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# VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCUS AUREUS: A COMPREHENSIVE STUDY

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**ABSTRACT**
**Objectives**
1. To identify antibiotics susceptibility pattern of Staph. aureus, focusing on the virulence factors contributing to its pathogenicity.
2. To assess the impact of antibiotic misuse on the emergence of antibiotic-resistant strains.

**Study Design:** Prospective cross-sectional design.
**Study Setting:** Institute of Medical Lab Technology Isra University Islamabad Campus in collaboration with Microbiology Department of Al Nafees Medical Collage & Hospital (ANMCH), Islamabad.
**Study Duration:** September 25th 2023 to January 31st, 2024.
**Sample Size:** Puss swabs were taken from 100 patients which were considered as positive for staph. aureus.
**Methodology:** Written informed consent was taken from patient visiting microbiology lab of Al-Nafees Hospital. Pus swab samples were collected from 100 individuals, and microbiological techniques were meticulously employed for the isolation and identification of *Staph. Aureus* strains. The assessment included the examination of key virulence factors such as coagulase, catalase, and toxins. Antibiotic susceptibility testing was conducted to determine resistance levels.
**Results:** The antibiotic susceptibility testing revealed varying degrees of resistance among the *Staph. aureus* strains. Notably, vancomycin and linezolid demonstrated the highest efficacy, while penicillin and amoxicillin exhibited lower effectiveness.
**Conclusion**: The research contributes valuable insights to the understanding of antibiotic resistance patterns, emphasizing the necessity for collaborative efforts in antimicrobial stewardship.

**Keywords:** Antibiotics Susceptibility, Antibiotics Resistance, Antimicrobial stewardship, *Staph. aureus*, Vancomycin, Coagulase and Gram Staining.

## INTRODUCTION

*Staphylococcus aureus* (*Staph. aureus*) is a Gram-positive bacterium that frequently inhabits the skin and mucous membranes of humans. It is predominantly found in the nasal passages but can also colonize the skin, intestines, and respiratory tract. [1] While often benign in healthy individuals, *Staph. aureus* is capable of causing a wide range of infections, from minor skin conditions to severe systemic diseases.[2] The pathogenicity of *Staph. aureus* is largely attributed to its ability to produce various virulence factors, including enzymes, toxins, and surface proteins, which enable it to evade the host immune system and persist within the host.[3]

One of the critical virulence factors produced by *Staph. aureus* is coagulase, an enzyme that converts fibrinogen to fibrin, forming a protective clot around the bacteria.[4] This mechanism is crucial for the bacterium's long-term colonization and infection persistence. Coagulase-positive strains of *Staph. aureus*is particularly associated with more severe infections such as sepsis and endocarditis, compared to coagulase-negative strains.[5]

Catalase is another significant enzyme that *Staph. aureus* employs to enhance its survival in the host environment. By converting hydrogen peroxide into water and oxygen, catalase helps the bacterium resist the oxidative burst produced by host immune cells.[6] Additionally, Staph. aureus produces several toxins, such as beta-toxin and delta-toxin, which contribute to tissue destruction and immune evasion. Beta-toxin is known for inducing tissue necrosis, while delta-toxin causes leukocyte lysis and tissue damage.[7]

The treatment of *Staph. aureus* infections are further complicated by the bacterium's resistance to multiple antibiotic classes, including beta-lactams, aminoglycosides, and macrolides.[8] The ability of *Staph. aureus* to develop resistance is primarily due to the misuse and overuse of antibiotics in both clinical and agricultural settings.[9] This selective pressure leads to the proliferation of antibiotic-resistant strains, posing a significant challenge for clinicians.

The rise of antibiotic-resistant *Staph. aureus* strains are a global public health concern. The misuse of antibiotics in both healthcare and agriculture significantly contributes to this problem. Inappropriate prescribing practices, such as the use of antibiotics for viral infections, accelerate the emergence of resistant strains.[10] Moreover, the widespread use of antibiotics in livestock production adds to the burden of resistance.

Healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) are the two main categories of MRSA strains. HA-MRSA is typically linked to infections acquired in healthcare facilities, while CA-MRSA is associated with infections in community settings.[11] Differentiating between these strains is crucial for developing effective treatment and infection control strategies.

