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Frequency of Isolation of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) from Pus Samples and its Antibiotic Susceptibility

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ABSTRACT

Objective: To determine the frequency of isolation of methicillin-resistant *Staphylococcus aureus* from pus samples and the in-vitro efficacy of commonly used antimicrobial drugs against them.

Study Design: Cross-sectional study

Place and Duration of Study: Department of Microbiology, Army Medical College, National University of Medical Sciences, Rawalpindi, from May 2018 to Apr 2019.

Methodology: The pus samples were subjected to standard microbiological procedures. The Kirby Bauer disc diffusion method was used to determine the antibiotics susceptibility pattern of the isolates, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines-2018.

Results: Of the total 499 *Staphylococcus aureus*, 214(42%) were Methicillin-Resistant *Staphylococcus aureus*. The resistance against antibiotics were found in penicillin 214(100%), Trimethoprim/Sulfamethoxazole 205(96%), Erythromycin 177(82.7%), Ciprofloxacin 160(75.3%), Chloramphenicol 131(61%), Gentamicin 88(40.9%), Tetracycline 52(24%), Fusidic Acid 35(16%), Clindamycin 33(15.3%), Minocycline 11(5.2%), Vancomycin and Linezolid (0%).

Conclusion: There is a high frequency of MRSA in our setup with very limited treatment options.

Keywords: Antibiotic susceptibility, Methicillin-resistant *staphylococcus aureus, Staphylococcus aureus.*

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* is a massive problem and a challenge to clinicians around the world.[1] It has contributed to the limited treatment options and increase in the expenses of treatment of patients. Over time, its resistance to antimicrobials is rising, becoming one of the leading causes of mortality and morbidity worldwide.[2] It is a common cause of both healthcare-associated infections and community-acquired infections.[3] It can cause infections ranging from local wound abscesses to life-threatening infections, so its surveillance is an important measure to be taken in hospitals.

There is a continuous increase in the global prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA).[4] It has overcome most of the therapeutic options. Initially, *Staphylococcus aureus* responded well to the beta-lactam group of antibiotics. Unfortunately, it has become resistant to most antibiotics used to treat ordinary infections over decades. The methicillin resistance is mediated by *the mec*-A gene on DNA cassette chromosomes.[5] This gene produces penicillinbinding protein 2a (PBP2a), which has a low binding affinity for all the betalactam antibiotics substituting the native PBPs and allowing continuous cell wall assembly.[6,7]

In Pakistan, the resistance to antimicrobials is determined conventionally by disc diffusion. Newer modalities are rare. The limitations are due to a lack of technical expertise; reagents and equipment are costly and are restricted to reference laboratories only. As we have seen, resistance against all the drugs but the last line of drugs in therapy also confronts microbial resistance. Therefore, knowing the frequency of MRSA and its susceptibility pattern is very important for clinicians to treat patients more effectively. Therefore, this study aimed to determine the frequency of MRSA and its susceptibility pattern due to the reckless increase in the resistance in the clinical specimens received from the affiliated teaching hospital.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Army Medical College, National University of Medical Sciences, from May 2018 to April 2019. Permission was taken from the Institutional Review Board and Ethical Committee (Certificate Ser No ERC/ID/16 Dated 14/02/20) for this study. After obtaining consent, pus swabs were processed for culture and sensitivity from the Pak Emirates Military Hospital Rawalpindi wards and

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outpatient departments. The sampling technique was non-probability consecutive sampling.

Inclusion Criteria: Males and females of all ages presenting in outpatient and inpatient departments with complaints of pus discharge during the study period were included.

Exclusion Criteria: Patients with any co-morbidities were excluded from the study.

