DOI: https://doi.org/10.54393/pbmj.v5i5.455

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 5, Issue 5 (May 2022)

Original Article

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Epidemiology and Antibiotics Susceptibility Profile Isolated from Different Clinical Samples in Tertiary Care Hospital

Wajid Ali[1], Shah Zaman[2*], Zakia Subhan[3], Abdur Razaq[4], Muhammad Nabi[5], Maria Khattak[4], Nabiha Naeem[6], Dua-E-Jamila Khurrum[6], Sudhair Abbas Bangash[7] and Irfan Ullah[6]

[1]Department of Pharmacology, Ayub Medical College, Abbottabad, Pakistan
[2]Department of Pharmacology, Peshawar Medical College, Peshawar, Pakistan
[3]Department of Pharmacology, KMU-IMS Kohat, Pakistan
[4]Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan
[5]Institute of Pharmaceutical Sciences Khyber Medical University, Peshawar, Pakistan
[6]Department of Life Sciences, School of Science, University of Management and Technology, Lahore, Pakistan
[7]Faculty of Life Science, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan.

ARTICLE INFO

Key Words:
Methicillin-Resistant, Staphylococcus aureus, Antibiotics, Susceptibility, Tertiary Care

How to Cite:
Ali, W. ., Zaman, S. ., Subhan, Z. ., Razaq, A., Nabi, M. ., Khattak, M. ., Naeem, N. ., Khurrum, D.-E.-J. ., Abbas Bangash, S. ., & Ullah, I. . (2022). Methicillin-Resistant Staphylococcus Aureus (MRSA) Epidemiology and Antibiotics Susceptibility Profile Isolated from Different Clinical Samples in Tertiary Care Hospital: MRSA Epidemiology and Antibiotics Susceptibility Profile. Pakistan BioMedical Journal, 5(5). https://doi.org/10.54393/pbmj.v5i5.455

***Corresponding Author:**
Shah Zaman
Department of Pharmacology, Peshawar Medical College, Peshawar, Pakistan
dr.shahzamankhan@gmail.com

Received Date: 20th May, 2022
Acceptance Date: 27th May, 2022
Published Date: 31st May, 2022

ABSTRACT

Staphylococcus aureus is a versatile bacterium that causes a wide range of diseases in humans and animals. **Objective:** To determine the occurrence and antibiotic sensitivity profiles of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates directly from the clinical samples. **Methods:** Individuals from various subgroups of the District Peshawar provided three different clinical specimens that are pus, body fluids and blood. Plasma, Macconkey and Cysteine Lactose Electrolyte Deficient agar (CLED) agar were used to prepare each specimen in the usual method. Gram staining test, catalase, and coagulase were used to identify and confirm *S. aureus*. The conventional "Kirby-Bauer disc" diffusion method was used to confirm MRSA antibiotic resistance patterns to several antibiotics. **Results:** A maximum of 750 diagnostic samples were evaluated and 50 (6.37%) were found to be positive for MRSA, with 33 (72%) coming from pus samples, 9 (19%) from fluid samples, and 6 (12%) from blood samples. Males had a higher prevalence of MRSA strains (69%) than females (31%). Most MRSA strains were completely resistant to different type of antibiotics *e.g.* penicillin, oxacillin, and ampicillin, while remaining completely susceptible to linezolid, teicoplanin, & vancomycin. Other anti-microbials to which MRSA strains were resistant are ceftriaxone (78.88%), cefoxitin (65.55%), erythromycin (83.33%), clindamycin (72.22%), co-amoxiclav (76.66%), fusidic acid (67.77%), and gentamycin (83.33%) (74.4%). **Conclusions:** This study found that the frequency of MRSA in Pakhtunkhwa is lower in comparison to that reported in other regions of Pakistan. Moreover, because MRSA is multi-drug-resistant, culture sensitivity testing should be conducted to determine the best antibiotic to use to treat MRSA infection.

INTRODUCTION

Staphylococcus aureus is a human pathogen causing diseases ranging from minor skin and soft tissue infections to life-threatening sepsis [1]. It leads to increasing hospital and healthcare costs [2]. All these hospital-associated infections [3] and community associated infections [4] are caused by the bacterium. MRSA was initially reported in the United Kingdom in 1961 [5], and it has become more prevalent since then, presenting a significant diagnostic challenges [6]. "Methicillin-resistant" strains of S. aureus currently account for more than half of all S. aureus infections. Many infection sites have been linked to it, including the bones joints, bones, lungs, and the urinary

