

Emerging Trends of Methicillin-Resistant Staphylococcus Aureus: A Tertiary Care Hospital-Based Study

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Author's Contribution

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ABSTRACT

Objective: To analyze the frequency and antimicrobial susceptibility patterns of Methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care hospital in Lahore, Pakistan.

Methodology: A retrospective study was conducted from October 2020 to December 2021 in a tertiary care hospital in Lahore, Pakistan. The clinical specimens meeting the inclusion criteria underwent processing in the microbiology section of a tertiary care hospital in Lahore, Pakistan, following the CLSI 2021 standards. Identification of all Methicillin-resistant Staphylococcus aureus (MRSA) isolates was conducted using standard laboratory procedures. Antibiotic susceptibility testing was performed utilizing the modified Kirby-Bauer disc diffusion method, with E-strips employed for vancomycin assessment.

Results: A total of 65 Staphylococcus aureus specimens were isolated. Among these, 49% were identified as Methicillin-resistant Staphylococcus aureus (MRSA). The majority of MRSA isolates (56%) were found in males, and a significant proportion (59%) fell within the age range of 21-60 years. Pus specimens accounted for the highest frequency (78%) among all samples. Notably, all MRSA isolates exhibited 100% resistance to beta-lactam drugs and combination therapies such as penicillin, cephalosporin, carbapenem, and amoxicillin-clavulanic acid. Conversely, Linezolid demonstrated complete sensitivity, while Chloramphenicol showed a sensitivity rate of 93%. Fluoroquinolones and aminoglycosides displayed a sensitivity of up to 50%. Additionally, the minimum inhibitory concentration (MIC) for vancomycin indicated sensitivity across all study isolates.

Conclusion: The study concluded that MRSA is continuously on the rise, with increasing resistance and limiting the antibiotic options for the treatment of these superbugs.

Keywords: Beta-Lactam drugs, Cephalosporin, MRSA, Tertiary care Hospitals, Lahore.

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Introduction

Staphylococcal infections are amongst the top causes of global mortality and morbidity, leading to hospital-acquired as well as community-acquired infections. ⁽¹⁾ These include bloodstream infections, skin and soft tissue

infections, pneumonia, and post-operative wound infections. ⁽²⁾ Treatment of staphylococcal infections is becoming difficult due to the drastic changes in the drug susceptibility profiles worldwide ⁽³⁻⁵⁾ and rising resistance against beta-lactam antibiotics. ⁽⁶⁾ As a consequence, the treatment of serious life-threatening infections caused by

Methicillin-resistant *Staphylococcus aureus* (MRSA), leads to prolonged hospital stay and increased cost of care. Once established, MRSA is very difficult to eradicate as it spreads more readily than other strains once introduced into hospitals. In many countries, 75 % of all *S. aureus* isolates in hospitals are MRSA, and infected or colonized patients are the major source of transmission. ^(7, 8)

MRSA is difficult to control and eradicate and has emerged due to injudicious antimicrobial chemotherapy. Hence, the knowledge of their antibiotic sensitivity pattern and prevalence is necessary for the selection of appropriate treatment. With this objective, the current study was conducted in a tertiary care hospital in Lahore to analyze the antimicrobial susceptibility patterns of MRSA.

Methodology

The study was initiated in a tertiary care hospitals of Lahore during the period of October 2020 and December 2021. All clinical samples obtained during that time frame that met the inclusion criteria were processed in the microbiological department of the pathology laboratory and included in the study. After inoculation on blood and MacConkey agar, specimens were incubated at 35-37 °C for 18 to 24 hours. Biochemical tests were conducted on gram-stained bacterial colonies that had beta hemolysis on blood agar or colonies that were tinted a golden yellow. *Staphylococcus aureus* was confirmed by performing DNase and coagulase assays on Gram-positive, catalase-positive bacteria that showed bunches on Gram stain. ⁽⁹⁾

Kirby Bauer disc diffusion method was used to assess the antimicrobial susceptibility patterns, as per Clinical Laboratory Standards Institute (CLSI, 2021) guidelines. Following antibiotics were analyzed: Oxacillin, penicillin, gentamicin, amikacin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, and erythromycin and clindamycin chloramphenicol, linezolid. ⁽¹⁰⁾ Cefoxitin was used to interpret the sensitivity of cephalosporin's, carbapenem and combination drugs. The zone of inhibition was measured and interpreted according to CLSI 2021 guidelines. ⁽¹⁰⁾ Vancomycin susceptibility was done by E test (Epsilometer) techniques and the results were interpreted based on determination of MIC of vancomycin in µg/ml after 48 h of incubation usually 2 µg/ml to 16µg/ml concentration. Data was compiled on Microsoft Excel.

