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Comparative Analysis Among MSSA and MRSA: Association with Demographic Factors and Mannitol Fermentation Characteristics

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Abstract - ***Staphylococcus aureus*** **is a pathogen of clinical significance that causes a broad range of infections, and the appearance of methicillin-resistant strains has become a significant therapeutic problem. The current research focused on the comparative analysis of the methicillin-resistant** ***S. aureus*** **(MRSA) and methicillin-sensitive** ***S. aureus*** **(MSSA) isolates regarding the demographic features and the mannitol fermentation profiles. Forty eight clinical isolates were examined by conventional microbiological techniques, such as Mannitol Salt Agar (MSA) and biochemical identification. Methicillin resistance was identified by cefoxitin disc diffusion method under the Clinical and Laboratory Standards Institute (CLSI) guidelines. Out of the isolates, 39 (81.25) were MRSA with only 9 (18.75) being MSSA. The results show that most of the patients in the study are dominated by MRSA. Whilst the majority of isolates fermented mannitol, some of the MRSA strains had non-fermenting behavior. Demographic determined that prevalence was higher in the age group (21-40 years old) with minor male preponderation. The research results in a conclusion that though MSA can help in the initial diagnosis of S. aureus, it cannot be depended upon to distinguish MRSA and MSSA. The constant monitoring with the application of standardized antimicrobial susceptibility testing is crucial to the infection control.**

Keywords: MRSA, MSSA, mannitol fermentation, Mannitol Salt Agar, cefoxitin disc diffusion, Clinical and Laboratory Standards Institute.

I.INTRODUCTION

Mannitol fermentation is one of the fundamental phenotypic characteristics typically employed during the primary identification of the Staphylococcus aureus in clinical microbiology (Forbes et al., 2016). The high salt content of Mannitol Salt Agar (MSA) is a selective and differential medium because high levels of sodium chloride (7.5%), inhibit the growth of most non-staphylococcal organisms but allows the growth of halotolerant staphylococci (Winn et al., 2017). Differentiation is performed on the fermentation capability of the organism using mannitol, leading to the formation of an acid and the change of the phenol red indicator to yellow (Forbes et al., 2016). Traditionally, *S. aureus* can be viewed as a reliable fermenter of mannitol, but the recent reports about atypical non-fermenters have also emerged as a significant public health issue in the world (Becker et al., 2014).The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the significant global concerns in terms of public health (Lee et al., 2018; Chambers et al., 2009). The main mechanism of resistance to b-lactam antibiotics in MRSA is the presence of the mecA gene, which encode a modified penicillin binding protein (PBP2a) with a decreased affinity of b-lactam antibiotics (Hiramatsu et al., 2002; Peacock et al., 2015). This resistance mechanism is a significant obstacle to the range of therapeutic options and leads to the increasing morbidity, mortality, the increase of the length of hospitalization, and the rise of healthcare expenses (Lowe, 1998; Tong et al., 2015). Methicillin-sensitive *Staphylococcus aureus* (MSSA), in its turn, is sensitive to b-lactam antibiotics and is typically linked to better clinical outcomes (David et al., 2010). Thus, rather precise discrimination of MRSA and MSSA is essential to the proper antimicrobial treatment and control of infection (CLSI, 2023; Brown et al., 2005).Phenotypic tests, including the use of cefoxitin disc diffusion test suggested by the Clinical and Laboratory Standards Institute (CLSI) are popular to identify methicillin resistance accurately (CLSI, 2023; Brown et al., 2005). Other than microbiological