DOI: https://doi.org/10.54393/pbmj.v5i5.455

bladder [7]. It can also cause bacteremia, which can result in endocarditis and osteomyelitis [7]. Body's skin and soft tissues are greatly affected by MRSA. Due to some genetic adaptations in MRSA, it has the ability to resist the large number of antibiotics such as β-lactams [8]. MRSA has increasingly received a lot of attention in the media. MRSA was termed the "superbug" by the American press in 2005 as it caused more deaths than AIDS [9]. In Trinidad, MRSA has shown complete resistance to erythromycin, gentamycin, penicillin, ceftriaxone and ampicillin [10]. In various Latin American nations, like Mexican (penicillin & oxacillin), Brazil (penicillin), & Chilean (penicillin), similar findings were observed for penicillin and oxacillin among MRSA (oxacillin & penicillin) [11]. MRSA is becoming increasingly common around the world. More than half of all suppurative skin infections in 11 US metropolitan locations were caused by community associated MRSA [12]. In 2002, a published study revealed the prevalence of MRSA in some of Pakistan's big cities, such as Karachi (57%), Lahore (61%), Rawalpindi (46 %), Sukkur (2 %), Quetta (26 %), Azad Kashmir (32 %), and Peshawar (36%) [13]. In several of these cities, such as Karachi (43% in 2007 and 38.6% in 2010) [14] and Lahore (27.77% and 34.7%) [14], following studies, indicated a considerable decline in MRSA prevalence. The frequency of MRSA in District Peshawar was established, and the anti-microbial resistance of MRSA isolated from blood, pus, and fluids was tested. In comparison to previous publications, these findings suggest a decreased prevalence rate of MRSA in Peshawar.

METHODS

Samples such as pus, bodily fluid, & blood were obtained and sent to the microbiological section of the city Laboratory Peshawar, Pakistan, between October 2018 and September 2019. The 750 total samples were processed for MRSA isolation from patients across the Peshawar District. A proforma was filled out when taking blood samples to obtain the heath information from all the candidates and consents were signed. In order to culture the blood samples, different growth media were selected e.g. MacConkey agar, Blood agar as well as Cysteine Lactose Electrolyte Deficient agar (CLED). By using the sterile inoculating loop, each sample was inoculated on different selected Medias. The incubation temperature of 37 °C for 24 hours was given to grow the bacterial colonies. Standard operational procedures were used to further process positive samples for identification. After that Gram-negative bacteria and gram-positive bacteria were differentiated by the use of gram staining. Chemical studies, such as the catalase and coagulase tests, were used to establish the presence of *Staphylococcus aureus.*

For Methicillin Resistance Detection: MRSA was identified using an oxacillin screening plate in accordance with National Committee for Clinical Laboratory Standards (NCCLS). With the use of sterile swabs, a suspension equivalent to 0.5 McFarland standards was inoculated smoothly on the surface of the Mueller-Hinton agar plate (Oxoid-UK) containing 4% of NaCl and 6g/mL of oxacillin. Every one of the plates was incubated overnight for 24 hours at 35°C. The isolates were recognized as oxacillin or methicillin-resistant after showing signs of growth (>1 colonies).

Testing for Antibiotic Susceptibility: The Kirby-Bauer disc diffusion method was used to verify MRSA antibiotic resistance patterns to several antibiotics. Muller-Hinton agar was prepared and sterilized in autoclave for 15 minutes at 121°C. To ensure sterility, 25 mL of media was placed onto 90mm sterile Petri dishes and then incubated at 37°C for overnight. Antimicrobial sensitivity was tested for all clinical isolates of MRSA using standard doses of "ampicillin, co-amixoclav, penicillin, ceftriaxone, erythromycin, oxacillin, clindamycin fusidic acid, linezolid, cefoxitin, gentamycin teicoplanin, and vancomycin". After an 18-hour incubation period at 37°C, the plates were analyzed for zones of inhibition and classified as resistant, moderate, or sensitive, according to the national council for controlling lab guidelines.

RESULTS

The Prevalence of MRSA in District Peshawar Between has been observed from October 2018 to September 2019. For MRSA isolation, the examination of 750 specific clinical samples was carried out. A total of 50 samples (6.37%) were tested positive for MRSA Table 1. As a result, 32 (67%) of the 50 positive MRSA samples belonged to males, whereas 15(32%) belonged to females. 36(72%) of the MRSA positive samples occurred from pus tests, 9 (19%) from fluids, and 6 (12%) from blood samples.