Inclusion Criteria

Patients with a history of no antibiotic use and symptoms of illness, as well as specimens yielding Cefoxitin-

resistant *Staphylococcus aureus* (MRSA) were included in the study.

All the coagulase negative staphylococcus species and Cefoxitin sensitive staphylococcus aureus (MRSA) were excluded from the study. The study was approved from Institutional Ethical Review Board, Azra Naheed Medical College with reference no IRB/ANMC/2020/008.

Results

A total of 65 staphylococcus aureus were isolated from the specimens, out of which 32 were confirmed as Methicillin resistant staphylococcus aureus (MRSA), making a percentage of 49%.

Males (56%) were affected more by Methicillin resistant staph aureus infections as compared to females. (Figure: 1). 59% of the MRSA isolates (n=19) obtained were in the age range of 21-60 years, as shown in figure 2.

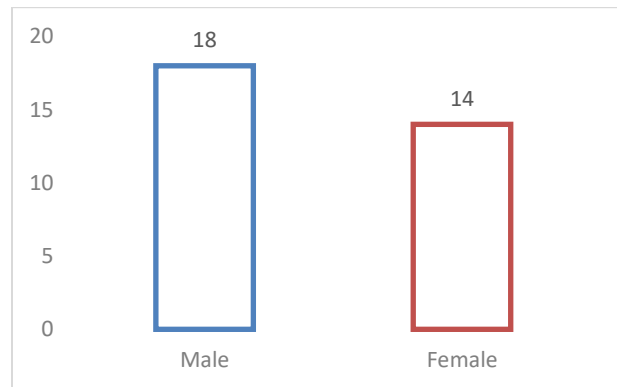


Figure 1. The demographic distribution, predominantly in terms of gender of patients affected by Methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

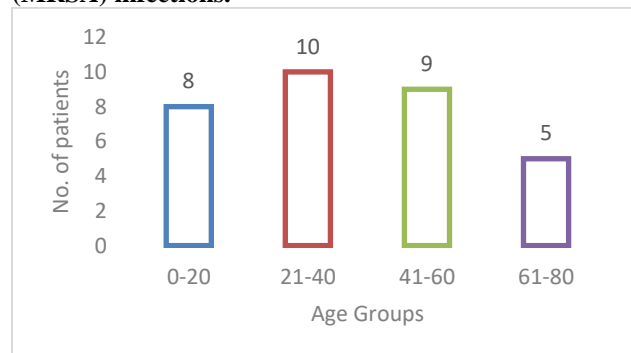


Figure 2: The demographic breakdown specifically in terms of age of patients with Methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Staphylococcus was identified in diverse specimens, with 78% of Methicillin-resistant *Staphylococcus aureus* (MRSA) being isolated from pus samples.

Based on the sensitivity pattern observed, all isolates obtained exhibited 100% resistance to beta-lactam drugs and combination drugs (including penicillin, cephalosporin, carbapenem, and amoxicillin-clavulanic acid). Conversely, Linezolid demonstrated 100% sensitivity, while Chloramphenicol showed 93% sensitivity. Fluoroquinolones and aminoglycosides displayed 50% sensitivity. Moreover, the minimum inhibitory concentration (MIC) of vancomycin for all isolates fell within the sensitive range refer to Figure 4.

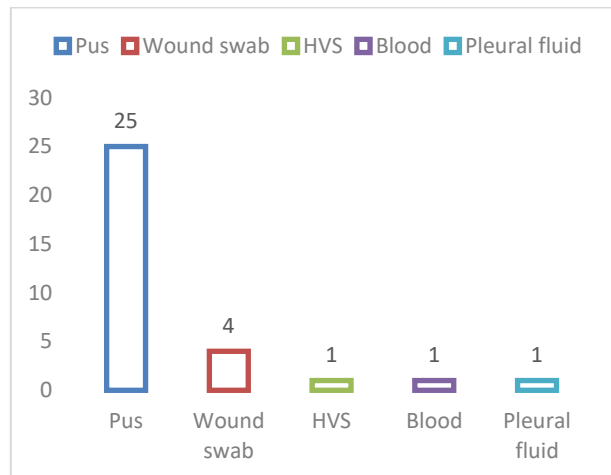


Figure 3. The distribution of Methicillin-resistant Staphylococcus aureus (MRSA) varies according to specimen type.