factors, demographic influences such as age and gender are also an etiological determinant of the epidemiology and dynamics of S. aureus infection transmission (Grundmann et al., 2006). The differences in the prevalence of MRSA and MSSA among populations are due to differences in host immunity, occupational exposure, healthcare contact, and behavior (David et al., 2010). Some studies have found an increased prevalence of MRSA rate amongst the young and middle aged adults with little gender dominance, especially in the age bracket of 21-40 years (Grundmann et al., 2006; David et al., 2010). These demographic connections are useful for identifying high-risk populations and developing targeted infection control strategies. Even though the most common method of preliminary identification of S. aureus is mannitol fermentation, its application in differentiating between MRSA and MSSA is limited, and both phenotypes can share the same fermentation patterns (Becker et al., 2014). Additionally, the relationship between the phenotypic traits, resistance patterns towards antimicrobials and demographic variables are not fully elucidated in most clinical settings (Lee et al., 2018). Through this, the given paper will compare MRSA and MSSA isolates in their fermentation of mannitol and demography besides establishing the suitability of the traditional approaches to phenotypic analysis in distinguishing these clinical pathogenicity agents. Such an integrated strategy will give essential information regarding accuracy of diagnosis, resistance. trend patterns, as well as the epidemiological patterns of the Staphylococcus infection with aureus.

II.MATERIAL AND METHODS

The isolates of *Staphylococcus aureus* were examined in order to test whether it could ferment mannitol, withstand methicillin, or demographically. Out of the 48 unique clinical isolates collected, they were of various sources such as wound swabs, pus and soft tissue infections. Only confirmed strains of Staphylococcus aureus were tested; there were no contaminated and duplicate samples. The isolates were identified with the help of the standard microbiological tests, including Gram staining, catalase, coagulase tests (Forbes et al., 2016; Winn et al., 2017). Mannitol Salt Agar (MSA), which is both selective and differentiating, was used to examine the mannitol fermentation (Winn et al., 2017). Pure cultures were inoculated on MSA and left to incubate under aerobic conditions between 18 to 24 hours in 37 degrees Celsius temperature. The fermentation of mannitol was indicated by the change of color of the phenol red indicator. According to Forbes and colleagues (2016), yellow was positive fermentation as a result of acid production, and red or pink was negative. This approach was an initial test to investigate the spectrum of fermentation capabilities of isolates. Methicillin resistance was determined based on the cefoxitin disc diffusion technique according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2023; Brown et al., 2005). Bacterial suspension that corresponded to 0.5 McFarland turbidity standard was taken to ensure even growth of bacteria in agar plates. They incubated the plates at temperatures of 35 to 37 degrees Celsius 18 to 24 hours after placing a cefoxitin disc (30 ug) on the plate. The inoculated surface was then covered and the zone of inhibition was recorded. Other isolates exhibited a zone diameter of 21 mm, which is a sign of methicillin resistant *Staphylococcus aureus* (MRSA). Some measured 22 mm and this was characteristic of Methicillin-sensitive *Staphylococcus aureus* (MSSA), as reported by CLSI in 2023. The demographic data collected included the age and gender in a study to investigate whether there are any patterns between MRSA, MSSA, and mannitol fermentation. Descriptive statistics were used to express this data in percentages and frequencies. Comparison of results was based on resistance pattern, fermentation characteristics and demographics through the use of tables and graphs to make the findings more understandable. To control the quality of the study, it was necessary to comply with all the lab protocols and ensure proper incubation conditions as recommended by Forbes et al., 2016.