Research from tertiary care hospitals in Egypt and Turkey reported MDR *Staph. aureus* prevalence rates of approximately 33% and 21%, respectively. The spread of MDR strains is driven by factors such as inadequate infection control measures and the global dissemination of resistant clones.[12] Addressing the issue of antibiotic resistance requires a comprehensive approach, including the judicious use of antibiotics, effective infection control measures, and the development of new antimicrobial agents.[13]

The Centers for Disease Control and Prevention (CDC) advocate for various strategies to control the spread of MRSA and similar strains.[14] These include hand hygiene, the use of personal protective equipment, thorough cleaning of patient care areas, and screening for MRSA colonization.[15] Additionally, the development of novel antibiotics is essential to ensure effective treatment options for patients with MDR Staph. aureus infections.

This study conducted at Al-Nafees Hospital in Islamabad aimed to evaluate the antibiotic susceptibility patterns of *Staph. aureus*, focusing on key virulence factors and the impact of antibiotic misuse. By analyzing pus swab samples and employing microbiological techniques, the study provided valuable insights into the evolving landscape of antibiotic resistance, emphasizing the need for continuous monitoring and adaptation of treatment strategies.[16] The findings underscore the importance of collaborative efforts in antimicrobial stewardship and public health interventions to combat the rise of MDR *Staph. aureus* strains.

## MATERIALS AND METHODS

This research employed a cross-sectional study design. The study is conducted at Islamabad Campus, in collaboration with the Department of Microbiology, Al Nafees Medical College and Hospital (ANMCH), Islamabad and carried out from September 25, 2023, to January 31, 2024. A total of 200 pus swab samples are included in this study. A non-probability convenient sampling technique is used to select the samples. Only samples showing growth of *Staphylococcus aureus* are included in the study and those samples that did not show growth of *Staphylococcus aureus* are excluded.

### Materials

Agar Plates are used for cultivating microorganisms, these plates provide a solid growth medium and the small, sterile paper discs infused with specific antibiotics, used to test bacterial susceptibility. A standardized agar medium for antibiotic susceptibility testing named Mueller-Hinton Agar is used along witha metal wire loop used to transfer microorganisms onto agar plates.A temperature-controlled chamber known as incubator is used for microbial growth.Zone of Inhibition Device is used to measure the diameter of inhibition zones around antibiotic discs as well as standard Interpretive ChartsGuidelines for interpreting antibiotic susceptibility test results.

### Methodology

### Ethical Considerations

Approval for the study was obtained from the Institute Review Board Committee of Al Nafees Medical College and Hospital, Islamabad.

### Data Protection

Collected data was stored on a password-protected personal computer, ensuring confidentiality.

### Collection of Specimens

Pus samples were collected from patients of both genders admitted to Al Nafees Hospital

### Inoculation of Samples

Samples were inoculated on blood agar, which serves as a nutrient-rich medium for the growth of *Staphylococcus aureus* due to its composition and provision of blood as a nourishing substrate.

### Preparation of Mueller-Hinton Agar

17 grams of Mueller-Hinton agar powder is weighted, 30 grams of peptone, and 3 grams of beef extract and dissolved in 1 liter of distilled water. The mixture is heated while stirring to dissolve completely, avoiding boiling and poured into autoclave bags or bottles and sterilize by autoclaving. Mixture was cooled to 45-50°C and poured into sterile Petri dishes.Allowed to solidify and store in a cool, dry place.

### Gram Staining

*Staphylococcus aureus* was identified using Gram staining. Bacterial cultures were spread on glass slides, air-dried, heat-fixed, and stained with gentian violet. The slides were then treated with Gram's iodine, decolorized with ethanol, washed, and air-dried. Under a microscope, Gram-positive *Staphylococcus aureus* appeared purple.

**Catalase Test**

The catalase test differentiates bacteria based on the presence of the catalase enzyme. *Staphylococcus aureus*, being catalase-positive, breaks down hydrogen peroxide into water and oxygen, observed as bubbling when hydrogen peroxide is added to the bacterial culture on a slide.

**Coagulase Test**

This test distinguishes *Staphylococcus aureus* from other staphylococci by detecting the coagulase enzyme. Both slide and tube methods were used, with clot formation in the test tube indicating a positive result for *Staphylococcus aureus*.

