LAB RESEARCH ARTICLE

Four year trend of antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital, Lahore

Faiqa Arshad,[1] Sidrah Saleem,[2] Romeeza Tahir,[3] Ayesha Ghazal,[4] Aneela Khawaja,[5] Shah Jahan[6]

Abstract

Objective: To determine the susceptibility pattern of methicillin-resistant *staphylococcus aureus* to different antibiotics.

Method: The descriptive study was conducted at the Microbiology Department of the University of Health Sciences, Lahore, Pakistan, from August 2016 to July 2019, and comprised *staphylococcus aureus* samples that were processed and identified using colony morphology on blood agar, gram stain, catalase, coagulase and deoxyribonuclease testing. Screening for methicillin-resistant *staphylococcus aureus* was done using cefoxitin disc 30µg and oxacillin disc 1µg. Antimicrobial susceptibility was tested using the modified Kirby-Bauer disc diffusion method in line with the Clinical and Laboratory Standards Institute guidelines 2019. Data was analysed using SPSS 24.

Results: Of the 2704 strains processed, 402(14.86%) were found to be methicillin-resistant *staphylococcus aureus*. Of them, 204(50.74%) were recovered from pus, while 10(2.48%) were recovered from urine. All 402(100%) isolates were sensitive to vancomycin and linezolid, and resistant to penicillin, followed by erythromycin 306(76.11%) and sulfamethoxazole/ trimethoprim 295(73.38%). Overall, lower resistance was seen with doxycycline 145(36.06%) and clindamycin 160(39.80%). Inducible clindamycin resistance was seen in 142(35.23%) isolates.

Conclusion: An efficacious susceptibility pattern of methicillin-resistant *staphylococcus aureus* was seen with vancomycin and linezolid, moderate susceptibility with doxycycline and clindamycin, while high resistance was observed for penicillin, erythromycin and trimethoprim/sulfamethoxazole.

Keywords: Antimicrobial resistance, Antibiotic susceptibility, Methicillin-resistant *staphylococcus aureus*, MRSA.
(JPMA 72: 305; 2022) **DOI: https://doi.org/10.47391/JPMA.20-509**

Introduction

Antimicrobial resistance (AMR) is becoming the most serious modern risk to human health, gradually threatening the usefulness of antibiotics. Globally, methicillin-resistant *staphylococcus aureus* (MRSA) is the most challenging pathogen.[1] Controlling antibiotic resistance is a universal phenomenon linking various fields: the ecosystem, the food cycle, the cattle farming, and, indeed, therapeutic medication.[2] Antimicrobial consumption without antimicrobial stewardship (AMS) is a major driver of antibiotic resistance.[3]

Clinical and epidemiological aspects of MRSA require appropriate interpretation to identify AMR. The therapeutic cost and duration of infections is a serious concern in well-developed countries, and the financial burden on the economies of under-developed countries is often beyond their means, which further increases AMR.[4] In recent years, developing multidrug-resistant (MDR) strains of *staphylococcus aureus* (MDRSA) has made treatment of staphylococcus (S.) aureus infections more prolonged, troublesome and distressing.[5]

The hospital-acquired and/or nosocomial varieties of MRSA have developed an evolutionary resistance to a number of antibiotics due to the variation of antibiotic target site, change in membrane permeability, production of enzymes that inactivate antibiotics, and extrusion of antibiotic from the bacterial cell by efflux pumps.[6] On the other hand, community-acquired MRSA is commonly associated with skin and soft tissue infections, usually spread by skin contact.[7,8]

The current study was planned to assess the antimicrobial susceptibility pattern of MRSA among clinical specimens in a tertiary care hospital.

Materials and Methods

The descriptive study was conducted from January 2016 to December 2019 at the Microbiology Department of the University of Health Sciences (UHS), Lahore, Pakistan, after approval from the institutional ethics review committee. Clinical specimens received from inpatients were processed according to standard

[1,2,4]Department of Microbiology, [3,6]Department of Immunology, University of Health Sciences, Lahore, [5]Department of Pathology, Rahbar Medical and Dental College, Lahore, Pakistan.
Correspondence: Faiqa Arshad. Email: drfaiqaarshad08@gmail.com

operating guidelines in the UHS microbiology laboratory. Methicillin-resistant strains isolated from all the processed *S. aureus* cultured from clinical specimens were included, while repeat samples of the same patient were excluded.

