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MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM TERTIARY CARE HOSPITAL IN FAISALABAD

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ABSTRACT

Methicillin-Resistant Staphylococcus aureus (MRSA) is a major cause of healthcare-associated infections worldwide posing significant challenges due to its resistance to multiple antibiotics. This study focuses on the molecular characterization of S. aureus isolates collected from a tertiary care hospital in Faisalabad, Pakistan. A total of 200 clinical samples were obtained from various infection sites including blood, wound and skin lesions. The samples were cultured on Mannitol Salt agar. Phenotypically identification revealed that total 112 samples were declared as confirmed *S. aureus* colonies on the basis of gram stain and biochemical test. Antibiotic sensitivity testing (AST) performed by using Cefoxitin disc diffusion and modified Kirby-Bauer disk diffusion assays to assess the susceptibility pattern of isolated *S. aureus* against Methicillin and other antibiotics respectively. Out of which 55 (49.1%) were confirmed methicillin resistant strains. Molecular analysis was conducted to detect the presence of the mecA gene, a key determinant of methicillin resistance through PCR, DNA extraction and agarose gel electrophoresis techniques which suggested the prevalence of gene was (78.1%) in the methicillin resistant strains of *S. aureus*. The isolates showed significant resistance to frequently utilized antibiotics such as penicillin, cephalosporin and fluoroquinolones hence rendering these medications ineffectual for treating MRSA infections.

INTRODUCTION

Staphylococcus aureus (S. aureus) is a Gram-positive bacterium that causes a variety of infectious illnesses, including skin infections, bacteremia, endocarditis, pneumonia and food poisoning. *S. aureus* represents a significant human pathogen responsible for a diverse array of infectious diseases in both healthcare and community environment.[1]*S. aureus* possesses a range of different virulent strains that enable it to provoke infections in hosts and renowned for its capacity to develop resistance to multiple antibiotics. During last years, the circulation of Methicillin resistant Staphylococcus aureus (MRSA) strains has been reported worldwide. [2] The unique morphological traits of S. aureus facilitate its identification as it is a Gram positive cocci appears round under the microscope. The term 'Staphylococcus' originated from the Greek language combining the words for 'bunch of grapes' (staphyle) and 'berries' (kokkos).[3] The cells have a diameter that varies between 0.5 and 1.0 μm. S. aureus is a facultative anaerobe indicating that the bacteria may thrive in both oxygen rich and oxygen-deprived environments. The agar medium exhibited sizable colonies that were either yellow or white. It is shown that they synthesize carotenoids that are chemicals responsible for imparting a yellow hue to bacterial colonies. The antibiotic sensitivity profile of S. aureus is experiencing a dynamic shift sadly revealing a reduction in sensitivity over the previous few decades. Instead of increasing sensitivity S. aureus has gradually acquired resistance to many medications especially in the instance of methicillin-resistant Staphylococcus aureus (MRSA).[4] The overwhelming tendency is antibiotic resistance instead sensitivity. In the beginning S. aureus exhibited susceptibility to medicines such as penicillin and methicillin however resistance developed shortly after the medications were introduced.[5] MRSA infections can result in many severe and sometimes fatal consequences mostly owing to its resistance to methicillin and other conventional treatments.[6] The ability of S. aureus to colonize in the skin and mucous membranes especially in the nasal passages make it possible for the bacteria to enter the body through wounds, incisions or surgical sites.[7] The prevalence and persistence of MRSA in hospital settings are highlighted by the global epidemiology which suggests hospital-acquired MRSA (HA-MRSA) a serious public health issue.[8]HA-MRSA is a major contributor to morbidity, mortality and healthcare expenses worldwide continues to rank among the most prevalent infections. The prevalence of HA-MRSA varies in developed countries like the US and many European countries but it typically accounts for 20–50% of all hospitalized S. aureus infections.[9]

RESEARCH METHODOLOGY

Before conduct the study official permission was sought from the Institutional Biosafety and Bioethics Committee (IBBC) of the Riphah International University, Faisalabad (RIUF)and Allied Hospital Faisalabad and took permission to enroll the case. It was Cross-sectional study. The study duration was 6 months from 01 July 2024 to 01 December 2024. A total of 200 different clinical samples including blood samples, wound swabs and skin lesions were collected. Clinical samples were collected at Allied Hospital Faisalabad and analyzed at Meezan Laboratory, Faisalabad.