**Antibiotic Susceptibility Testing**

The Kirby-Bauer disk diffusion method was used for antibiotic susceptibility testing. Mueller-Hinton agar plates were prepared, and a standardized bacterial inoculum was spread over the plates. Antibiotic discs were placed on the inoculated agar.Sterile forceps were used to place antibiotic discs, including gentamicin (10µg), erythromycin (15µg), doxycycline (30µg), clindamycin (2µg), rifampin (5µg), linezolid (30µg), vancomycin (30µg), ciprofloxacin (5µg), penicillin (10µg), and oxacillin (30µg), onto the inoculated agar surface. After gentle pressing to ensure adhesion, the discs were spaced at least 24 mm apart to prevent overlapping zones of inhibition. and the plates were incubated at 35-37°C for 16-18 hours. Zones of inhibition were measured and interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

## RESULTS

**Measurement and Interpretation of Results**

The study conducted in 2023 rigorously evaluated the antibiotic susceptibility patterns of *Staphylococcus aureus* isolated from pus swabs at a tertiary care hospital in Islamabad. Utilizing data from the Clinical and Laboratory Standards Institute (CLSI), the research provides an in-depth analysis of various antibiotics' effectiveness, offering crucial insights into their performance in clinical settings. After incubation, zones of inhibition around each antibiotic disc were measured using a ruler. The sizes were compared to CLSI guidelines to classify each antibiotic as sensitive, intermediate, or resistant based on the measured zone diameters.

**Vancomycin's Remarkable Efficacy**

Vancomycin emerged as the most effective antibiotic, exhibiting a remarkable success rate of approximately 80%. This glycopeptide antibiotic's unique molecular structure and potent action against a broad range of bacterial strains make it a cornerstone in treating severe infections, especially those caused by resistant Staphylococcus aureus. The high sensitivity of Vancomycin reinforces its pivotal role in clinical practice, particularly for challenging and persistent infections (see Graph 1).

**Linezolid's Strong Performance**

Linezolid, a synthetic antibiotic, demonstrated a commendable success rate of approximately 78%, making it the second most effective antibiotic in the study. Linezolid's performance solidifies its position as a reliable treatment option, especially in scenarios where first-line antibiotics fail or are contraindicated. The study underscores Linezolid's importance as a critical alternative in the antibiotic arsenal, ensuring effective treatment against various bacterial infections (see Graph -1).

**Penicillin's Reduced Effectiveness**

Contrasting with the newer antibiotics, Penicillin showed a lower success rate of 70%. Despite its historical significance, this result highlights the diminished effectiveness of Penicillin in the face of emerging resistant strains of *Staphylococcus aureus*. The study emphasizes the need for cautious use of Penicillin, advocating for a more selective and judicious approach to its prescription. These finding calls attention to the evolving nature of antibiotic resistance and the necessity for ongoing reassessment of traditionally used antibiotics (see Graph 2).

