Original Research Article   **Open Access**

PREVALENCE AND ANTIBIOTIC RESISTANT PATTERN OF STAPHYLOCOCCUS AUREUS IN CLINICAL ISOLATES FROM PESHAWAR: A SINGLE CENTER STUDY

KHAN WA[1], ULLAH J[*2], ULLAH MJ[2], ULLAH K[3], RABNAWAZ M[2], ULLAH I[2], AHMAD N[4], SHUMAILA[5], ULLAH A[2]

[1]*Pakistan Red Crescent Society, National Headquarter Islamabad, Pakistan*
[2]*Department of Allied Health Sciences, Iqra National University Peshawar Pakistan*
[3]*Department of Pathology, Aga Khan Hospital Karachi, Pakistan*
[4]*Department of Surgery, Lady Reading Hospital Peshawar, Pakistan*
[5]*Department of Health and Biological Sciences, Abasyn University Peshawar, Pakistan*
Correspondence Author Email Address: jamshidawar0071@gmail.com

Abstract *The health authorities recognize S. aureus as a severe public health problem because of its varied pathogenicity while antibiotics lose their effectiveness against this pathogen. The main goal of this research was to analyze S. aureus resistance patterns among hospital patients from Peshawar's tertiary care facility in Pakistan. The research work was carried out in the Pathology section of Rehman Medical Institute from July 2022 to December 2022. The study processed 400 samples were obtained. The research team performed morphological as well as biochemical identification of the isolates followed by antibiotic susceptibility testing through Kirby-Bauer disk diffusion testing methods. The positive results for bacteria reached 78.25% from 400 collected samples and S. aureus emerged as one of the 10% types of isolates found. The study showed resistance towards Cefoxitin, Levofloxacin, and Erythromycin 74.35%, 69.23%, and 64.10% respectively. The bactericidal activities of Vancomycin, Linezolid, as well as Rifampicin, demonstrated high efficiency in combating bacterial pathogens. The research demonstrates antibiotic-resistant S. aureus strains have appeared in this region demonstrating the necessity of enhancing antibiotic-use strategies and steady monitoring for S. aureus resistance patterns to stop global S. aureus infection threats.*

Introduction

Public health teams identify the gram-positive bacterium *Staphylococcus aureus* as an important threat because of its wide variety of infections that progress from skin surface infections to severe systemic diseases. Research indicates that 30% of people carry these bacteria without symptoms, yet they enable the bacterium to spread throughout healthcare facilities and community areas (Ahmad-Mansour *et al.,* 2021). The discovery of methicillin-resistant *S. aureus* (MRSA) in 1961 accelerated the problem of the bacteria developing into a significant pathogen that spread within hospitals and communities (Lawal *et al.,* 2021; Wu *et al.,* 2019). The resistance mechanism of MRSA arises from the mecA gene that produces the PBP2a protein, which makes the bacterium immune to β-lactam antibiotics. Medical treatment is challenging because resistance from patients surpasses 50% of treatments that use macrolides, fluoroquinolones, lincosamides, and aminoglycosides (Khatoon *et al.,* 2018; Zaky *et al.,* 2020).

The emergence of both VISA and VRSA strains of *S. aureus* creates advanced therapeutic hurdles for physicians (Lalitha *et al.,* 2020). The pathogenic capabilities of S. aureus intensify through biofilm formation because this mechanism helps the bacteria avoid immune response while blocking the entry of medication into the infection site (Lalitha *et al.,* 2020). Treatment of community-associated MRSA strains is challenging because these strains possess distinct virulence factors that make them difficult to combat (Hwang, 2023). Healthcare strategies require immediate approval of antimicrobial stewardship alongside constant monitoring of resistance patterns to reduce the global effects of *S. aureus* infections (Setiabudy, 2023).

Materials and Methods

The research followed a descriptive cross-sectional design conducted in the Pathology Department of Rehman Medical Institute (RMI) Peshawar from July 2024 to December 2024. All participants who had their blood samples tested in this department constituted the examined population. A total of 400

random blood cultures were collected and processed comprising patients of different ages, and both female and male patients. Individuals who received antibiotics before 72 hours of blood culture analysis were excluded from the experiment.

Specialized growth media within Bactec blood culture vials served as the collection medium for blood culture specimens. The laboratory followed standard protocols to create several bacterial culture media for identification purposes. Morphological identification was done by ZN staining while biochemical identification was performed by catalase, followed by tube and slide coagulase and DNase testing Figure 1. The Kirby-Bauer disk diffusion method was used to analyze antibiotic susceptibility according to the CLSI (2017) protocol Figure 2. A sterile saline suspension of bacterial colonies was used to prepare the Inocula until the solution reached a McFarland standard of 0.5. Antibiotic disks were applied to inoculated Mueller Hinton agar plates using an oxide antimicrobial susceptibility disk dispenser. Temperature-controlled conditions of 35-37°C maintained the plates for a timeframe extending from 18 h to 24 h. Bacterial sensitivity was determined by measuring inhibition zones using millimeter values.