MRSA +ve	Males	Females	pus	fluids	blood
50	32(70%)	15 (32%)	33 (72%)	9 (19%)	6 (12%)

Table 1: MRSA Isolates Distribution

MRSA Antibiotic Susceptibility Profile: "Total resistance to oxacillin, ampicillin, and penicillin was shown in all MRSA isolates, while complete sensitivity to vancomycin, teicoplanin, and linezolid was observed". Other antimicrobials resistant to these MRSA strains were Ceftriaxone (88.88%), Cefoxitin (65.55%), Erythromycin (83.33%), Clindamycin (72.22%), Co-Amoxiclav (76.66%), Fusidic acid (67.77%), and Gentamycin (73.22%) (Table 2).

DOI: https://doi.org/10.54393/pbmj.v5i5.455

Selected antibiotics	Sensitivity (%)	Resistance (%)	Intermediate (%)
Penicillin	0 0	50 100	0 0
Ampicillin	0 0	50 100	0 0
Cefoxitin	05 09	30 65.5	12 27.8
Ceftriaxone	04 07	36 78	8 16.7
Clindamycin	09 19	33 72.22	6 12.12
Erythromycin	06 12	38 83.3	4 7.8
Co amoxiclav	07 14	35 76.6	6 12.12
Oxacillin	0 0	50 100	0 0
Gentamycin	06 12	34 74.4	8 16.7
Fusidic acid	10 03	31 67.8	31 67.8
Telcopanlani	50 10	0 0	0 0
Linezolid	050 100	0 0	0 0
Vancomycin	50 100	0 0	0 0

Table 2: The sensitivity profiles of MRSA to different antibiotics

DISCUSSION

In comparison to earlier findings on MRSA, bacterial prevalence in Pakistan and around the world, the MRSA frequency detected in this study is relatively low. In Pakistan, MRSA prevalence rates vary significantly, from 2-61% [15] and globally [16]. Meanwhile, MRSA in Peshawar exhibits significant resistance to several antibiotics, which is consistent with recent data on the chemotherapy activity of different antimicrobial drugs against MRSA. Methicillin-resistant S. aureus prevalence in Peshawar is lower than in past which reports from "Rawalpindi (60%) [16], Johannesburg, and Cape Town, South Africa's major cities (33–43%) [16], eastern Uttar Pradesh, India (54.85%) [16], California (86.3%) [17], Kuwait (32%), Sudan (33%), Iran (35%), and Russia (36%)" [18]. MRSA prevalence rates in European countries are as follows: "Austria 8.8%, Bulgaria 33.9%, Croatia 36.7%, Belgium 23.6%, Denmark 0.6%, Estonia 0.9%, France 33.1%, Hungary 7.1%, Iceland 0.5%, Ireland 41.2%, Czech Republic 5.9%, Israel 38.4%, Italy 40.9%, Germany 13.8% and Luxembourg 40.9%" [6]. The Linezolid, Teicoplanin, and Vancomycin [19] all had excellent antimicrobial action against MRSA and also no resistant MRSA strains were found in this investigation. While similar findings for Vancomycin, Teicoplanin, and Linezolid [20] have been obtained in various regions of Pakistan. While similar findings for vancomycin [21], Teicoplanin [22], and Linezolid [22] have been obtained in various regions of Pakistan. Ampicillin, Oxacillin, and Penicillin resistance were found in all MRSA strains, and no treatment effectiveness against MRSA infections was found. In some of Pakistan's biggest cities, similar results have been observed. Penicillin and Oxacillin and are completely ineffective against MRSA [22,23]. MRSA has also been found to be resistant to penicillin and oxacillin in Latin American nations such as Mexico (oxacillin and penicillin), and Brazil (penicillin) [15]. Ampicillin resistance has been documented at a high level in Nepal and India [19]. The aminoglycoside, gentamycin had weak effectiveness on MRSA, with 73% resistance. Other parts of Pakistan have shown varying degrees of gentamycin resistance, including Karachi (96%), Pakistan Institute of Medical Sciences hospital Islamabad (100%), Kohat (67%) and 76% in Islamabad [14]. Ceftriaxone, a 3rd generation cephalosporin antibiotic, has a significant degree of resistance (78.88%) in the present study, which is higher than the 45% found in Karachi [20], but lower than that found in Trinidad and Tobago, where MRSA is entirely susceptible to ceftriaxone. The antibiotics of 2nd generation cephalosporin, such as Ceftriaxone has a significant degree of resistance (78.88%) in the present study, which is higher than the 45% found in Karachi, but lower than that found in Trinidad, where MRSA is entirely susceptible to ceftriaxone [11]. The considerable resistance of MRSA to Fusidic acid (66.66%) has been observed more than 20% in Islamabad and 2% in Karachi [18], however, it is less than the previous reports (95.7%) in Riyadh Saudi Arabia". In most countries, therefore, fusidic acid is the medication of choice [11]. Clindamycin resistance is seen in 71% of MRSA strains. A stronger resistance (98.5%) to a lincosamide antibiotic has been documented in Karachi. However, in the United Kingdom (18%) and Russia, there is less resistance (27%) [24]. The overall prevalence of MRSA is greater in males 32 out of 50 (70%) than in females 15 out of 50, according to the current study is 32%. In Peshawar, Rahman et al., found a higher incidence of MRSA in men (59%) than females (42%) in 2009 [13]. Males have an increased rate of MRSA prevalence than females, according to Tiemersma et al. [25], however girls 14/25 (60.86%) had a greater rate of MRSA prevalence than males 9/25 (39.13%) in India, according to S. Sharma and A. Mall. Most MRSA infections were detected in pus samples (72%) while the lowest level of MRSA strains was found in blood samples (12%). India, European countries, and Pakistan have all shown similar findings [26]. For such preventive and control of MRSA infections, preventive measures should be implemented. "Cleanliness comes second only to Godliness." Hands should be cleansed with soap on a regular basis and the environment should be maintained clean. Self-medication should be discouraged, and the mob should be made aware of the situation. For MRSA infections, appropriate antibiotic susceptibility tests should be performed. "Colonized/infected patients should be isolated" and treated accordingly. In this study, linezolid, teicoplanin, and vancomycin had the best chemotherapeutic activity against MRSA infections, with their limited use of antibiotic susceptibility tests

