that have reported a high burden of MRSA.

More critically, the study's finding of an 11% prevalence of Vancomycin-Resistant *S. aureus* (VRSA)

among the isolates is a significant and alarming discovery. While the MIC values for these strains ranged from 4µg/ml to 8µg/ml and are considered intermediate or resistant according to CLSI guidelines, their presence indicates a serious and emerging threat. This finding is particularly notable because VRSA has been reported as rare in Pakistan and this is one of the first studies to document its presence in the Kohat region. The emergence of VRSA is a direct consequence of the misuse and overuse of vancomycin, which is a last-resort antibiotic. This highlights the urgent need for a review of antimicrobial prescribing practices and the implementation of robust infection control protocols in local hospitals. The documented cases of vancomycin resistance present a grave challenge for clinicians, as treatment options for these patients are severely limited.

Conclusion and Recommendations

This study provides crucial data on the prevalence of antimicrobial resistance in surgical infections in the Kohat district. The high rates of MRSA and, more importantly, the documented presence of VRSA, underscore a critical public health issue. These findings necessitate immediate action to curb the spread of these multidrug-resistant pathogens. Based on these results, we strongly recommend the following:

1. **Strict Antibiotic Stewardship:** Hospitals should develop and enforce clear guidelines for the rational use of vancomycin and other potent antibiotics.
2. **Enhanced Surveillance:** Continuous surveillance is required to monitor the trends of antimicrobial resistance, particularly for vancomycin, in local healthcare facilities.
3. **Infection Control:** Stricter infection control measures, including hand hygiene and prompt isolation of infected patients, are essential to prevent the spread of resistant strains.

Limitations

This study has a few limitations. The sample size was relatively small, which may not fully represent the overall prevalence in the broader region. Additionally, the study was conducted at a single hospital, which might limit the generalizability of the findings to other healthcare settings. Further research with a larger sample size and a broader geographical scope is needed to better understand the true burden of vancomycin resistance in Pakistan.

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