Results

A total of n=400 single selected non-repetitive samples were collected to determine antimicrobial resistance in which 313 (78.25%) were positive and 87 (21.75%) showed no growth Figure 3. Male patients were 255 (63.75%), (p = 0.016) higher than female patients 145 (36.25%). The total staphylococcal positive samples in males and females were 27/255(10.58%) and 12/145(8.27%) shown in Figure 4. Both male and female patients were distributed in different age groups shown in Table 1 in which the highest number of patients that showed infections was under ten years. The pathogen distribution examined in this study displayed a substantial statistical difference (Chi-square = 73.1, p < 0.001) because *Salmonella Typhi* (14%) followed by skin contaminants (13%) and *Staphylococcus aureus* was (10%) Table 2. The susceptibility tests demonstrated Vancomycin's total effectiveness while Linezolid and Rifampicin showed excellent effects on the bacteria (100%, p < 0.001) Figure 5. The hospital needs to enhance antibiotic care management through strategic planning and ongoing antimicrobial resistance pattern monitoring due to emerging resistance against three important antibiotics: Cefoxitin (74.35%), Levofloxacin (69.23%), and Erythromycin (64.10%) respectively.
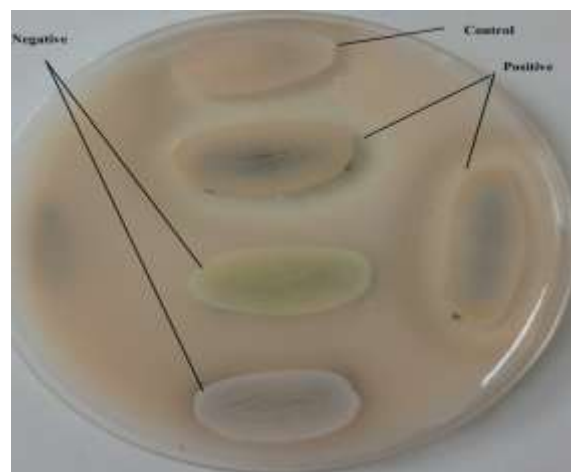
Figure 1. Positive and negative Staphylococcus aureus on DNase media

Figure 2. Antibiotic resistance behavior on Kirby-Bauer disk diffusion method
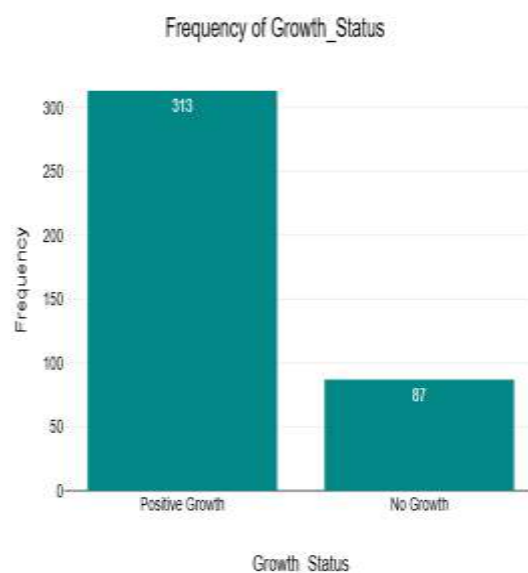
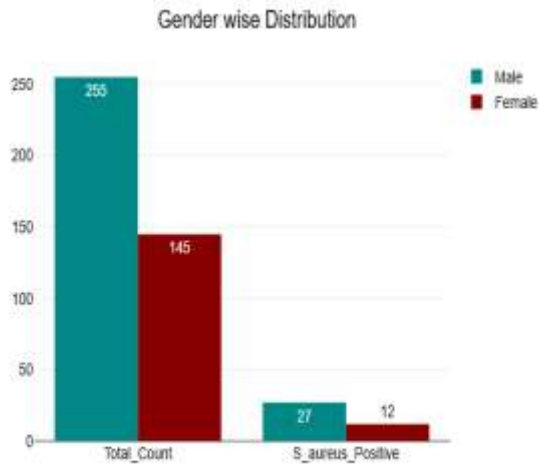**Figure 3. Frequency of positive and nagetive staphylococcal infection**

Table 1: Age wise distribution

Age Group	Count (%)
<10 Years	120 (30%)
11-20 Years	85 (21.25%)
21-30 Years	75 (18.75%)
31-40 Years	70 (17.50%)
>41 Years	50 (12.50%)
Total	400 (100%)

Table 2: Pathogen Distribution

Pathogen	Percentage
Skin contamination	13%
Salmonella Typhi	14%
Staphylococcus aureus	10%
Escherichia coli	9%
Others	54%
Total	**100%**