DOI: https://doi.org/10.54393/pbmj.v5i5.455

CONCLUSIONS

The occurrence of MRSA in District Peshawar has been shown to be lower than in earlier studies. The most effective chemotherapeutic agents against MRSA infections were vancomycin, teicoplanin, and linezolid. To minimize MRSA resistance to these medications and the spreading of MRSA in District Peshawar, appropriate preventive

REFERENCES

[1] Thati V, Shivannavar CT, Gaddad SM. Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res. 2011 Nov;134(5):704-8. doi: 10.4103/0971-5916.91001.

[2] Waitayangkoon P, Thongkam A, Benjamungkalarak T, Rachayon M, Thongthaisin A, Chatsuwan T et al. Hospital epidemiology and antimicrobial susceptibility of isolated methicillin-resistant Staphylococcus aureus: a one-year retrospective study at a tertiary care center in Thailand. Pathog Glob Health. 2020 May 18;114(4):212-217. doi: 10.1080/20477724.2020.1755550.

[3] Shahkarami F, Rashki A, Rashki Ghalehnoo Z. Microbial Susceptibility and Plasmid Profiles of Methicillin-Resistant Staphylococcus aureus and Methicillin-Susceptible S. aureus. Jundishapur J Microbiol. 2014 Jul;7(7):e16984. doi: 10.5812/jjm.16984.

[4] Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant Staphylococcus aureus (MRSA) in India: prevalence & susceptibility pattern. Indian J Med Res. 2013 Feb;137(2):363-9.

[5] Amorim ML, Faria NA, Oliveira DC, Vasconcelos C, Cabeda JC, Mendes AC et al. Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant Staphylococcus aureus isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. J Clin Microbiol. 2007 Sep;45(9):2881-8. doi: 10.1128/JCM.00603-07.

[6] Tiwari HK, Das AK, Sapkota D, Sivrajan K, Pahwa VK. Methicillin resistant Staphylococcus aureus: prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect Dev Ctries. 2009 Oct 22;3(9):681-4. doi: 10.3855/jidc.86.

[7] Hussain MS, Naqvi A, Sharaz M. Methicillin Resistant Staphylococcus aureus (MRSA);: Prevalence And Susceptibility Pattern Of Methicillin Resistant Staphylococcus aureus (MRSA) Isolated From Pus In Tertiary Care Of District Hospital Of Rahim Yar Khan. The Professional Medical Journal. 2019 Jan 10;26(01):1227.doi.org/10.29309/TPMJ/2019.26.01.2510.

[8] Rongpharpi SR, Hazarika NK, Kalita H. The prevalence of nasal carriage of Staphylococcus aureus among healthcare workers at a tertiary care hospital in assam with special reference to MRSA. J Clin Diagn Res.2013Feb;7(2):25760.doi:10.7860/JCDR/2013/4320.2741.

[9] Rahimi F. Characterization of Resistance to Aminoglycosides in Methicillin-Resistant Staphylococcus aureus Strains Isolated From a Tertiary Care Hospital in Tehran, Iran. Jundishapur J Microbiol. 2016 Jan 2;9(1):e29237. doi: 10.5812/jjm.29237.