Data collection method

Sample collection was executed carefully using regulated standards to assure the reliability and authenticity of the specimens obtained. All clinical specimens including blood samples, skin lesions, and wound swabs, were collected from different wards of Allied hospital including Emergency wards, Neonatal Intensive Care Unit, Medical wards and Surgery wards. All clinical samples were delivered into the Laboratory to commence the workflow.[10] The sample processing technique employed in the investigation formulated to guarantee precise detection of pathogens found in the blood specimens obtained from study participants.[11]

Procedure

For the filling of electrophoresis chamber sufficient amount of 0.5 X TAE buffer was used.1g of agarose powder weighed with the help of weighing balance dissolved in 100 ml of 0.5 X TAE buffer in conical flask and heated in

microwave oven for at least 2 minutes.[12] Casting tray with suitable comb was applied. Agarose gel mixture was set with room temperature and 5µl of ethidium bromide was added in the conical flask act as a dye. Agarose gel mixture was added in the electrophoresis tray carefully and removing all the bubbles. Then tray with solidified gel putted into the electrophoresis tank filled with TAE buffer. [13]10µl of DNA products were added in the wells along with ladder in one well. Electric current was applied to the chamber with the set requirements of 100V temperature and for 40 minutes.[14] With the passage of time movement of DNA proteins were seen with assisted by the dye moving from cathode towards anode pole. After the procedure completed results were seen and gel was observed under the gel documentation system.[15]

Interpretation

Typically ladder is use as the molecular weight marker which helps to analyze the size of DNA fragment. Positive control of MRSA was used with mecA gene and negative control of MSSA without mecA gene.

Normally band size of mecA gene is 500-700 (basepair) bp indicated presence of mecA gene while when there is absence of band indicated absence of mecA gene.

Data analysis

Data entry and analysis were done using the Statistical Package for Social Sciences (SPSS) version 26 IBM and Microsoft Excel.

RESULTS&DISCUSSION

Sample distribution

Total 200 samples collected from different wards of Allied hospital including males and females. Out of 200 blood samples 43, skin lesions 79 and wound swabs 78 were collected. Patients were distributed among gender, age and residential background. Total 112 confirmed *S. aureus* were detected on the basis of biochemical testing profiles including catalase and coagulase testing which was followed by Antimicrobial Susceptibility testing (AST). Out of which 55 were confirmed methicillin resistant *S. aureus* isolates. For molecular characterization of MRSA was done to detect the mecA gene prevalence in the isolates by followed DNA extraction, Polymerase Chain Reaction and Agarose Gel Electrophoresis. Results revealed that out of 55 isolates 43 isolates were mecA gene.

Demographic Data Findings

The analysis of demographic data is essential for interpreting the clinical epidemiology, distribution as well as incidence rates caused by MRSA infections. The results generally emphasize 36 patient age, gender, medical manifestations, previous medical history and settings for health care.

Gender group distribution of participants

The distribution of men and women in the sample of 200 participants indicates that 110 (55%) were male and 90(45%) were female. This signifies a higher percentage of males were the participated in study. The gender distribution of study participants is presented in following Table1.

Table 1 *Gender distribution of patients included in the study*

Gender distribution	Male	Female
Frequency	100	90
Percentage	55%	45%

Clinical samples including blood samples, wound swabs and skin lesion collected from males and females from different wards of Allied hospital Faisalabad. Total 43 blood samples were collected from NICU and emergency ward including 30 males and 13 females. Total 79 skin lesion samples collection were conducted in medical, surgical and OPD patients including 58 females and 21 males. In case of wound swabs total 78 samples were collected from emergency ward, medical and surgical ward including 59 males and 19 females.