All the samples were inoculated on blood agar and MacConkey agar, prepared according to manufacturers' instructions (Oxoid, UK). The plates were incubated aerobically at 35oC overnight. Primary identification of *S. aureus* was done by examining the colony morphology on blood agar plates. This included size, shape, surface, margins, consistency, elevation, colour, translucency and presence or absence of haemolysis. Microscopically, clustered gram-positive cocci were processed through biochemical reactions i.e., catalase, coagulase and deoxyribonuclease (DNAase) for the confirmation of *S. aureus*.

Screening was done through the Modified Kirby Bauer disc diffusion method using cefoxitin 30µg and oxacillin 1µg discs (Oxoid) on all isolates of *S. aureus* in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines 2019.[9] MRSA ATCC 33591 and methicillin-sensitive staphylococcus aureus (MSSA) ATCC 25923 were used as positive and negative controls, respectively.

Antibiotic susceptibility was determined by modified disc diffusion method as per the CLSI 2019 Guidelines.[9] Antibiotic discs of penicillin (PEN)10U, vancomycin (VAN) 30µg, erythromycin (E) 15µg, clindamycin (DA) 2µg, linezolid (LZD) 30µg, ciprofloxacin (CIP) 5µg, gentamicin (CN) 10µg, sulfamethoxazole/trimethoprim (SXT) were applied.[9]

Inducible DA resistance was identified during susceptibility testing through D-test by placing E and DA discs at a distance of 15-20mm, as per the CLSI 2019 Guidelines.[9] A D-shaped zone of inhibition around DA proximal to E specified inducible DA resistance. Resistance to both E and DA discs with circular zone of inhibition was labelled as constitutive DA resistance.[9]

Data was analysed using SPSS 24. Quantitative variables, like the specimens from which MRSA was isolated, were presented as frequencies and percentages. Pie-charts were used to show the breakup distribution, while line-chart was used to highlight the four-year trend. P<0.05 was considered statistically significant, while p<0.001 was taken as highly significant.

Results

Of the 2704 strains processed, 402(14.86%) were found to be methicillin-resistant *staphylococcus aureus*. MRSA was prevalent the highest in 2017 and the lowest in 2018 (Table-1).

Table-1: Distribution of methicillin-resistant staphylococcus aureus (MRSA) among total staphylococcus (S.) aureus isolates from 2016-2019 (n=2704).

Year	S. aureus	MRSA	
		Negative (%)	Positive (%)
2016	723	628 (86.86)	95 (13.13)
2017	603	494 (81.92)	109 (18.07)
2018	789	691 (87.57)	98 (12.42)
2019	589	489 (83.02)	100 (16.97)
Total	2704	2302 (85.13)	402 (14.86)

Table-2: Resistance pattern of methicillin-resistant staphylococcus aureus (MRSA) isolates to different antibiotics from 2016 to 2019 (n=402).

Antibiotics	2016 n=95 (%)	2017 n=109 (%)	2018 n=98 (%)	2019 n=100 (%)	Total n=402 (%)
PEN	95 (100)	109 (100)	98 (100)	100 (100)	402 (100)
E	65 (68.42)	85 (77.98)	73 (74.4)	83 (83)	306 (76.11)
DA	40 (42.10)	44 (40.36)	34 (31.19)	42 (42)	160 (39.80)
CN	43 (45.26)	55 (50.45)	51 (52.04)	50 (50)	199 (49.50)
CIP	58 (61.05)	67 (61.46)	61 (62.24)	68 (68)	254 (63.18)
DOX	35 (38.94)	44 (40.36)	39 (39.79)	25 (25)	145 (36.06)
SXT	69 (72.63)	80 (73.39)	78 (79.59)	68 (68)	295 (73.38)

PEN: Penicillin, E: Erythromycin, DA: Clindamycin, CN: Gentamicin, CIP: Ciprofloxacin, DOX: Doxycycline, SXT: Sulfamethoxazole/ Trimethoprim.

Of the MRSA isolates, 204(50.74%) were recovered from pus, while 10(2.48%) were recovered from urine (p<0.05) (Figure-1).

