ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Methicillin-resistant *Staphylococcus aureus* causing ocular infections in a tertiary care center in north India

Shariq Wadood Khan[1], Adil Raza[2], Shaik Mohammed Zakir[3], Haris Manzoor Khan[4], Mohammad Shahid[5]

[1]Senior Resident, [2]Associate Professor, [4]Chairman and Professor, Department of Microbiology, [3]Associate Professor, Retina Section, Institute of Ophthalmology, J. N. Medical College, AMU, Aligarh, India, [5]Professor, Department of Microbiology, Immunology and Infectious Diseases, College of Medicine and Medical Sciences, Kingdom of Bahrain

Submission: 04-07-2022 Revision: 02-10-2022 Publication: 01-11-2022

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is often the cause of serious ocular infections that are difficult to treat, and these ocular infections are a significant public health problem. **Aims and Objective:** The study aimed to determine the prevalence and antimicrobial susceptibility pattern of MRSA in ocular infections. **Materials and Methods:** The patients were recruited from the outpatient and inpatient departments from July 2018 to February 2021 from the Institute of Ophthalmology and study was done in the Department of Microbiology, JNMC, AMU, Aligarh (UP). MRSA was identified by Cefoxitin disk diffusion method. Polymerase chain reaction was used for detection of mecA gene. **Results:** Seventy-six *S. aureus* were found in 350 patients with clinically suspected various bacterial infections. Among all *S. aureus*, 41 (53.94%) were MRSA. Maximum 10 (24.4%) MRSA were isolated from lacrimal apparatus infection followed by 7 (17%) in lid infection, and 6 (14.6%) in keratitis. All MRSA isolates were positive for mec A gene. MRSA isolates showed 85.3% sensitivity to amikacin and 100% to vancomycin. Fluoroquinolones showed resistance to the majority of MRSA strains. **Conclusion:** MRSA is the main pathogen of ocular infections, resistant to a wide range of antibiotics. The monitoring of antibiotic sensitivity patterns, regular surveillance of associated ocular diseases, and strict antibiotics policy should be followed in an institute.

Key words: *Staphylococcus aureus*; MRSA; Ocular infections; Antimicrobial susceptibility

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v13i11.46419

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The eye is impervious to nearly all external agents. Microorganisms accumulation is prevented by continuous tear flow and blink reflex, which wash out the eye surface substances. Tears contain lactoferrin, lysozyme, defensins, and secretory immunoglobulins, reducing ocular surface bacterial colonization.[1] *Staphylococcus aureus* is the common pathogen in ocular infections such as keratitis, conjunctivitis, lacrimal apparatus, and lid infections.[2] Methicillin-resistant *Staphylococcus aureus* (MRSA) is a special strain of *S. aureus* resistant to β-lactams antibiotics. MRSA was first isolated in 1961 in the UK and is now widespread all over the world, particularly in the health-care setting.[3-5] It causes a high mortality rate across the globe due to the rapid progression of the disease and multidrug resistance.[6,7] MRSA isolates are commonly multidrug-resistant and resistant to various classes of antibiotics such as erythromycin, fluoroquinolones, tetracycline, and aminoglycosides.[8,9] There have been various reports from different parts of the world like the USA and India showing an increase in the prevalence of ocular MRSA infections in current years, while other studies demonstrate a reliably stable prevalence in Taiwan.[10-12] We described the prevalence of ocular infections caused by MRSA and their antibiotic susceptibility pattern recovered from patients with ocular infection in a tertiary care center in North India.

Address for Correspondence:
Dr. Adil Raza, Associate Professor, Department of Microbiology, J. N. Medical College, AMU, Aligarh, Uttar Pradesh India.
Mobile: +91-9760596119. **Email:** adilbrdmc@gmail.com

Aims and objectives

The study aimed to determine the prevalence and antimicrobial susceptibility pattern of MRSA in ocular infections.

MATERIALS AND METHODS

The patients were recruited from the outpatient and inpatient departments from July 2018 to February 2021. The study was approved by the Institutional Ethics Committee. All the specimens collected from clinically diagnosed patients were transported to the laboratory for further processing. Identification of organisms was done by conventional methods. Antimicrobial susceptibility was done as recommended by the Clinical and Laboratory Standards Institute using the Kirby-Bauer disc diffusion method. Cefoxitin disk diffusion methods were used for the detection of MRSA.