Age distribution of participants

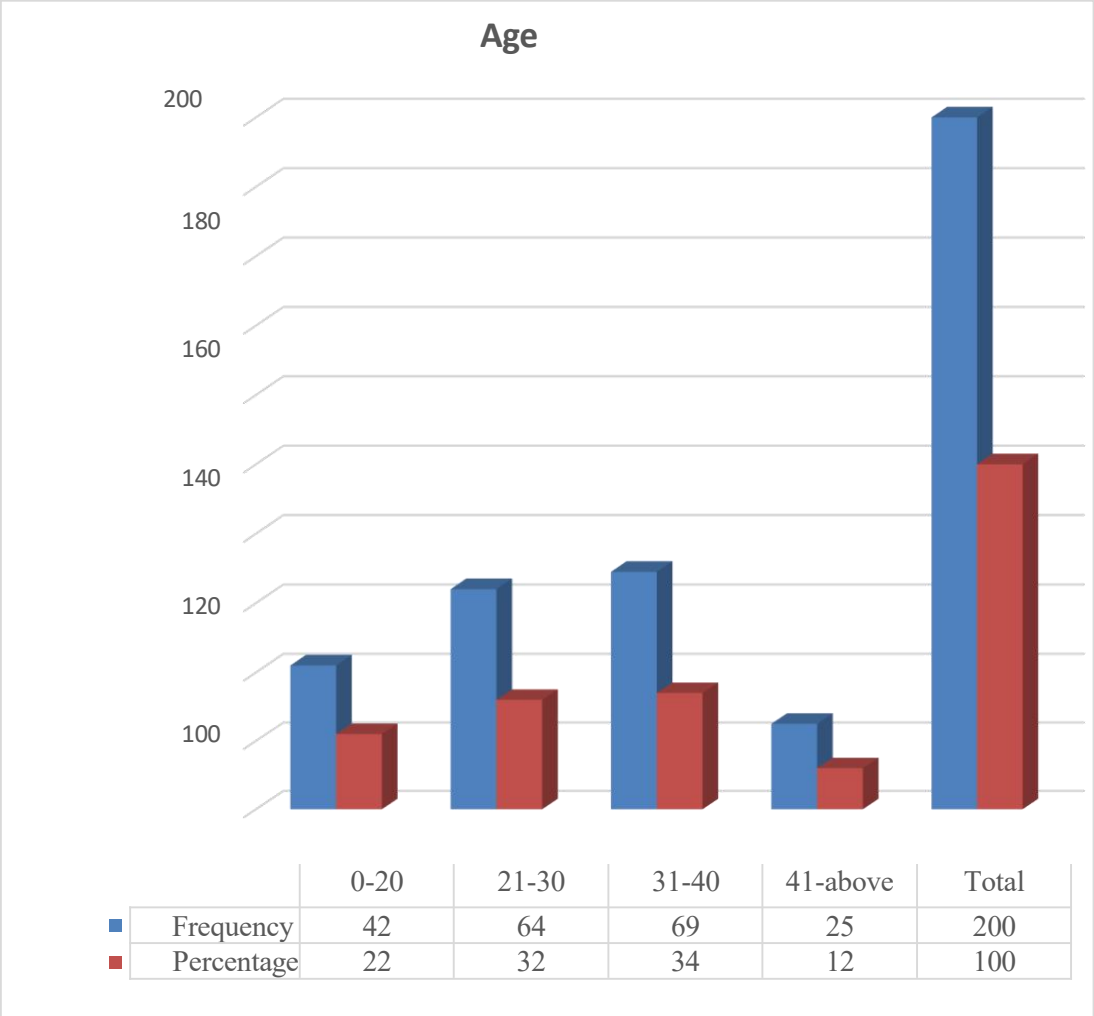
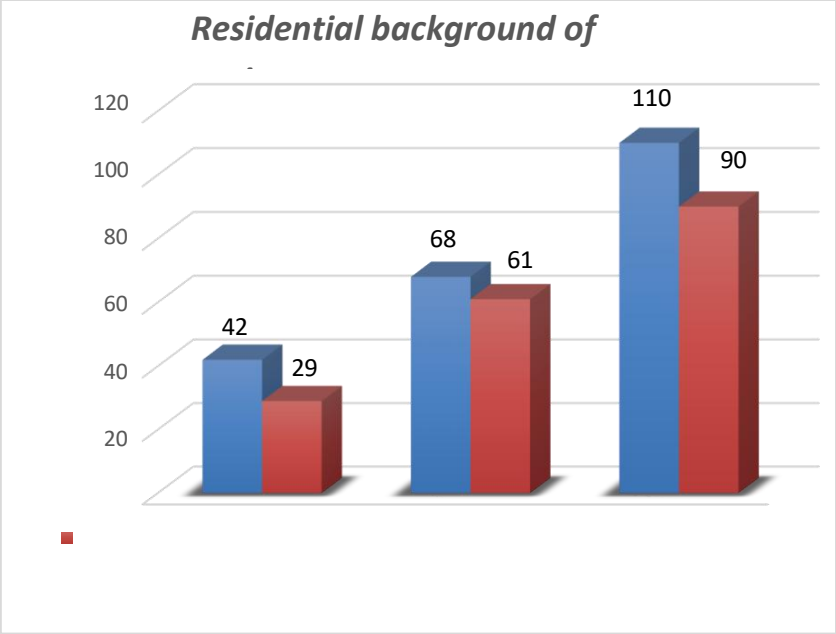