All 402(100%) isolates were sensitive to VAN and LZD, and resistant to PEN, followed by E 306(76.11%) and SXT 295(73.38%). Overall, lower resistance was seen with doxycycline (DOX) 145(36.06%) and DA 160(39.80%) (Table-2). Inducible DA resistance was seen in

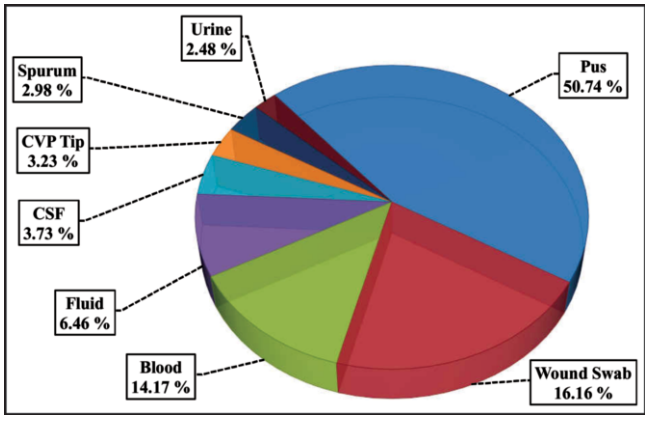
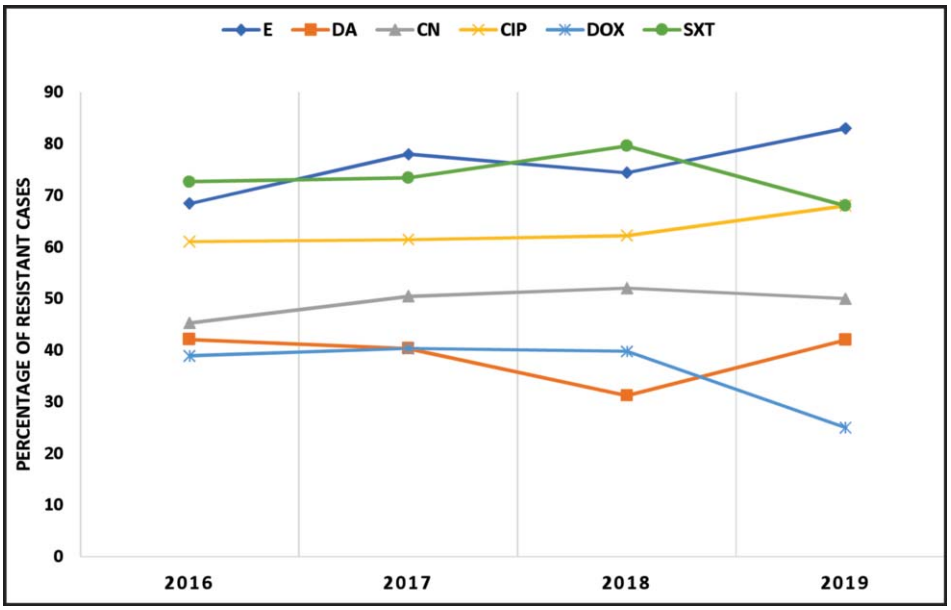


Figure-1: Breakup of methicillin-resistant *staphylococcus aureus* (MRSA) isolates from different clinical samples in four years (2016-2019) (n=402).



E: Erythromycin, DA: Clindamycin, CN: Gentamicin, CIP: Ciprofloxacin, DOX: Doxycycline, SXT: Sulfamethoxazole/Trimethoprim.

Figure-2: Pattern of drug susceptibility over four years from 2016 to 2019 for all methicillin-resistant staphylococcus aureus (MRSA) isolates (n=402).

142(35.23%) isolates, while constitutive DA resistance was present in 95(23.63%) (p<0.001) (Figure-2).