Figure 4. Gender wise distribution of culture positive and negative patients
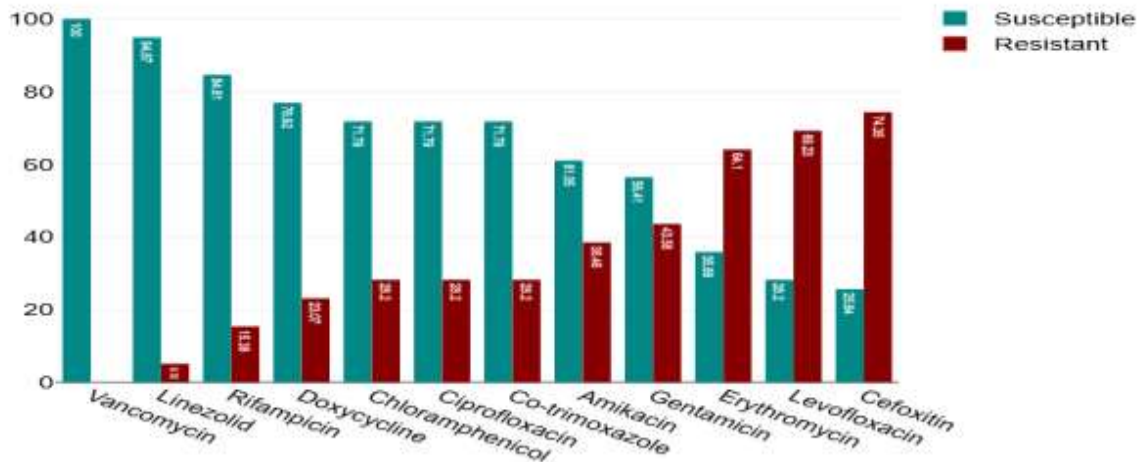

Figure 5. Antibiotic Susceptibility behavior on desk diffusion method

Discussion

A cross-sectional study performed at a Peshawar tertiary care hospital generated important data about bacterial pathogen occurrences as well as their antimicrobial resistance patterns for *Staphylococcus aureus*. A total of 78.3% of blood culture samples showed growth in the hospital along with S. aureus which emerged as one of the leading bacterial isolates with a frequency of 10% of the total samples. *S. aureus* is a major cause of bloodstream infections (septicemia) worldwide (Coombs and Others, 2020; Singh and Others, 2018). The studies show that pediatric patients under ten years old along with male patients make up the majority of reported cases therefore pediatric populations need specific intervention strategies.

Resistance testing demonstrated Vancomycin and Linezolid and Rifampicin maintained perfect effectiveness yet Cefoxitin presented moderate resistance (74.35%), while Levofloxacin showed 69.23% resistance and Erythromycin demonstrated 64.10% resistance to the bacteria. The research confirms previous studies showing increasing *S. aureus* isolate resistance in developing regions with active antibiotic misuse issues (Ansari and Others, 2014; Mnyambwa and Others, 2021). The study conducted by Naik *et al*., (2020). showed comparable findings to this research by reporting high resistance levels against standard antibiotic treatments thus demonstrating a critical need for proper antibiotic management practices (Naik and Others, 2020).

A critical healthcare concern about methicillin-resistant *Staphylococcus aureus* (MRSA) can be seen directly through the study results. Cefoxitin resistance serves as a public health warning since MRSA infections create higher mortality rates and worsen illness severity (Singh, 2018; Tarai *et al.,* 2013). Various investigations in different areas show that *S. aureus* clinical isolates commonly

demonstrate MRSA resistance at rates surpassing 50% according to scientific findings (Castro and Others, 2016; Mnyambwa and Others, 2021). The widespread occurrence of MRSA requires continuous monitoring to support best infection control practices in healthcare facilities.

The research demonstrates positive findings about the antibiotic efficacy of Vancomycin and Linezolid since these drugs function as primary options to address severe *S. aureus* infections that include MRSA. The increasing detection of vancomycin-intermediate *S. aureus* (VISA) strains in medical facilities represents an important threat to antibacterial treatment strategies according to research findings by Bhattacharya (Bhattacharya and Others, 2013). The antibiotic susceptibility patterns detected at Peshawar strongly emphasize ongoing antibiotic monitoring programs for developing treatment protocols that stop resistance strain spread.

The study results about male patient infections match findings in other studies which demonstrate gender-based differences in *S. aureus* infection rates. The research conducted by Kengne et al. (2020) supports this finding by demonstrating male patients experience increased numbers of *S. aureus* skin and soft tissue infections (Kengne and Others, 2020). The findings support several explanations about male behavioral patterns as well as health conditions that create susceptibility to infections.