III.RESULT

Mannitol Fermentation

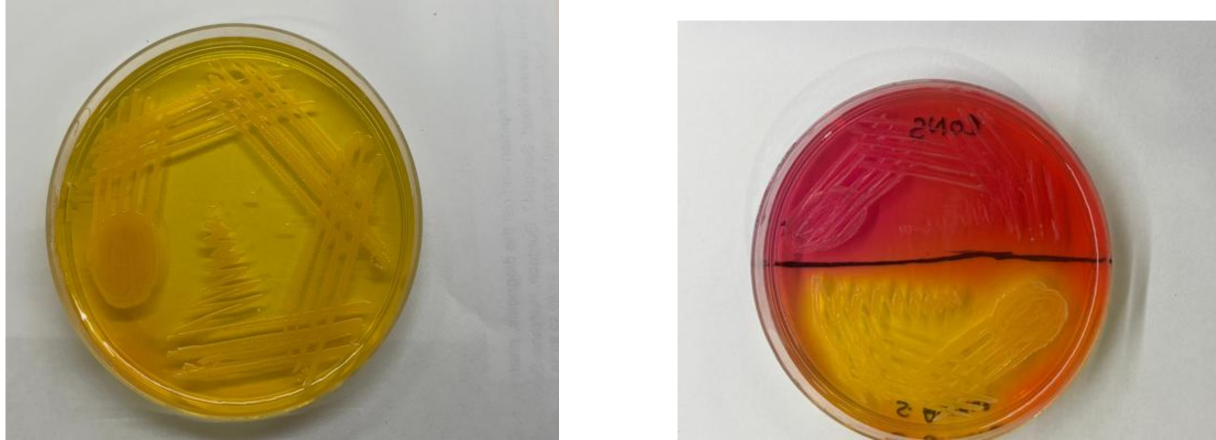

Figure 1: MSA plates displaying yellow mannitol coloniesred /pink colonies for non-fermenting isolates and positive isolates

This (Figure 1) shows a Mannitol Salt Agar (MSA) plate that indicates a clear demonstration of mannitol fermentation. Based on the features of colonies and color change, the medium is divided into two distinct regions. The plate's lower half is yellow, indicating a successful mannitol fermentation. The fermentation of mannitol produces acids, which is the source of this color shift. It causes the The pH will decrease and the phenol red indicator will be yellow. This is commonly attributed to both MRSA and MSSA strains of Staphylococcus aureus. Conversely, the red-pink color at the top of the plate means that no fermentation of mannitol has occurred. The medium pH is also not affected as the organisms in this region do not produce acid. The same phenotype is manifested by non-fermenting staphylococci like Staphylococcus epidermidis or other coagulase-negative staphylococci (CoNS). This image has the differential property of MSA in which the yellow stains represent mannitol. The non-fermenting isolates are denoted using red/pink stains and the positive isolates (which are supposed to be S. aureus) are used to assist in initial identification and classification.

IV.MRSA AND MSSA DISTRIBUTION

The identified strains of Staphylococcus aureus that are resistant to b-lactam are clinically severe MRSA and MSSA. Also, MRSA harbors the mecA gene, a gene that encodes the penicillin-binding protein PBP2a, thereby making it resistant to methicillin and other similar antibiotics. Consequently, the phenotypes of multidrug resistance have been reported often. MSSA on the other hand do not have this gene thus remain vulnerable to b-lactam antibiotics, enhancing the treatment effectiveness. These differences between MSSA and MRSA are the keys to the correct use of antibiotics and infection control measures. There were 48 clinical isolates of Staphylococcus aureus in this investigation. The Methicillin-resistant and methicillin-sensitive isolates were separated into groups based on.

Cefoxitin disc diffusion outcome. The distribution of the MRSA and MSSA isolates is presented in (Table 1). The prevalence of MRSA was 39 (81.25) out of 48 isolates with 9 (18.75) being less than.

Table 1: Distribution of Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Isolates Using Cefoxitin Disc Diffusion

Type of Isolate	Number (n)	Percentage (%)
MRSA	39	80.26%
MSSA	09	18.75%
Total	48	99.9 %

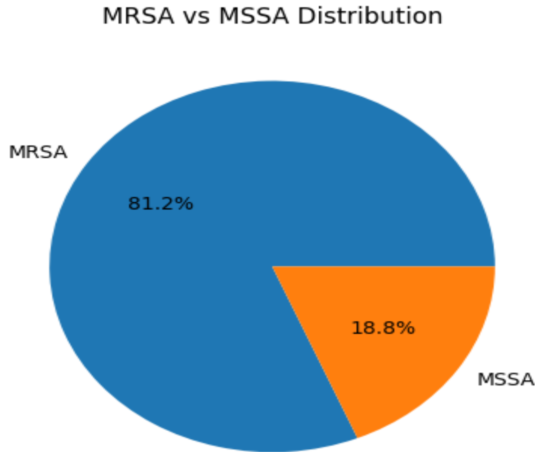

Figure 2: Distribution of MRSA and MSSA Isolates

Table 2: Demographic distribution of antibiotics

Antibiotic	Male(%)	Female(%)
Pencillin	0	0
Cefoxitin	0	0
Erythromycin	26.10%	25.00%
Clindamycin	47.63%	50.00%
Ciprofloxacin	30.44%	31.25%
Gentamicin	56.53%	62.50%
Amikacin	65.23%	68.75%
Tetracycline	43.47%	50.00%
Cotrimoxazole	52.27%	56.25%
Doxycycline	60.88%	62.50%
Linezolid	99.91%	99.98%
Vancomycin	99.93%	99.93%
Teicoplanin	99.70%	99.78%
Rifampicin	69.55%	75.00%
Chloramphenicol	73.91%	75.00%
Levofloxacin	39.13%	43.65%
Azitromycin	34.77%	37.53%