Samples were cultured on blood agar aerobically at 37°C and incubated for 48 hours. 8-Isolates were identified based on morphology, gram stain and biochemical tests such as catalase test, tube coagulase and DNase test following standard microbiology techniques described in Manual of Determinative Bacteriology by Bergey *et al.*[9]

A standard Kirby Bauer disc diffusion technique was used to determine antibiotic susceptibility pattern Clinical and Laboratory Standard Institute (CLSI) guidelines 2018.[10] The phenotypic identification of MRSA was made based on Cefoxitin. The isolates with a zone of inhibition of Cefoxitin ≤21mm were considered resistan.[11] The saline suspension of colonies of *Staphylococcus aureus* equivalent to 0.5 McFarland turbidity standards was prepared and inoculated on the Muller Hinton agar.

Antibiotic discs of Clindamycin (2µg), Erythromycin (15µg), Gentamicin (10µg), Tetracycline (30µg), Minocycline (30µg), Ciprofloxacin (5µg), Penicillin (10µg), Chloramphenicol (30µg), Linezolid (3µ0g), Fusidic Acid (10µg) Trimethoprim/Sulfamethoxazole and (30µg) Cefoxitin discs were applied on the plates. The plates were inverted and incubated at 37°C for 24 hours. After overnight incubation, plates were examined to read the zone of inhibition by Vernier caliper, and results were interpreted according to CLSI 2018. The minimum inhibitory concentration of vancomycin was determined by the agar dilution method.

Statistical Package for Social Sciences (SPSS) version 21.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency & percentages.

RESULTS

In positive samples, 105(49%) were males, and 109(51%) were females. The resistance to Penicillin was in 214(100%), 205(96%) were resistant to Trimethoprim /Sulfamethoxazole, and 177(82.7%) showed resistance to Erythromycin. The resistant to Ciprofloxacin came out to in 160(75%) followed by Chloramphenicol 131 (61%), Gentamicin 88(41%), Tetracycline (24.2%), Clindamycin (15.3%), Fusidic Acid (16.2%) and Minocycline 11(5.1%) and no resistance to Linezolid was found. The MIC of vancomycin came out to be two ug, according to CLSI revelatory criteria, not resistant to MRSA. The graphical representation of the resistance pattern of antimicrobials in pus isolates is shown in Figure-1. Concluding the above facts, maximum resistance was demonstrated by Penicillin & Trimethoprim /Sulfamethoxazole, followed by others. Linezolid and Vancomycin is still an option, as they are highly sensitive.

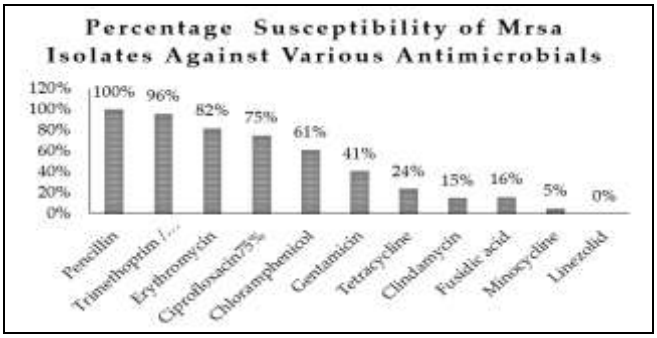

Figure-1: Percentage of Resistance of Antibiotics in Pus Samples (n=214)

The resistance pattern of MRSA was based on various departments of the hospital. 88(40.9%) MRSA-positive patients were those who visited the Outpatient Department, 47(21.9%) patients were from the General Surgery Ward, and 37(17.2%)were those from the Dermatology Department. 24(11.2%) patients were reported from the Department of Medicine. Some untreatable cases of about 18(8.4%) were also recorded from the intensive care unit. Considering the above facts, the highest population came to the outpatient department for follow-up purposes and recurrence of infection. The susceptibility pattern of MRSA-positive patients in Various Hospital Departments is shown in Figure-2.