Figure 1 *Age-wise distribution of patients*

Residential Background of Participants

A total of 200 patients were included in the study 129 residing in urban areas and71 residing in rural areas. The distribution of study participants according to location is presented in Figure 2.



Sample distribution of participants

Sample collection from the participants demonstrates the types of specimens retrieved for *S. aureus* detection along with their frequencies and corresponding percentages mention in below figure 3.

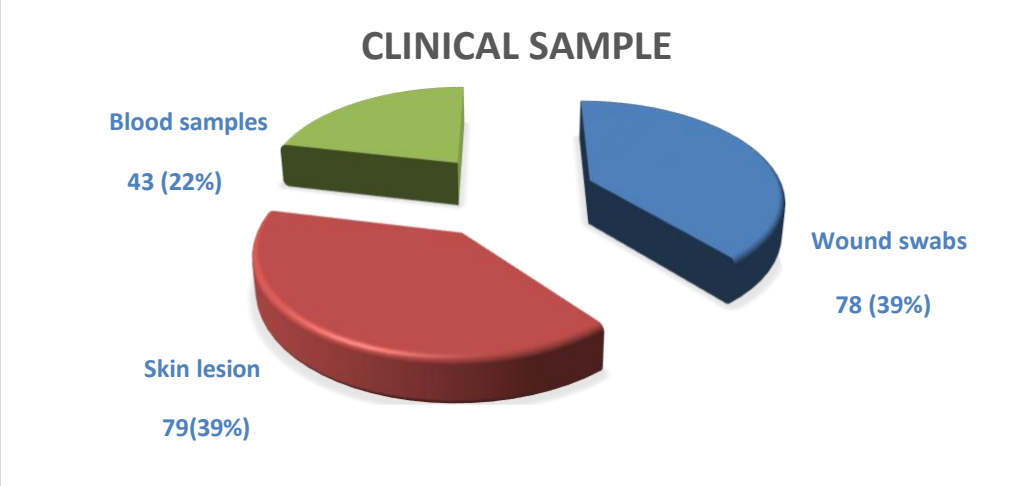


Figure 3 *Distribution of various clinical specimens*

Prevalence of *S. aureus* among clinical samples

Prevalence of *S. aureus* among clinical samples were analyzed. Among the200allclinical samples analyzed by culturing on Mannitol salt agar 160 (80%) samples showed microbial growth while 40 (20%) samples exhibited no growth. 130 samples revealed the characteristics of *Staphylococcus* species including yellow colonies with mucoid and clear appearance after incubation period of culture plates

Antibiotic Sensitivity Patterns in *S. aureus*

Evaluating the antibiotic sensitivity profiles of *S. aureus* is crucial for optimal clinical management and infection control.