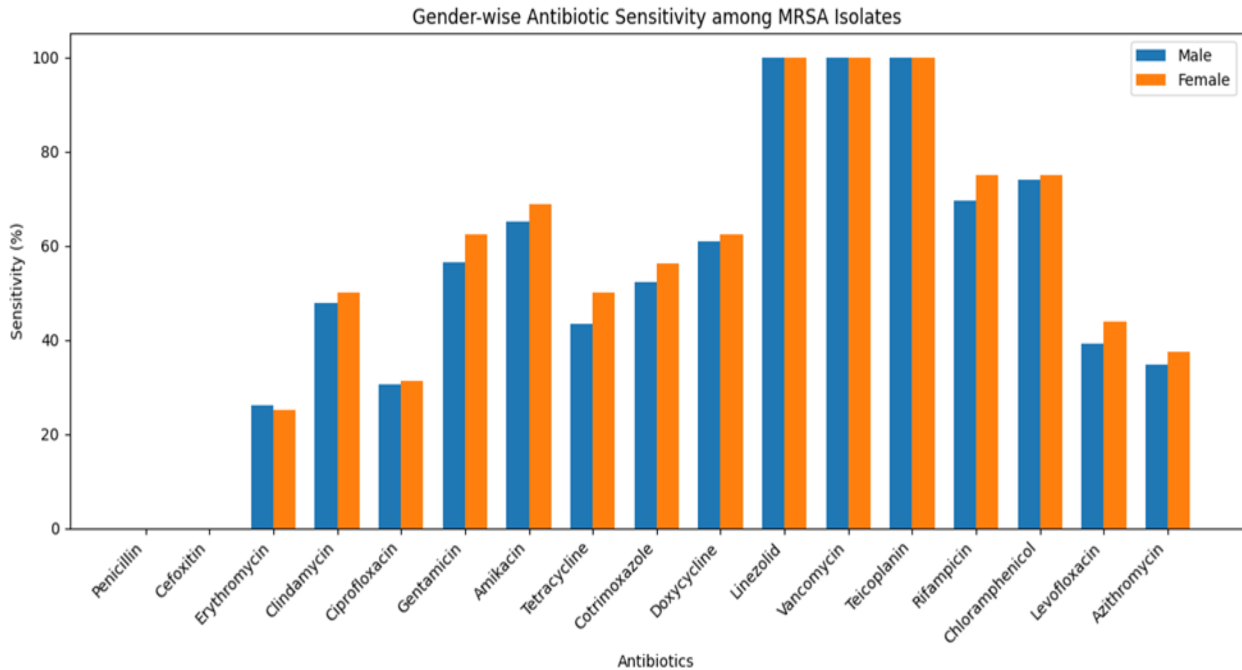

Figure 3: Gender wise antibiotics sensitivity among MRSA isolates

This (Figure 3) shows the influence of gender on the susceptibility of the MRSA isolates to medications and a tendency of multidrug resistance with insignificant differences between the male and female patients. According to the MRSA phenotype, cefoxitin and penicillin resistant isolates of both sexes and females are totally resistant (0% sensitive). Nonetheless, linezolid, vancomycin and teicoplanin have a sensitivity of 100 percent in both genders and, therefore, can also be used as the first-line therapies.

Amikacin, gentamicin, rifampicin, and chloramphenicol are some of the moderately sensitive antibiotics; they have a sensitivity of between 55 and 75.All of them, erythromycin, ciprofloxacin, tetracycline, cotrimoxazole, doxycycline, levofloxacin and azithromycin are less sensitive and more resistant. The female isolates are slightly more susceptible to most antibiotics than the male isolates but the differences are not significant to the extent of being clinically meaningful. On the whole, the graph demonstrates the prevalence of resistant MRSA strains and the necessity of such treatment as glycopeptides and linezolid to be effective in treating the infection.