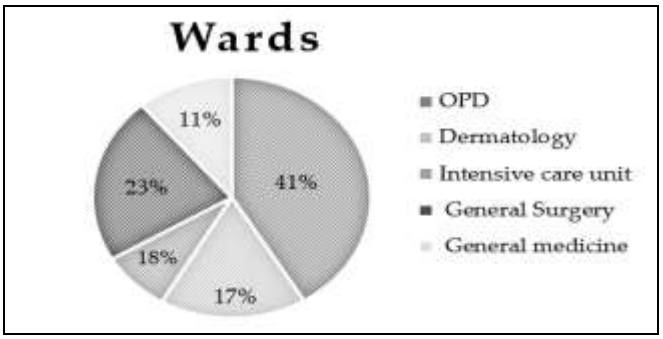

Figure-2: Antibiogram of Methicillin resistant Staphylococcus aureus MRSA positive patients in various departments (n=214)

DISCUSSION

MRSA was first reported in the early 1960s.[12] The emergence of MRSA has resulted due to the excessive use of β-lactams.[13] Therefore, there arises a need for rapid testing and early detection of MRSA in clinical settings.[14]

A study conducted in Khyber teaching hospital Peshawar in 2016 stated the frequency of MRSA in their setup as 35%.[15] Similarly, a study conducted in Karachi in 2017 showed the percentage of MRSA as 50%.[16] A study conducted in Lahore showed the prevalence of methicillin-resistant *Staphylococcus aureus* as 55% among total *Staphylococcus aureus* isolated from pus samples.[17] Another study conducted in Peshawar in 2018 stated that the frequency of MRSA in their setup is 57%.[18]

The international statistics vary because of better infection control. Europe showed a quite variation in MRSA infection ranging from 1-50%. The frequency of MRSA in Europe is less than 5% in Denmark, Iceland, Netherlands, Sweden and Norway. Some European countries showed above 25% MRSA prevalence, such as Italy, United Kingdom, Romania, Spain, Turkey and Greece.[19] In the USA, there is a lower trend of hospitalization due to MRSA.[20] The prevalence of MRSA is more in Asia than the other continents. In East Asian countries, i.e., Hong Kong and Indonesia, the prevalence rate is 28%, almost 75% in Korea, while in South Asian countries, i.e. India, it is estimated as 43%.[21]

To prevent increasing antimicrobial resistance at the national level or internationally, there is a need to develop an antibiogram with the cooperation of clinicians and clinical microbiologists to avoid antibiotic resistance. In addition, the Irrational use of antibiotics should be discouraged by making strict policies. No over-the-counter selling of antimicrobials without a physician's prescription, proper hygienic conditions and continuous antibiotic resistance surveillance are efficient ways to prevent MRSA infection.

CONCLUSION

There is a high frequency of MRSA in our setup with very limited treatment options.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

FR & MM: Data acquisition, data analysis, critical review, approval of the final version to be published.

AZ & SS: Study design, data interpretation, critical review, approval of the final version to be published.