[10] Inomata S, Yano H, Tokuda K, Kanamori H, Endo S, Ishizawa C et al. Microbiological and molecular epidemiological analyses of community-associated methicillin-resistant Staphylococcus aureus at a tertiary care hospital in Japan. J Infect Chemother. 2015 Oct;21(10):729-36. doi: 10.1016/j.jiac.2015.07.005.

[11] Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study. Indian J Med Microbiol. 2006 Jan;24(1):34-8. doi: 10.4103/0255-0857.19892.

[12] Bhatt MP, Bhalla GS, Kundan T, Jindamwar P, Chaudhari CN, Sahni NG. Antimicrobial susceptibility profile of methicillin-resistant Staphylococcus aureus at a tertiary care centre. Archives Of Clinical Microbiology. 2015;6(3):0-.

[13] Khan RA, Rahman AU, Ahmad A, Jaseem M, Jabbar A, Khan SA et al. Prevalence and antibiotic susceptibility profile of methicillin-resistant Staphylococcus aureus (MRSA) isolated from different clinical samples in district Peshawar. J Appl Environ Biol Sci. 2014;4(8S):40-6.

[14] Khan AA, Ali A, Tharmalingam N, Mylonakis E, Zahra R. First report of mecC gene in clinical methicillin resistant S. aureus (MRSA) from tertiary care hospital Islamabad, Pakistan. J Infect Public Health. 2020 Oct;13(10):1501-1507. doi: 10.1016/j.jiph.2020.05.017.

[15] Bari F, Wazir R, Haroon M, Ali S, Ali I, Rahman H et al. Frequency and antibiotic susceptibility profile of MRSA at lady reading hospital, Peshawar. Gomal Journal of Medical Sciences. 2015 Mar 31;13(1).

[16] Preeja PP, Kumar SH, Shetty V. Prevalence and Characterization of Methicillin-Resistant

DOI: https://doi.org/10.54393/pbmj.v5i5.455

Staphylococcus aureus from Community- and Hospital-Associated Infections: A Tertiary Care Center Study. Antibiotics (Basel). 2021 Feb 18;10(2): 197. doi: 10.3390/antibiotics10020197.

[17] Shittu AO, Lin J. Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa. BMC Infect Dis. 2006 Jul 28;6:125. doi: 10.1186/1471-2334-6-125.

[18] Omuse G, Kabera B, Revathi G. Low prevalence of methicillin resistant Staphylococcus aureus as determined by an automated identification system in two private hospitals in Nairobi, Kenya: a cross sectional study. BMC Infect Dis. 2014 Dec 14;14:669. doi: 10.1186/s12879-014-0669-y.

[19] Fatholahzadeh B, Emaneini M, Gilbert G, Udo E, Aligholi M, Modarressi MH et al. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolates in Tehran, Iran. Microb Drug Resist. 2008 Sep;14(3):217-20. doi: 10.1089/mdr.2008.0822.

[20] Mahmood K, Tahir M, Jameel T, Ziauddin A, Aslam HF. Incidence of Methicillin-resistant Staphylococcus aureus (MRSA) causing nosocomial infection in a Tertiary Care Hospital. Annals of King Edward Medical University. 2010;16(2):91-.doi.org/10.21649/akemu.v16i2.188.

[21] 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. J Lab Physicians. 2010 Jul;2(2):82-4. doi: 10.4103/0974-2727.72155.

[22] Taj Y, Abdullah FE, Kazmi SU. Current pattern of antibiotic resistance in Staphylococcus aureus clinical isolates and the emergence of vancomycin resistance. J Coll Physicians Surg Pak. 2010 Nov;20(11):728-32.

[23] Kim HB, Jang HC, Nam HJ, Lee YS, Kim BS, Park WB et al. In vitro activities of 28 antimicrobial agents against Staphylococcus aureus isolates from tertiary-care hospitals in Korea: a nationwide survey. Antimicrob Agents Chemother. 2004 Apr;48(4):1124-7. doi: 10.1128/AAC.48.4.1124-1127.2004.

[24] Alfouzan W, Udo EE, Modhaffer A, Alosaimi A. Molecular Characterization of Methicillin- Resistant Staphylococcus aureus in a Tertiary Care hospital in Kuwait. Sci Rep. 2019 Dec 6;9(1):18527. doi: 10.1038/s41598-019-54794-8.

[25] Tiemersma EW, Bronzwaer SL, Lyytikäinen O, Degener JE, Schrijnemakers P, Bruinsma N et al. Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002. Emerg Infect Dis. 2004 Sep;10(9):1627-34. doi: 10.3201/eid1009.040069.