Table -3 Age-Based Stratified Analysis of Antibiotic Susceptibility Trends

Antibiotic	0-20 yrs (%)	21-40 yrs (%)	41-61 yrs (%)	>60 yrs (%)
Penicillin	0.%	0.%	0.%	0.%
Cefoxitin	0.%	0.%	0.%	0.%
Erythromycin	29.9%	28%	25%	20%
Clindamycin	55%	50%	45%	40%
Ciprofloxacin	35%	32%	30%	25%
Gentamicin	65%	60%	55%	50%
Amikacin	75%	70%	65%	60%
Tetracycline	55%	50%	45%	40%
Cotrimoxazole	60%	55%	50%	52%
Doxycycline	65%	60%	58%	52%
Linezolid	99.9%	99.9	99.9%	99.9%
Vancomycin	99.9%	99.9%	99.9%	99.9%
Teicoplanin	99.9%	99.9%	99.9%	99.9%
Rifampicin	78%	72%	68%	65%
Chloramphenicol	80%	75%	70%	68%
Levofloxacin	45%	42%	38%	35%
Azithromycin	40%	38%	35%	30%

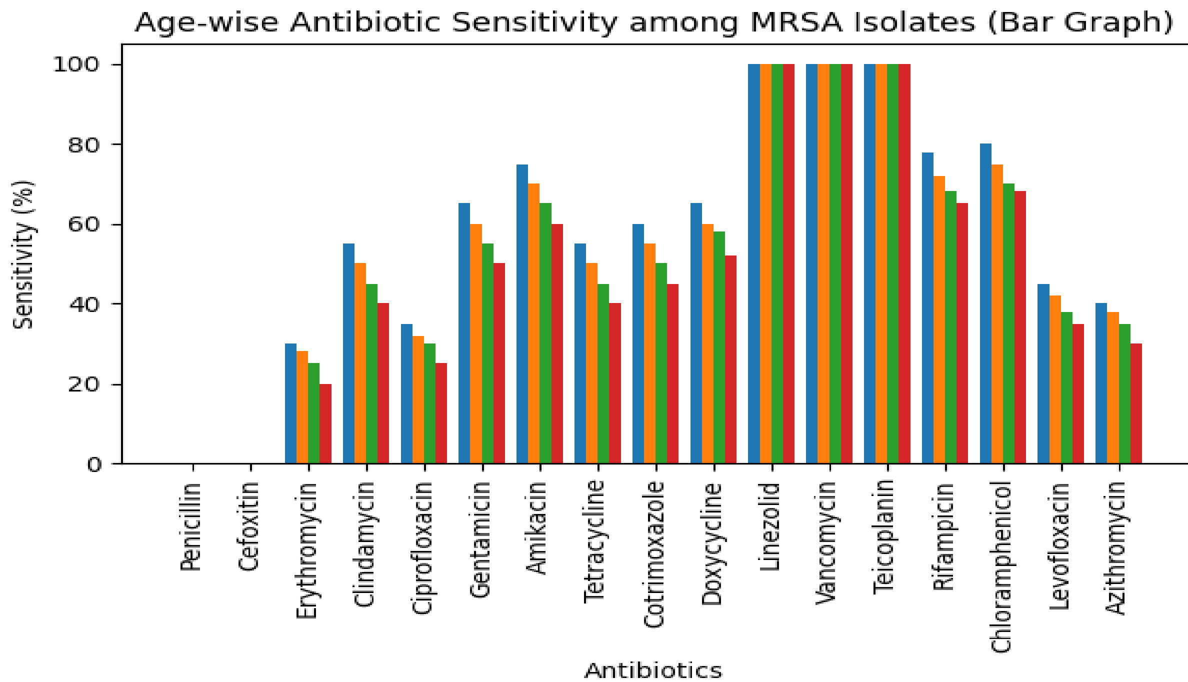

Figure 4:Antibiotics by Age MRSA isolate sensitivity

This(figure4) shows that antibiotic sensitivity generally declines with age. The 0–20 age group is the most sensitive, and the >60 age group has the least resistance; in other words, the older the patient, the more resistant they are. Linezolid, vancomycin, and teicoplanin are 100% sensitive to all age groups, while cefoxitin and penicillin are 100% resistant. A reduced level of efficacy is noted with macroclides and fluoroquinolones, whereas the sensitivity to aminoglycosides and chloramphenicol is modest. One of the variables that has been found to be important in this trend as a cause of antibiotic resistance in MRSA isolates is age.