NA & SA: Conception, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. World Health Organization. WHO Antimicrobial Resistance: Global Report of Surveillance; 2014, [Internet] available at: http://www.who.int/drugresistance/documents/surveillancereport/en/. (Accessed on October 28, 2020)
2. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Crit Care 2017; 21(1): 211. doi: 10.1186/s13054-017-1801-3.
3. Kong EF, Johnson JK, Jabra-Rizk MA. Community-Associated Methicillin-Resistant Staphylococcus aureus: An Enemy amidst Us. PLoS Pathog 2016; 12(10): e1005837. doi: 10.1371/journal.ppat.1005837.
4. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea. Can J Infect Dis Med Microbiol 2019; 2019(1): 8321834. doi: 10.1155/2019/8321834.
5. Mahdiyoun SM, Kazemian H. Frequency of Aminoglycoside-Resistance Genes in Methicillin-Resistant Staphylococcus aureus (MRSA) Isolates from Hospitalized Patients. Jundishapur J Microbiol 2016; 9(8): e35052. doi: 10.5812/jjm.35052.
6. Nowrouzian FL, Karami N, Welinder-Olsson C, Ahrén C. Virulence gene typing of methicillin-resistant Staphylococcus aureus as a complement in epidemiological typing. J Microbiol Methods 2013; 93(3): 173-176. doi: 10.1016/j.mimet.2013.03.020.
7. Thimmappa L, Bhat A, Hande M, Mukhopadhyay C, Devi E, Nayak B. Risk factors for wound infection caused by Methicillin Resistant Staphylococcus aureus among hospitalized patients: a case control study from a tertiary care hospital in India. Afr Health Sci 2021; 21(1): 286-294. doi: 10.4314/ ahs.v21i1.37.
8. CLSI, M100: performance standards for antimicrobial susceptibility testing: twenty-third informational supplement, Clinical Laboratory Standard Institute, Wayne, PA, USA, 2018, [Internet] available at: https://clsi.org/media/1930/100ed28_sample.pdf
9. Bergey DH, Buchanan RE, Gibbons NE: Bergey's manual of determinative bacteriology. Williams and Wilkins USA, 1974, [Internet] available at: https://www.biodiversitylibrary.org/item/41848#page/7/mode/1up
10. CLSI, M100: performance standards for antimicrobial susceptibility testing: twenty-third informational supplement, Clinical Laboratory Standard Institute, Wayne, PA, USA, 2018, [Internet] available at: https://clsi.org/media/1930/100ed28_sample.pdf
11. Sadiq A, Samad M, Saddam, Basharat N, Ali S, Roohullah, et al. Methicillin-Resistant Staphylococcus aureus (MRSA) in Slaughter Houses and Meat Shops in Capital Territory of Pakistan During 2018-2019. Front Microbiol 2020; 11: 577707. doi: 10.3389/fmicb.2020.577707.
12. Wertheim HF, Melles DC, Vos MC, Leeuwen W, Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 2005; 5(12): 751-762.

13. Saeed A, Ahsan F, Nawaz M, Iqbal K, Rehman KU, Ijaz T. Incidence of Vancomycin Resistant Phenotype of the Methicillin Resistant Staphylococcus aureus Isolated from a Tertiary Care Hospital in Lahore. Antibiotics (Basel) 2019; 9(1): 3-5. doi: 10.3390 /antibiotics9010003. Erratum in: Antibio (Basel) 2020; 9(2): 10-15.
14. Pillai MM, Latha R, Sarkar G. Detection of methicillin resistance in Staphylococcus aureus by polymerase chain reaction and conventional methods: a comparative study. J Lab Physicians 2012; 4(2): 83-88. doi: 10.4103/0974-2727.105587.
15. Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad N, et al. High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. Springerplus 2016; 5(1): 600. doi: 10.1186/s40064-016-2277-3.
16. Siddiqui T, Muhammad IN, Khan MN, Naz S, Bashir L, Sarosh N. MRSA: Prevalence and susceptibility pattern in health care setups of Karachi. Pak J Pharm Sci 2017; 30(Supple-6)): 2417-2421.
17. Ghias W, Sharif M, Yazdani FA, Rabbani M. Isolation & identification of Methicillin and Vancomycin resistance Staphylococcus aureus from pus samples of injured skin patients in Lahore, Pakistan. Biomed Lett 2016; 2(5): 103-112.
18. Khan F, Ahmad B, Ahmed J. Investigation of Staphylococcus aureus, prevailing in the environment of Khyber Teaching Hospital, Peshawar, Pakistan. Pak J Pharm Sci 2018; 31(2): 429-437.
19. Tiemersma EW, Bronzwaer SL, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N, et al; European Antimicrobial Resistance Surveillance System Participants. Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002. Emerg Infect Dis 2004; 10(9): 1627-1634. doi: 10.3201/eid1009.040069.
20. Klein EY, Mojica N, Jiang W, Cosgrove SE, Septimus E, Morgan DJ, et al. Trends in Methicillin-Resistant Staphylococcus aureus Hospitalizations in the United States, 2010-2014. Clin Infect Dis 2017; 65(11): 1921-1923. doi: 10.1093/cid/cix640.
21. Albusayes NN, Binkhamis K, Alselaimy RM. Prevalence and factors associated with methicillin-resistant Staphylococcus aureus colonization among clinical medical students. J Nat Sci Med 2019; 2(4): 226-227. doi: 10.4103/JNSM.JNSM_3_19