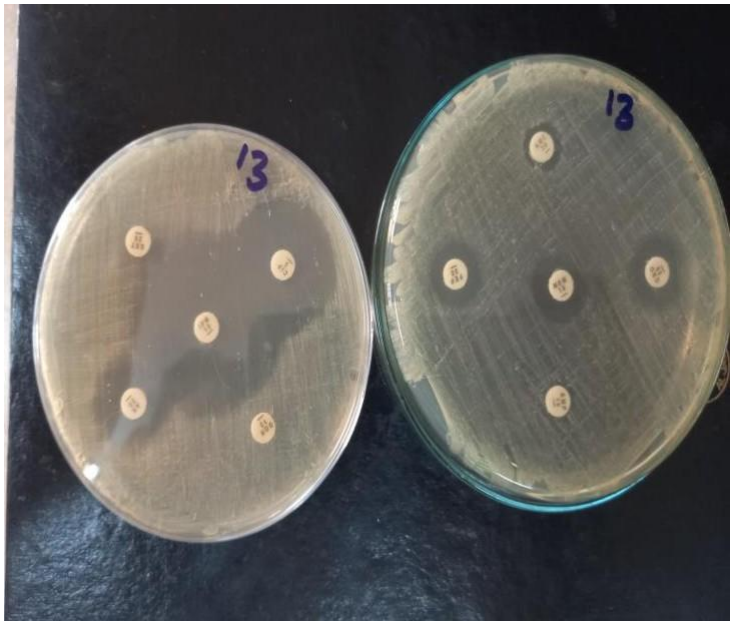


Figure 4 Inhibition zones of tested antibiotics against *S. aureus* isolates

Recognizing these sensitivity patterns is essential for directing suitable antibiotic therapy especially in situations necessitating prompt intervention in order to ensure patient survival (Martin, 2023).

Table 2 *Antibiotic sensitivity pattern of S. aureus*

Antibiotics	Resistance	Intermediate	Sensitive	Total
Chloramphenicol	40 (35.71%)	27 (24.10%)	45 (40.70%)	112 (100%)
Cefoxitin	55 (49.10%)	37 (33.03%)	20 (16.07%)	112 (100%)
Levofloxacin	53 (47.32%)	20 (17.85%)	39 (34.82%)	112 (100%)
Erythromycin	34(30.35%)	23 (20.53%)	55 (49.10%)	112 (100%)
Clindamycin	43 (38.39%)	28 (25%)	41 (36.60%)	112 (100%)

Ciprofloxacin	45 (40.17%)	14 (12.5%)	53 (47.32%)	112 (100%)
Gentamycin	47 (41.96%)	30 (26.78%)	35 (31.25%)	112 (100%)
Sulfamethoxazole	49 (36.60%)	17 (15.18%)	54 (48.2%)	112 (100%)
Doxycycline	44 (43.75%)	21 (18.7%)	42 (37.7%)	112 (100%)
Vancomycin	33 (34.8%)	18 (16.0%)	55 (49.1%)	(100%)