Polymerase chain reaction (PCR) for mecAgene

PCR amplification of conserved regions of mecA genes in resistant isolates were determined with the primers as described by McClure et al.[13] The Mec A gene was amplified in a total volume of 25 µl reaction mixture that contained 2.5µl DNA templates, 0.24 µM for the primers (mecA 15'GTAGAAATGACTGAACGTCCGATAA-3' and mecA2 5'CCAATTCCACATTGTTTCGGTCTAA-3'). The PCR was performed in Bio-Rad thermal cycler with cycling conditions consisted of an initial denaturation step at 94°C for 10 min, followed by 25 cycles of final denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension for 72°C for 75 s, and the process was completed with the final extension step at 72°C for 5 min. Analysis of amplified PCR product was done by gel electrophoresis.

RESULTS

A total of 76 *S. aureus* were found in 350 patients with clinically suspected different ocular infections. Among all *S. aureus*, 41 (53.94%) were MRSA.

MRSA isolates in various ocular diseases

Seventy-six isolates of *S. aureus* were obtained from various ocular infections. Out of these, 41 (53.94%) isolates were methicillin-resistant, and the remaining 35 (46.05%) were methicillin-susceptible. The maximum number of MRSA were isolated from lacrimal apparatus infections 10 (24.4%), followed by lid infection 7 (17%) and keratitis 6 (14.6%) (Table 1).

Detection of mecA gene

All of the 41 MRSA isolate were positive for mecA by PCR. 310 bp PCR product was obtained for mecA gene (Figure 1).

Antibiotic susceptibility pattern of MRSA isolates

MRSA isolates from the various ocular infections were tested for antimicrobial susceptibility. All MRSA isolates were susceptible to vancomycin, and significant isolates were also susceptible to chloramphenicol, tetracycline, and amikacin. Ciprofloxacin had very low rates of susceptibility, which are commonly used in conjunctivitis (Table 2).

Table 1: MRSA isolates in various ocular diseases

Ocular Disease	MRSA isolates (%)
Dacryocystitis	10 (24.4)
External hordeolum	07 (17.1)
Corneal ulcer	06 (14.6)
Conjunctivitis	05 (12.2)
Orbital cellulitis	04 (9.8)
Preseptal cellulitis	03 (7.3)
Endophthalmitis	03 (7.3)
Panophthalmitis	02 (4.9)
Post-surgical infection	01 (2.4)
Total	41

Table 2: Sensitivity pattern of commonly used antibiotics in ocular infections

Antibiotics	MRSA (n=41)	Percentage
Vancomycin	41	100.0
Chloramphenicol	40	97.6
Tetracycline	36	87.8
Amikacin	35	85.4
Moxifloxacin	30	73.2
Levofloxacin	28	68.3
Clindamycin	20	48.8
Gentamicin	18	43.9
Ofloxacin	13	31.7
Cotrimoxazole	13	31.7
Azithromycin	10	24.4
Ciprofloxacin	07	17.1

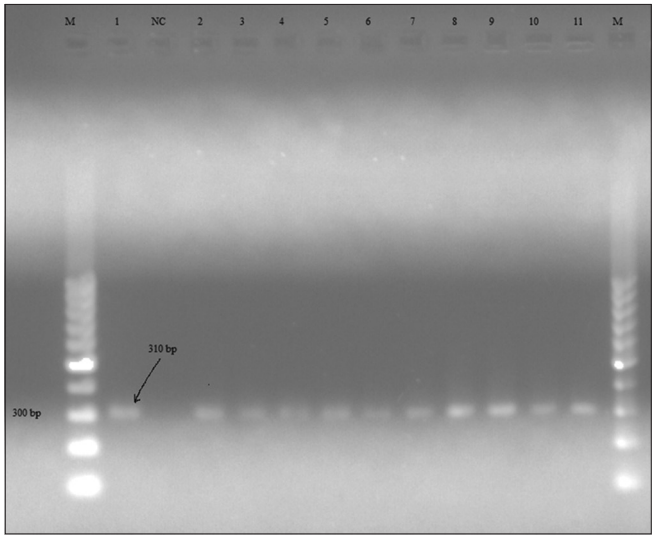

Figure 1: Agarose gel electrophoresis of MRSA isolates (PCR product of 310 bp indicating mecA gene), Lane M 100 bp DNA ladder, NC: Negative control

DISCUSSION

MRSA is a significant burden in various health-care settings. Our study shows that 53.9% of ocular *S. aureus* infections were MRSA. Different studies have depicted variations in MRSA rates in ocular specimens in different countries. About 34.9% prevalence rate of MRSA was reported in the USA, 25.4% MRSA was reported in the pediatric age group in China, and 50% MRSA in Italy.[14-16] Duration of study, types of samples, site of the specimen, and size of sample may be contributory factors in variations of prevalence at different places.