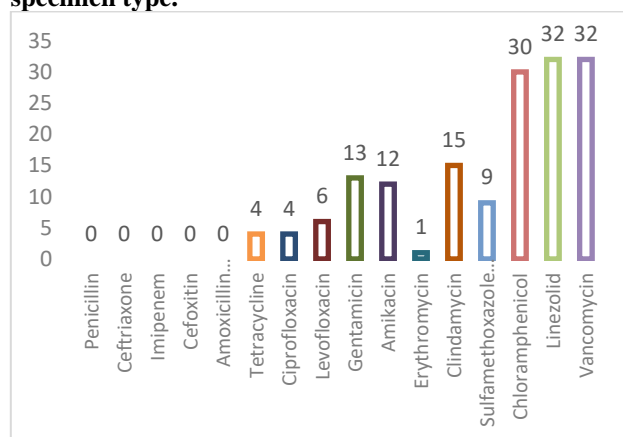


Figure 4: The susceptibility of Methicillin-resistant Staphylococcus aureus (MRSA) isolates to various antibiotics

Discussion

In this study, out of 65 Staphylococcus aureus isolates, almost half (49%) turned out to be MRSA. A study from Debre Markos Hospital reported similar magnitude of MRSA (49.7%), which is consistent with our study findings, ⁽¹¹⁾ whereas, slightly lower prevalence was observed in studies from Moi Teaching and Referral

Hospital of Kenya (37%) ⁽¹²⁾ South Africa (30.9%). ⁽¹³⁾ Bangladesh (72%). ⁽¹⁴⁾ Differences in the age groups, study subjects and specimen type could be attributed to the difference amongst various studies. Antibiotic misuse can also be associated with this substantial magnitude of MRSA. ^(15, 16)

Males (56% vs 44%) were affected more by Methicillin resistant staph aureus infections as compared to females. This subgroup analysis is comparable with a meta-analysis reported from India (60.4% vs 39.6%), thereby representing MRSA infections more common in male gender. ⁽¹⁷⁾ This is very similar to the findings of a work done by Harbath and colleagues and Kupfer et al. ^(18, 19) Behavioral and physiological factors, (including hand hygiene, nose picking and nail-biting) and working with livestock industry are considered as the major contributory factors to the unusual occurrence of MRSA in male population. ⁽²⁰⁾

Most of the MRSA isolates obtained (59%) in our study were falling in the age range of 21-60 years, consistent with a study conducted in Ethiopia. However, a rising trend has been observed in the prevalence of MRSA (25%) amongst population group of less than 20 years, as seen in a study by Wu et al, reporting MRSA in young Chinese population. ⁽²¹⁾ The major source of MRSA in the current study was pus specimen (78%), followed by 13% of wound swabs as observed in a study by Tebelay Dilnessa et al. ⁽²¹⁾

Looking at the susceptibility profile of the study isolates against anti-staphylococcal drugs, Penicillin, Cephalosporin, Carbapenem and Amoxicillin clavulanic acid were found to be 100% resistant. A similar picture was witnessed in India, ⁽⁸⁾ Trinidad & Tobago. ⁽²²⁾ Apart from high resistance to the reported β -lactams, trimethoprim/sulfa-methoxazole, clindamycin, erythromycin and gentamicin showed resistance of 72%, 53%, 97% and 59% respectively, which is higher in comparison to a systematic review from Tanzania showing 50% resistance in co-trimoxazole, 45% in clindamycin, 37% in erythromycin and 32% in gentamicin. Considering the fluoroquinolones, 81% resistance was observed against levofloxacin as compared to ciprofloxacin that showed 87% resistance. This is in concordance with a study from Assam, India showing 87.5% resistance to ciprofloxacin, and a study from Nigera showing 78.6% of the isolates showing resistance to the fluoroquinolone antibiotic ciprofloxacin. ⁽²³⁾