The identification of *Salmonella Typhi* at 14% among the leading isolates demands improved diagnostic programs for handling different bacterial infections effectively. The detection of *Salmonella* in blood cultures requires urgent implementation of suitable antibiotic treatments because it shows evidence of serious systemic infections (Bessa and Others, 2013). Different pathogens identified in the same patient group demonstrate how difficult it is to control infections inside hospitals so medical practices must use personalized antibiotic choices based on what each microorganism can survive.

References

Ahmad-Mansour, N., Loubet, P., Pouget, C., Dunyach-Remy, C., Sotto, A., Lavigne, J.-P., and Molle, V. (2021). Staphylococcus aureus toxins: an update on their pathogenic properties and potential treatments. *Toxins* **13**, 677.

Ansari, S., and Others (2014). Threat of drug resistant Staphylococcus aureus to health in Nepal. *BMC Infectious Diseases* **14**, 157.

Bessa, L. J., and Others (2013). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *International Wound Journal* **10**, 49-54.

Bhattacharya, S., and Others (2013). Vancomycin intermediate Staphylococcus aureus isolated from a tertiary care hospital in Kolkata. *IOSR Journal of Dental and Medical Sciences* **5**, 192-193.

Castro, A., and Others (2016). Prevalence of Staphylococcus aureus from nares and hands on health care professionals in a Portuguese Hospital. *Journal of Applied Microbiology* **121**, 131-138.

Coombs, G. W., and Others (2020). Australian Group on Antimicrobial Resistance (AGAR) Australian Staphylococcus aureus Sepsis Outcome Programme (ASSOP) Annual Report 2018. *Communicable Diseases Intelligence* **44**, 18.

Hwang (2023). Comparing the Phylogenetic Distribution of Multilocus Sequence Typing, Staphylococcal Protein A, and Staphylococcal Cassette Chromosome Mec Types in Methicillin-Resistant Staphylococcus Aureus (MRSA) in Korea from 1994 to 2020. *Antibiotics*.

Kengne, I., and Others (2020). Antibiotic sensitivity profile of Staphylococcus aureus isolated from HIV/AIDS patients presenting with pyoderma, at the Yaounde Central Hospital, Cameroon. *Pan African Medical Journal* **37**, 185.

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T.-F., and Alarcon, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon* **4**.

Lalitha, A. V., Rebello, G., Chettri, S., and Reddy, M. (2020). Demographic and Clinical Profile of Invasive Staphylococcal Infections in Children Admitted to Pediatric Intensive Care Unit: A Retrospective Study. *Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine* **24**, 890.

Lawal, O. U., Barata, M., Fraqueza, M. J., Worning, P., Bartels, M. D., Goncalves, L., Paixão, P., Goncalves, E., Toscano, C., and Empel, J. (2021). Staphylococcus saprophyticus from clinical and environmental origins have distinct biofilm composition. *Frontiers in microbiology* **12**, 663768.

Mnyambwa, N. P., and Others (2021). Antibiotic Susceptibility Patterns of Bacterial Isolates from Routine Clinical Specimens from Referral Hospitals in Tanzania: A Prospective Hospital-Based Observational Study. *Infection and Drug Resistance* **14**, 2947-2958.

Naik, S., and Others (2020). Speciation and antibiogram of Staphylococcus isolated in a tertiary care centre. *Indian Journal of Microbiology Research* **7**, 84-89.

Setiabudy (2023). Biofilm Formation in Staphylococcus aureus and Coagulase-Negative Staphylococcus. *Folia Medica Indonesiana*.

Singh, A., and Others (2018). Prevalence and Antibiogram of Methicillin Resistant Staphylococcus aureus Isolates in a Tertiary Care Centre of Western Uttar Pradesh. *Journal of Medical Science and Clinical Research* **6**, 153-158.

Wu, M., Tong, X., Liu, S., Wang, D., Wang, L., and Fan, H. (2019). Prevalence of methicillin-resistant Staphylococcus aureus in healthy Chinese population: A system review and meta-analysis. *PLoS One* **14**, e0223599.

Zaky, A. I., Mahmoud, R. M., M Abdelraheem, W. M., and Zaki, S. H. (2020). Detection of Methicillin resistance of Staphylococcus aureus among Minia University Hospital Patients. *Minia Journal of Medical Research* **31**, 56-58.

Declaration

Acknowledgment

Author's contribution

WA K, and JU, conducted research and wrote the initial draft of manuscript. MJ, UA, S, MR, and IU, NA and AU collected the literature and wrote the manuscript, and edited the manuscript in original. All authors have read and approved the final manuscript. The author have read and approved the final manuscript.

Conflict of Interest

The authors state that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial or personal manner concerning the development of this project, the gathering of data, the interpretation of the data, or the writing and publishing of this paper.

Funding

Data Availability statement

All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

These aspects are not applicable in this paper.

Consent for publication

Not applicable