Prevalence of Methicillin Resistant *S. aureus* (MRSA) isolates

Prevalence of MRSA isolates was calculated by analyzing the susceptibility of the, isolates against cefoxitin (30µg). Out of 112 isolates 55 isolates were marked as methicillin resistant *S. aureus* isolates on basis of this testing and results are presented in following figure 4.5. The European Antimicrobial Resistance Surveillance Network (Ayobami et al., 2020) reported a total incidence of 16.4% in Europe varying from under 1% in Scandinavian countries to around 30% in Greece and Italy with decreasing trends in numerous regions attributed to rigorous infection control measures. A United States study (CDC, 2020) indicated that MRSA accounted for 43% of invasive *S. aureus* ailments predominantly the *mecA* gene in community- associated instances. These findings emphasize the global heterogeneity in MRSA frequency and reinforce the necessity for comprehensive infection control and surveillance techniques to mitigate the dissemination of MRSA.

Figure 5 *Prevalence of MRSA isolates*

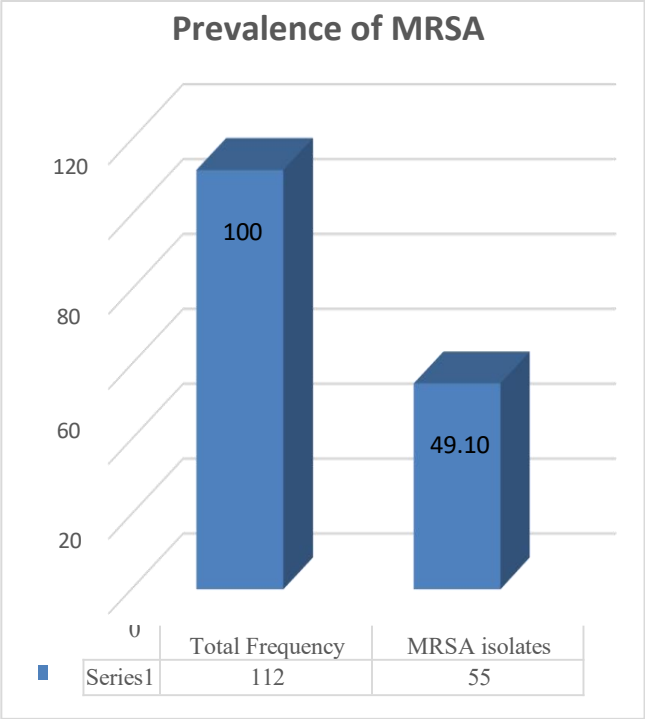


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Figure 6 Positive test for MRSA isolates with cefoxitin

Prevalence of mecA gene in MRSA isolates

A total of 55MRSA isolates were tested for the occurrence of *mecA* gene. Total 43 isolates of MRSA were marked positive for occurrence of *mecA* gene and 12 isolates of MRSA were marked as negative *mecA* gene isolates results are represented in below Table 4.4.

Table 3 *Prevalence of mecA gene in MRSA isolates*

Presence of gene	Percentage
Positive mecA gene	43 (78.1%)
Negative mec Agene	12 (21.8%)
Total MRSA Isolates	**55 (100%)**