In this study, the most common presentation of ocular MRSA infections was Dacryocystitis (24.4%), followed by lid disorder (17.1%) and a corneal ulcer (14.6%), which is consistent with the previous study done in south India, but Freidlinet al., reported conjunctivitis as a most common manifestation of ophthalmic MRSA in the USA.[2,7] This difference occurs, maybe due to different geographical locations of study.

In terms of antibiotic susceptibility, all MRSA isolates were sensitive to vancomycin.

In our study, different FQs have different degrees of susceptibility. It is also reported by Nithya et al., and Klos et al.[17,18] In our study, among fluoroquinolones moxifloxacin showed the highest efficacy (73.2%).The lowest susceptibility is found in ciprofloxacin (17.1%) and ofloxacin (31.7%). These antibiotics are frequently used in ocular infections.

Chloramphenicol showed good susceptibility (97.6%). Broad-spectrum antibiotics like fluoroquinolones have been used abundantly in recent years and replaced chloramphenicol usage, this could be a possible reason for the good susceptibility shown by MRSA against chloramphenicol. Croghan and Lockington, Harford et al., also showed excellent susceptibility of chloramphenicol against ocular MRSA isolates.[19,20]

Amikacin (85.4%) has a remarkable susceptibility rate compared to Gentamicin (43.9%) among aminoglycosides. Gentamicin is more commonly used as compared to amikacin, this could be a possible reason for the marked difference in the susceptibility of these two antibiotics. Cotrimoxazole (31.1%) and Azithromycin (24.4%) showed disappointing susceptibility for MRSA. This is consistent with the study by Harford et al, who reported 33.9 % susceptibility to azithromycin for MRSA from ocular isolates[20]

Limitations of the study

Due to limited period study and COVID 19 pandemic the sample size was limited.

CONCLUSION

Treatment of MRSA infections is challenging for clinicians. To prevent MRSA infections proper screening & treatment of MRSA carriers should be followed in an institute. Infection control practices should be followed & monitored and regular microbiological surveillance of associated ocular infections & antibiotic policy should be allowed.

ACKNOWLEDGMENT

Authors are highly thankful for the laboratory Supporting staff for their co-operation.

REFERENCES

1. Khosravi AD, Mehdinejad M and Heidari M. Bacteriological findings in patients with ocular infection and antibiotic susceptibility patterns of isolated pathogens. Singapore Med J. 2007;48(8):741-743.
2. Freidlin J, Acharya N, Lietman TM, Cevallos V, Whitcher JP and Margolis TP. Spectrum of eye disease caused by methicillin-resistant *Staphylococcus aureus*. Am J Ophthalmol.2007;144(2):313-315. https://doi.org/10.1016/j.ajo.2007.03.032
3. Hall G, Shrestha R and Vogel S. Methicillin/Oxacillin-Resistant *S. aureus* (MRSA/ORSA): Laboratory detection in 2004. BD Lab O Microbiology News and Ideas. p. 1-3.
4. Liu GY. Molecular pathogenesis of *Staphylococcusaureus* infection. Pediatr Res. 2009;65(5 Pt 2):71R-77R.
https://doi.org/10.1203/PDR.0b013e31819dc44d
5. Yang JA, Park DW, Sohn JW and Kim MJ. Novel PCR-restriction fragment length polymorphism analysis for rapid typing of *Staphylococcal* cassette chromosome mec elements. J Clin Microbiol 2006;44(1):236-238.
https://doi.org/10.1128/JCM.44.1.236-238.2006
6. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Methicillin-Resistant *Staphylococcusaureus*. Atlanta: Centers for Disease Control and Prevention; 2014.
7. Kalwaje EV, Munim F, Tellapragada C, Varma M, Edward LL and Mukhopadhyay C. Upsurge of MRSA bacteraemia in South Indian tertiary care hospital: An observational study on clinical epidemiology and resistance profile. Int J Infect Dis. 2012;6(1):e224.
https://doi.org/10.1016/j.ijid.2012.05.827
8. Shanmuganathan VA, Armstrong M, Buller A and Tullo AB. External ocular infections due to methicillin-resistant *Staphylococcusaureus* (MRSA). Eye (Lond). 2005;19(3):284-291.
https://doi.org/10.1038/sj.eye.6701465
9. Chuang CC, Hsiao CH, Tan HY, Ma DH, Lin KK, Chang CJ, et al. *Staphylococcusaureus* ocular infection: Methicillin-resistance, clinical features, and antibiotic susceptibilities. PLoS One. 2012;8(8):e42437.
https://doi.org/10.1371/journal.pone.0042437
10. Marangon FB, Miller D, Muallem MS, Romano AC and Alfonso EC. Ciprofloxacin and levofloxacin resistance among methicillin-sensitive *Staphylococcusaureus* isolates from keratitis and conjunctivitis. Am J Ophthalmol. 2004;137(3):453-458.