**Amoxicillin's Suboptimal Performance**

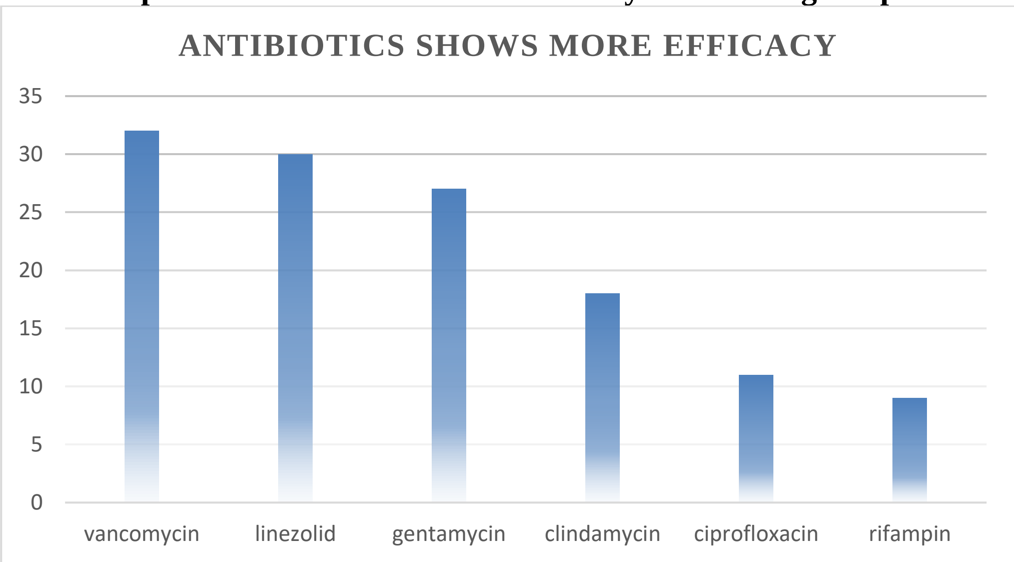
Amoxicillin, another widely used antibiotic, exhibited a success rate of 65%, positioning it as one of the less effective options in the study. This suboptimal performance prompts a reconsideration of Amoxicillin's use in certain clinical scenarios. The results highlight the importance of tailored antibiotic selection based on specific bacterial strains and their susceptibility profiles, advocating for more personalized treatment approaches to optimize patient outcomes (see Graph 2).

**Intermediate Response of Other Antimicrobial Agents**

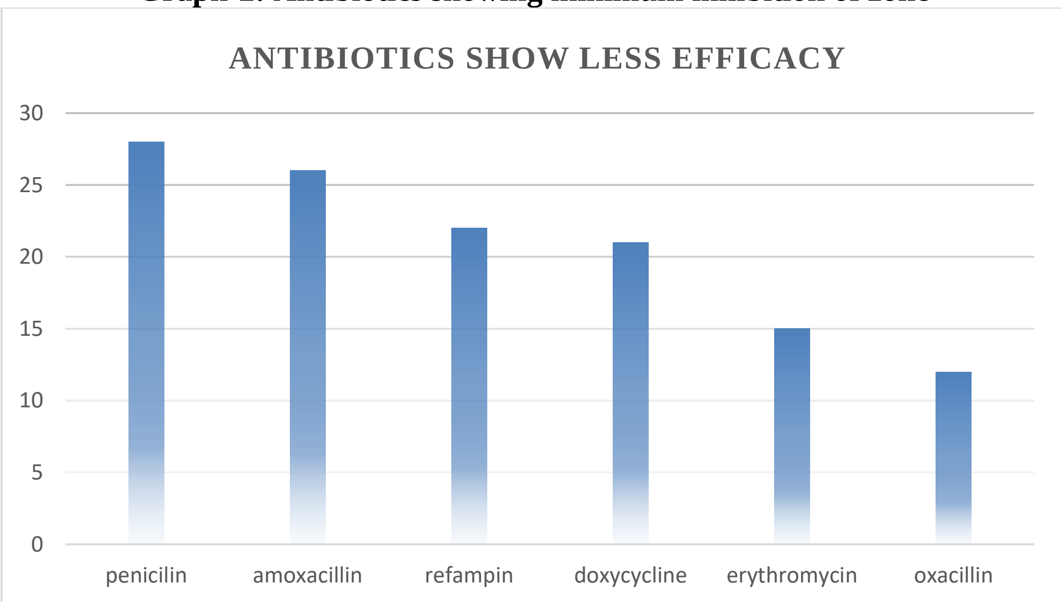
The remaining antimicrobial agents displayed an intermediate response against *Staphylococcus aureus*, with sensitivity levels ranging between full susceptibility and resistance. This nuanced finding underscores the complexity of bacterial interactions with antimicrobial agents and the necessity for individualized treatment strategies. Understanding these intermediate responses is crucial for optimizing antibiotic regimens, particularly in the context of evolving microbial resistance patterns.