V.DISCUSSION

The present investigation demonstrated a high prevalence of MRSA (81.25%) compared to MSSA (18.75%), indicating a substantial burden of antimicrobial resistance within the study population. The observation is correlated with the global tendencies that report the rising number of MRSA in healthcare and community settings. MRSA has been heavily prevalent because of different factors, which include improper use of antibiotics, hospital-acquired infection, and poor infection control methods. The fermentation analysis based on mannitol showed that the technique could be used as a first line identification tool of *S. aureus*, since fermentation of isolates resulted in typical yellow colonies on MSA. The existence of non-fermenting MRSA strains, however, underscores the weakness of using the phenotypic features alone in the process of identification. It was shown that the cefoxitin disc diffusion technique was a good method of identifying methicillin resistance, and acted as a good surrogate endpoint of mecA-mediated resistance. Regardless of these findings, the study has some weaknesses such as relatively small sample size and lack of methods of molecular confirmation like polymerase chain reaction (PCR). The use of molecular methods would increase the sensitivity of the resistance detection and give a greater understanding of the resistance mechanisms.

VI.CONCLUSION

This is in compliance with the study done by Chambers et al. (2009), and Lee et al. (2018), indicating the increasing public health importance of MRSA as an important pathogen associated with infections that are difficult to treat and for which limited therapeutic options are available (Tong et al., 2015). The current trend in MRSA prevalence could be attributed to the fact that antibiotics are still inappropriately used, nosocomial transmission, and lack of proper practice in infection control (David et al., 2010).Since most isolates turned yellow, the fermentation on mannitol salt agar was found to be a quick and practical method for initial diagnostic purposes in the identification of *S. aureus* and differentiation from *S. aureus* and other coagulase-negative staphylococci (CoNS). However, the failure of some isolates to ferment, indicating atypical isolates, shows that this phenotypic feature alone is the possible cause of mThe current study's MRSA/MSSA ratio reveals that the studied population has a significant level of antibiotic resistances identification (Becker et al., 2014).Mannitol fermentation must be performed in conjunction with further biochemical and antimicrobial susceptibility tests to ensure accurate identification. It is important to note that the study found that mannitol fermentation is ineffective for discriminating between MRSA and MSSA since their fermentation characteristics are quite similar.. This is in compliance with the relevance of standardized methods for testing antimicrobial susceptibility, particularly the cefoxitin disc diffusion method for identifying methicillin resistance (CLSI, 2023; Brown et al., 2005). For regular clinical microbiology labs to evaluate mecA-mediated resistance, cefoxitin is a commonly used and reliable surrogate resistance. In the demographic data, it is found that there is a slight male dominance and the *S. aureus* infections are increased in the 21-40 age range. This implies that the working population will be more susceptible to the virus since they have an increased exposure to the environment, working conditions, and social contacts. This is because, in the majority of cases, the human resources possess distinct values and interests (Grundmann et al., 2006; David et al., 2010). Gender differences can be attributed to variety of factors, including behavior, exposure potential, and health care availability. The identification of high-risk populations and provision of targeted infection control strategies can both benefit from these data. In general, the research paper demonstrates the importance of an advanced diagnostic method in developing a more

comprehensive idea of the epidemiology of Staphylococcus aureus infections. The transmission of MRSA should be prevented by constantly monitoring resistance, reasonable use of antibiotics, and proper infection control (Lee et al., 2018).The paper suggests that research in the future would be carried out to get more precise data, particularly by discovering mecA gene to provide more valuable information on the resistance mechanisms (Hiramatsu et al., 2002).

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