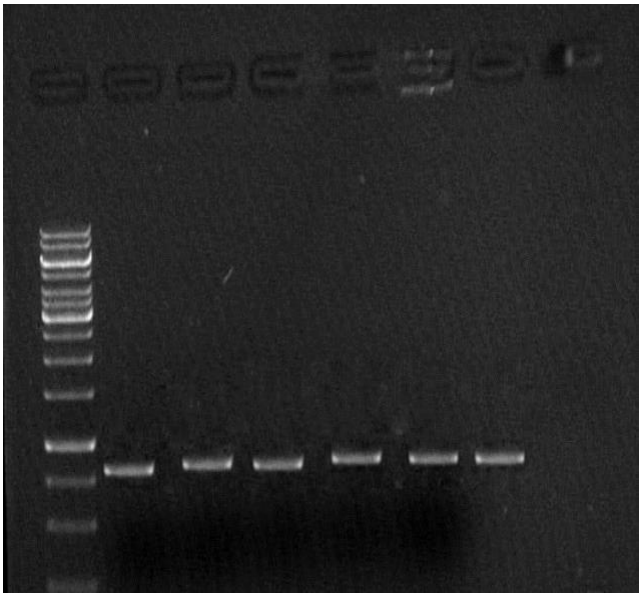

Figure7 *Gel electrophoresis of mecA gene in MRSA isolates*

Conclusion

The study found a high incidence prevalence of MRSA in the selected tertiary care hospital and showed the extent to which MRSA affects the clinical settings. About half of the S. aureus isolates were reported as methicillin-resistant indicating that these are still hard to contain infections in healthcare facilities. Due to its high prevalence, the need for reporting of infection 59 control measures and constant monitoring of antibiotic resistance is established. The research from this paper done under demographic study showed that the MRSA infections appeared to be higher among male patients than among the female patients suggesting that there could be differences in the incidence rates or hospitalization rates or risk factors. The analysis of age distribution profile showed that the MRSA was more common in patients of 31–40 years age group and the second common in 21–30 years age group. Those patients who were 40 years old and above had a high incidence of MRSA cases based on previous immunizations and contact with healthcare services. Furthermore, MRSA affected people in the metropolitan areas more than people in the rural areas probably because of frequent hospitalizations, frequent invasive procedures and probably because of increased exposure to MDOs. Molecular typing revealed a diverse spectrum of MRSA genotypes suggesting the presence of clonal expansion within the hospital and the emergence of the new strains from outside sources. This variety underlines the complexity of MRSA transfer and the need in frequent molecular sample investigation to control the outbreaks. Pattern of resistance to the antibiotics under test was also revealed using antibiotic susceptibility tests. The isolates have exhibited a very high degree of resistance towards commonly used antibiotics like the penicillins, cephalosporin, fluoroquinolones and macrolides and therefore that class of medications is useless when it comes to treating MRSA infections. However, aminoglycosides and tetracycline was identified to have medium resistance level hence limiting the treatment options. Significantly minimal resistance was detected against last resort medicines such vancomycin, linezolid and daptomycin which continue to be effective but must be utilized cautiously to avert the emergence of resistance. Multiple risk factors for MRSA infections were discovered including extended hospitalizations prior antibiotic use and hospitalization to ICUs. These variables enhance exposure to antibiotic-resistant strains and underscore the necessity for tailored infection control measures in high risk regions. The study also demonstrated indications of nosocomial transmission identifying healthcare professionals, invasive devices and insufficient infection control procedures as possible carriers for MRSA dissemination inside the hospital setting. This research emphasizes the complex patterns of MRSA infections and the immediate necessity for a multidisciplinary strategy to combat antibiotic resistance. Cooperation among healthcare practitioners, microbiologists, epidemiological experts and officials are crucial for formulating and executing effective MRSA control plans. Outreach efforts and educational programs can significantly improve community involvement in addressing antibiotic resistance. 60 By implementing a holistic strategy that encompasses monitoring, prevention and awareness rising in healthcare organizations can lessen the effects of MRSA and aid in worldwide initiatives to address the escalating challenges of antibiotic resistance.

Recommendation

Further studies towards the subject of MRSA should further aim on such particular areas of concerns to enhance therapeutic modalities for the illness and deal with resistance. To define how MRSA evolves resistance to drugs new resistance mechanisms must be

discovered through genetic studies. The findings of this research may help in the identification of new molecular agents for pharmacological intervention and ideas on how to overcome present therapy challenges. In addition, studies comparing outcomes of combination and other form of treatments are essential as a way of improving medical outcomes especially in reports of resistance or severe infections. Using less known forms of medication such as bacteriophage treatments, antimicrobial peptides or new drug cocktails may give sound tactics to deal with uncompromising MRSA strains. Further, the development of the rational approach to the problem of MRSA using the genetic data of the bacterium and patients may help to choose the most effective therapy regime concerning each case. The combination of these research areas will improve the approach to MRSA infection and reduce the future burden of resistance

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