Almost negligible resistance was observed against chloramphenicol (6.2%). Linezolid and vancomycin showed 0% resistance amongst the MRSA study isolates, similar to the study conducted in Nepal.⁽²⁴⁾ This is in contrast to a study from Ethiopia showing 29.4 % vancomycin resistant in MRSA. Similar trends were observed in studies from Trinidad⁽²⁵⁾ Argentina, Brazil and Mexico.⁽²⁶⁾

Variable trend against vancomycin have been reported in previous studies, ranging from 0% in Ethiopia, Karachi and Uganda^(8, 27) to 8 % in Iran, Malaysia and Nigeria,^(28, 29) not comparable to our study. Studies from United States and Latin America reported increased resistance to other classes of antibiotics, thereby necessitating the use of vancomycin for these infectious agents.⁽³⁰⁾ Prudent use of drugs and constant surveillance susceptibility testing of MRSA against vancomycin has been stated as a remedy to deal with these organisms.⁽³¹⁻³³⁾

Conclusion

The study concluded that the prevalence of MRSA is on a rise. The most significant remedy is the wise use of anti-staphylococcal drugs. Prompt diagnosis and appropriate management of infected patients is the key to contain MRSA infections. Along with this adherence to the infection control practices also play a substantial role in this regard.

References

1. Francois P, Pittet D, Bento M, Pepey B, Vaudaux P, Lew D, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. *Journal of Clinical Microbiology*. 2003;41(1):254-60.
2. Kaur H, Purwar S, Saini A, Kaur H, Karadesai S, Kholkute SD, et al. Status of methicillin-resistant *Staphylococcus aureus* infections and evaluation of PVL producing strains in Belgaum, South India. *JKIMSU*. 2012;1(2):43-51.
3. Alborzi A, Pourabbas B, Salehi H, Pourabbas B, PANJEH SM. Prevalence and pattern of antibiotic sensitivity of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* in Shiraz-Iran. 2000.
4. Bukhari M, Iqbal N, Naeem S, Qureshi G, Naveed I, Iqbal A, et al. A laboratory study of susceptibility of methicillin resistant *Staphylococcus aureus* (MRSA). *Pakistan Journal of Medical Sciences*. 2004;20(3):229-33.
5. Krishna B, Patil AB, Chandrasekhar M. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in a south Indian city. *Southeast Asian journal of tropical medicine and public health*. 2004;35:371-4.
6. Orrett FA. Antimicrobial sensitivity pattern of aerobic bacteria blood isolates: experience at a university hospital in Trinidad. *International journal of antimicrobial agents*. 2001;17(1):75-7.
7. Kesah C, Ben Redjeb S, Odugbemi T, Boye CB, Dosso M, Ndinya Achola J, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clinical Microbiology and Infection*. 2003;9(2):153-6.
8. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. *BMC infectious diseases*. 2016;16(1):1-9.
9. Collee JG, Mackie TJ, McCartney JE. Mackie & McCartney practical medical microbiology: Harcourt Health Sciences; 1996.
10. M100 Performance Standards for Antimicrobial Susceptibility Testing. (2021), 31st Edition.
11. Kahsay A, Mihret A, Abebe T, Andualem T. Isolation and antimicrobial susceptibility pattern of *Staphylococcus aureus* in patients with surgical site infection at Debre Markos Referral Hospital, Amhara Region, Ethiopia. *Archives of public Health*. 2014;72(1):1-7.
12. Akoru C, Kuremu RT, Ndege SK, Obala A, Smith JW, Bartlett M. Prevalence and anti-microbial susceptibility of methicillin resistant *Staphylococcus aureus* at Moi teaching and referral hospital eldoret. *Open Journal of Medical Microbiology*. 2016;6(1):9-16.
13. Shuping LL, Kuonza L, Musekiwa A, Iyaloo S, Perovic O. Hospital-associated methicillin-resistant *Staphylococcus aureus*: A cross-sectional analysis of risk factors in South African tertiary public hospitals. *PloS one*. 2017;12(11):e0188216.
14. Hasan R, Acharjee M, Noor R. Prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in methicillin resistant *S. aureus* (MRSA) strains isolated from burn wound infections. *Tzu Chi Medical Journal*. 2016;28(2):49-53.
15. Anguzu J, Olila D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *African health sciences*. 2007;7(3).
16. Cusini A, Rampini SK, Bansal V, Ledergerber B, Kuster SP, Ruef C, et al. Different patterns of inappropriate antimicrobial use in surgical and medical units at a tertiary care hospital in Switzerland: a prevalence survey. *PloS one*. 2010;5(11):e14011.
17. Nagaraju U, Raju BP. Methicillin-resistant *Staphylococcus aureus* in community-acquired pyoderma in children in South India. *Indian Journal of Paediatric Dermatology*. 2017;18(1):14.
18. Harbarth S, Sax H, Fankhauser-Rodriguez C, Schrenzel J, Agostinho A, Pittet D. Evaluating the probability of previously unknown carriage of MRSA at hospital admission. *The American journal of medicine*. 2006;119(3):275. e15-. e23.
19. Kupfer M, Jatzwauk L, Monecke S, Möbius J, Weusten A. MRSA in a large German University Hospital: Male gender is a significant risk factor for MRSA acquisition. *GMS Krankenhaushygiene interdisziplinär*. 2010;5(2).
20. Humphreys H, Fitzpatrick F, Harvey BJ. Gender differences in rates of carriage and bloodstream infection caused by