**GRAPHS:**

**Graph-1**: **Antibiotics with their efficacy rates among samples**




**Graph-2: Antibiotics showing minimum inhibition of zone**



**Figures:**

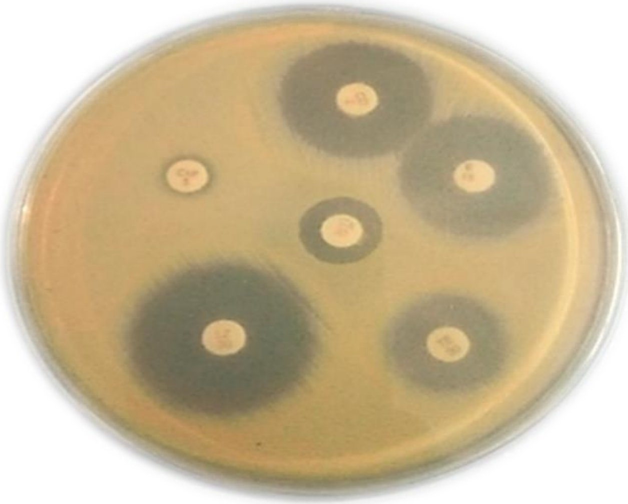
**Fig-1 Colonies of *Staph. aureus* on blood agar**

**Fig- 2:Hemolysis of *Staph. aureus* on blood ager**


**Fig-3:Positive Catalase test showing bubbles formation**

**Fig-4:*Staph. aureus* showing Coagulase positive test**

**Fig-5Antibiotics susceptibility patternof *Staph. aureus* on MHA**



## DISCUSSION

*Staphylococcus aureus* is a highly adaptable and resilient pathogen responsible for a wide spectrum of human infections, ranging from minor skin issues to severe, life-threatening conditions such as endocarditis and sepsis. The emergence of antibiotic-resistant strains has created a global health crisis, underscoring the importance of understanding the bacterium's antibiotic susceptibility patterns and pathogenic mechanisms.

In our study, we found that *S. aureus* demonstrated significant resistance to penicillin, with 95% out of 100 isolates showing resistance. This indicates that penicillin has become largely ineffective against this bacterium, reflecting a broader trend seen in previous studies where complete resistance to penicillin was observed.[17] Historically, penicillin revolutionized bacterial infection treatment, but its overuse has led to widespread resistance, making it nearly obsolete against *S. aureus* today.[18]

Similarly, our findings revealed that *S. aureus* exhibited resistance to amoxicillin in all cases studied. This aligns with other research showing high levels of resistance to both amoxicillin and ampicillin due to the production of β-lactamases by the bacterium.[19] This resistance complicates the management of *S. aureus* infections, indicating a pressing need for alternative treatment strategies.

Rifampicin, traditionally effective against various bacterial infections, was also found to be less effective against *S. aureus* in our study.[20] This reduced efficacy limits the available treatment options and raises concerns about the growing resistance.

On a more positive note, our study identified vancomycin as the most effective antibiotic against *S. aureus*, with 100% sensitivity observed. Vancomycin, a glycopeptide antibiotic, disrupts bacterial cell wall synthesis, proving its continued efficacy.[21] Additionally, linezolid showed significant effectiveness, suggesting it as a viable treatment option for *S. aureus* infections. Gentamicin, an aminoglycoside antibiotic that inhibits bacterial protein synthesis, also demonstrated substantial activity against *S. aureus*, ranking as the third most effective antibiotic in our study.[22]

Our findings, corroborated by other studies, emphasize the critical need for healthcare professionals to stay informed about antibiotic resistance patterns. This knowledge is essential for making informed treatment decisions and addressing the challenge of increasing antibiotic resistance in clinical settings.

## CONCLUSION

Recent research sheds light on the emergence of anti-infection resistance in *Staph. aureus*, a significant concern in the medical community.Penicillin, once a stalwart against *Staph. aureus* infections, now faces a substantial rate of resistance.Similarly, amoxicillin, ampicillin, and rifampicin demonstrate limited efficacy against *Staph. aureus* strains, emphasizing the pressing need for alternative therapeutic approaches.While some antibiotics retain effectiveness against *Staph. aureus*, the spectrum is narrowing.Vancomycin, renowned for its ability to disrupt bacterial protein synthesis, stands out as an effective treatment option against *Staph. aureus*.Linezolid and gentamicin also demonstrate responsiveness in combating *Staph. aureus* infections.However, addressing the challenge of antibiotic resistance in *Staph. aureus* demands ongoing global research efforts to ensure the availability of effective treatment strategies in the face of evolving resistance patterns.

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