Discussion

AMR has to be controlled, otherwise in the coming decades, death toll could be as high as one person every three seconds.[10]

Non-curable infections can be dangerous because of resistance to existing antibiotics along with stasis in the promotion of new antibiotics to an unacceptable level worldwide. An up-to-the-minute class of antimicrobials is the need of hour to combat the emerging pressures of resistant strains causing ailments both in public and healthcare settings.[11]

The current study showed prevalence of MRSA isolates cultured from 2704 *S. aureus* isolates in 4 years. The overall prevalence was 14.86%, ranging from 12.42-18.07% per year. The prevalence of MRSA has fluctuated from 5-70% in previous years and in different countries.[12-15] Studies in Pakistan have shown the prevalence range from 12% to 60%.[16,17]

MRSA isolates were recovered mostly from pus, followed by wound swab, blood, fluids, CSF, central venous catheter (CVC) tip, sputum and urine. Some studies have reported greater frequency of MRSA isolated from pus and wound swabs.[16,17] while others have reported majority of MRSA isolated from urine samples[18] and from endotracheal secretions and CVCs.[19] In the current study, all the isolates were sensitive to VAN and LZD, and resistant to PEN, followed by E and SXT. Overall, lower resistance was seen with DOX and DA. Other studies have also reported resistance to SXT and/or cotrimoxazole (COT) and CIP (60-80%) each, followed by E (50-70%), and CN (30-60%).[16,17,19] Resistance to PEN has been documented as 100% by two studies.[20,21] Sensitivity to VAN and LZD of all the 402 MRSA isolates was 100% in the current study. Similar findings have been reported by studies conducted in the region as well as globally.[22,23]

In the current study, 35.32% isolates revealed inducible DA resistance, which can be compared with earlier studies.[24,25] Data in Pakistan has shown inducible DA resistance 30-70%.[26,27]

Conclusion

An efficacious susceptibility pattern of MRSA was seen with VAN and LZD, moderate susceptibility with DOX and DA, while high resistance was observed for PEN, E and SXT. Antibiogram for MRSA has to be streamlined periodically. This can be helpful in formulating antibiotic stewardship policy, implementation of adequate infection control and prevention strategies, and in upgrading patient care in terms of duration and cost of hospitalisation and medication.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Capurro G. "Superbugs" in the Risk Society: Assessing the Reflexive Function of North American Newspaper Coverage of Antimicrobial Resistance. SAGE Open 2020; 10: 2158-440.
2. Miranda-Novales MG, Flores-Moreno K, López-Vidal Y, Rodríguez-Álvarez M, Solórzano-Santos F, Soto-Hernández JL, et al; UNCAR Network. Antimicrobial resistance and antibiotic consumption in Mexican hospitals. Salud Publica Mex 2020; 62: 42-9.
3. Langford BJ, Quirk J, Carey S, Daneman N, Garber GE. Influencing duration of antibiotic therapy: A behavior change analysis in long-term care. Am J infect Control 2019; 47: 1409-14.
4. Watkins RR, Holubar M, David MZ. Antimicrobial resistance in methicillin-resistant Staphylococcus aureus to newer antimicrobial agents. Antimicrob Agents Chemother 2019; 63:

e01216-19.
5. Nisbet M. The Superbug Crisis: False Beliefs about Antibiotics Are a Global Threat. Skeptical Inquirer 2017; 41: 21-8.
6. Mao P, Peng P, Liu Z, Xue Z, Yao C. Risk Factors and Clinical Outcomes Of Hospital-Acquired MRSA Infections In Chongqing, China. Infect Drug Resist 2019; 12: 3709-17.
7. Sastalla I, Datta SK. Antibiotic Education: Not Just Another Brick in the Cell Wall. Cell Host Microbe 2015; 18: 520-2.
8. Neradova K, Fridrichova M, Jakubu V, Pomorska K, Zemlickova H. Epidemiological characteristics of methicillin-resistant Staphylococcus aureus isolates from bloodstream cultures at University Hospital in the Czech Republic. Folia Microbiol (Praha) 2020; 65: 615-22.
9. Clinical Laboratory and standard institute (CLSI). Performance Standards for Antimicrobial susceptibility testing. 29th ed. USA: CLSI; 2019.
10. Kong EF, Johnson JK, Jabra-Rizk MA. Community-Associated Methicillin-Resistant Staphylococcus aureus: An Enemy amidst Us. PLoS Pathog 2016; 12: e1005837.
11. Ray MD, Boundy S, Archer GL. Transfer of the methicillin resistance genomic island among staphylococci by conjugation. Mol Microbiol 2016; 100: 675-85.
12. Lim WW, Wu P, Bond HS, Wong JY, Ni K, Seto WH, et al. Determinants of methicillin-resistant Staphylococcus aureus (MRSA) prevalence in the Asia-Pacific region: A systematic review and meta-analysis. J Global Antimicrob Resist 2019; 16: 17-27.
13. Simango C, Mulea L. Prevalence and antimicrobial susceptibility of methicillin resistant Staphylococcus aureus isolated from clinical specimens in Harare, Zimbabwe. Central Afr J Med 2019; 65: 1-5.
14. Murray H, Leifso K. Just the Facts: Methicillin-resistant Staphylococcus aureus and soft tissue abscess in the emergency department. CJEM 2020; 22: 149-51.
15. Ibrahim ESH, El-Baghdady KZ, Abd El-All SM, Warda MAA, Prince AM, Ibrahim MK. Prevalence of multidrug resistance in the Egyptian methicillin-resistant Staphylococcus aureus isolates. Afr J Biol Sci 2020; 16: 43-52.
16. Khawaja A, Arshad F, Jamil R. METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS; PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL. Prof Med J 2019; 26: 601-7.
17. Taj FA, Javed F, Saeed MS, Khan S, Masood B, Khan AA,et al. Prevalence and Sensitivity Pattern of MRSA against Gentamycin, Vancomycin &Ciprofloxacin, in Indoor and OPD Patients of Holy Family Hospital. J Rawal Med Coll 2019; 23: 115-8.
18. Bhowmik D, Chetri S, Paul D, Chanda DD, Bhattacharjee A. Detection and molecular typing of methicillin-resistant Staphylococcus aureusfrom northeastern part of India. Med J Armed Forces India 2019; 75: 86-9.
19. Ajmal AN, Mir F, Aslam M, Hafeez R, Attique R. Nosocomial Methicillin-Resistant Staphylococcus aureus frequency in a tertiary care hospital, Lahore, Pakistan. Biomedica 2017; 25: 97-100.
20. Al Bshabshe A, Joseph MRP, Awad El-Gied AA, Fadul AN, Chandramoorthy HC, Hamid ME. Clinical Relevance and Antimicrobial Profiling of Methicillin-Resistant Staphylococcus aureus (MRSA) on Routine Antibiotics and Ethanol Extract of Mango Kernel (Mangifera indica L.). Biomed Res Int 2020; 2020: 4150678.
21. Jafari-SA, Farhadi F, Ezdiyadi M, Tarbiat-ND. Study of antibiotic resistance pattern in methicillin-resistant Staphylococcus aureus isolated from clinical samples of hospitals in Tabriz–Iran. Int J Biomed Public Health 2018; 1: 71-5.
22. Dat VQ, Vu HN, Nguyen HT, Hoang LB, Viet DV, Bui CL, et al. Bacterial bloodstream infections in a tertiary infectious disease hospital in Northern Vietnam: aetiology, drug resistance, and treatment outcome. BMC Infect Dis 2017; 17: 493-7.
23. Ceballos S, Aspiroz C, Ruiz-Ripa L, Zarazaga M, Torres C; Spanish study group on clinical LA-MRSA. Antimicrobial resistance phenotypes and genotypes of methicillin-resistant Staphylococcus aureus CC398 isolates from Spanish hospitals. Int J Antimicrob Agents 2020; 55: 105907.
24. Che Hamzah AM, Yeo CC, Puah SM, Chua KH, A Rahman NI, Abdullah FH, et al. Tigecycline and inducible clindamycin resistance in clinical isolates of methicillin-resistant Staphylococcus aureus from Terengganu, Malaysia. J Med Microbiol 2019; 68: 1299-305.
25. Shidiki A, Pandit BR, Vyas A. Characterization and Prevalence of Clindamycin Resistance Staphylococcus aureus from Clinical samples of National Medical College and Teaching Hospital, Nepal. Asian J Pharm Clin Res 2019; 12: 90-2.
26. Razzak S, Faisal H, Detho AB, Durrani MA, Saeed I. D-Test as a tool to detect the frequency of clindamycin resistance in community acquired and hospital acquired methicillin resistant Staphylococcus aureus infections (MRSA). The Prof Med J 2020; 27: 1-4.
27. Khan AA, Farooq J, Abid M, Zahra R. Assessment of inducible clindamycin resistance and Hyper Variable Region (HVR) of mecA gene in clinical staphylococci. Pak J Med Sci 2020;36: 136-40.