- methicillin-resistant *Staphylococcus aureus*: are they real, do they matter and why? *Clinical Infectious Diseases*. 2015;61(11):1708-14.
21. Rathinam KK, Immanuel JM. Prevalence of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in India: A Systematic Review and Meta-Analysis.
 22. Pai V, Rao VI, Rao SP. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* [MRSA] isolates at a tertiary care hospital in Mangalore, South India. *Journal of laboratory physicians*. 2010;2(02):082-4.
 23. Umar AI, Manga SB, Baki AS, Uba A. Molecular characterization and epidemiology of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples in Sokoto, Nigeria. *Adesh University Journal of Medical Sciences & Research*. 2023;1-8.
 24. Khanal LK, Sah AK, Adhikari RP, Khadka S, Sapkota J, Rai SK. Prevalence and molecular characterization of methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) in a tertiary care hospital. *Nepal Medical College Journal*. 2023;25(1):32-7.
 25. Orrett FA, Shurland S. Prevalence of bacterial pathogens and susceptibility patterns from clinical sources in Trinidad. *The West Indian Medical Journal*. 2000;49(3):205-9.
 26. Aires de Sousa M, Miragaia M, Santos Sanches I, Ávila S, Adamson I, Casagrande ST, et al. Three-year assessment of methicillin-resistant *Staphylococcus aureus* clones in Latin America from 1996 to 1998. *Journal of clinical microbiology*. 2001;39(6):2197-205.
 27. Ojulong J, Mwambu T, Joloba M, Bwanga F, Kaddu-Mulindwa D. Relative prevalence of methicilline resistant *Staphylococcus aureus* and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. *Tanzania journal of health research*. 2009;11(3).
 28. Rajendra Goud N, Agarval D, Nadagoudar PH, Gaddad S. Antibiotic sensitivity pattern of community-associated methicillin-resistant *S. aureus* in High Schools, Bangalore city, Karnataka, South India. *Int Med J Students Res*. 2011;1(1):27-35.
 29. Olowe O, Eniola K, Olowe R, Olayemi A. Antimicrobial susceptibility and betalactamase detection of MRSA in Osogbo, SW Nigeria. *Nature and Science*. 2007;5(3):44-8.
 30. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *The Lancet*. 1997;350(9092):1670-3.
 31. Guerin Fo, Buu-Hoï A, Mainardi J-L, Kac G, Colardelle N, Vaupré S, et al. Outbreak of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to glycopeptides in a Parisian hospital. *Journal of Clinical Microbiology*. 2000;38(8):2985-8.
 32. Kim M-N, Hwang SH, Pyo Y-J, Mun H-M, Pai CH. Clonal spread of *Staphylococcus aureus* heterogeneously resistant to vancomycin in a university hospital in Korea. *Journal of clinical microbiology*. 2002;40(4):1376-80.
 33. Van Griethuysen A, Van't Veen A, Buiting A, Walsh T, Kluytmans J. High percentage of methicillin-resistant *Staphylococcus aureus* isolates with reduced susceptibility to glycopeptides in The Netherlands. *Journal of clinical microbiology*. 2003;41(6):2487-91.