https://doi.org/10.1016/j.ajo.2003.10.026

11. Asbell PA, Sahm DF, Shaw M, Draghi DC and Brown NP. Increasing prevalence of methicillin resistance in serious ocular infections caused by *Staphylococcus aureus* in the United States: 2000 to 2005. J Cataract Refract Surg. 2008;34(5):814-818.
12. Hsiao CH, Chuang CC, Tan HY, Ma DH, Lin KK, Chang CJ, et al. Methicillin-resistant *Staphylococcus aureus* ocular infection: A 10-year hospital-based study. Ophthalmology. 2012;119(3):522-527.

    https://doi.org/10.1016/j.ophtha.2011.08.038
13. McClure J, Conly J, Lau V, Elsayed S, Louie T, Hutchins W, et al. Novel multiplex PCR assay for detection of the *Staphylococcal* virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from-resistant Staphylococci. J Clin Microbiol. 2006;44(3):1141-1144.

    https://doi.org/10.1128/JCM.44.3.1141-1144.2006
14. Asbell PA, Sanfilippo CM, Sahm DF and DeCory HH. Trends in antibiotic resistance among ocular microorganisms in the United States from 2009 to 2018. JAMA Ophthalmol. 2020;138(5):439-450.

    https://doi.org/10.1001/jamaophthalmol.2020.0155
15. Zheng XY, Choy BN, Zhou MM and Zhao ZY. Antibiotic resistance pattern of *Staphylococcus aureus* isolated from pediatrics with ocular infections: A 6-year hospital-based study in China. Front Pediatr. 2021;9:728634.

    https://doi.org/10.3389/fped.2021.728634
16. Francesco P, Veronica F, Biagio S, Gianluigi F, Francesco F, Maria CT, et al. Prevalence and antibiotic resistance patterns of ocular bacterial strains isolated from pediatric patients in university hospital of Campania "Luigi Vanvitelli," Naples, Italy. Int J Microbiol. 2020;2020:1-6.

    https://doi.org/10.1155/2020/8847812
17. Nithya V, Rathinam S, Karthikeyan RS and Lalitha P. A ten year study of prevalence, antimicrobial susceptibility pattern, and genotypic characterization of methicillin resistant *Staphylococcus aureus* causing ocular infections in a tertiary eye care hospital in South India. Infect Genet Evol. 2019;69:203-210.

    https://doi.org/10.1016/j.meegid.2019.01.031
18. Klos M, Pomorska-Wesolowska M, Romaniszyn D, Chmielarczyk A and Wojkowska-Mach J. Epidemiology, drug resistance, and virulence of *Staphylococcus aureus* isolated from ocular infections in polish patients. Pol J Microbiol. 2019;68(4):541-548.

    https://doi.org/10.33073/pjm-2019-056
19. Croghan C and Lockington D. Management of MRSA-positive eye swabs and the potential advantages of chloramphenicol availability in the United Kingdom. Eye (Lond). 2018;32(1):157-159.

    https://doi.org/10.1038/eye.2017.257
20. Harford DA, Greenan E, Knowles SJ, Fitzgerald S and Murphy CC. The burden of methicillin-resistant *Staphylococcus aureus* in the delivery of eye care. Eye (Lond). 2022;36(7):1368-1372.

    https://doi.org/10.1038/s41433-021-01643-6

Authors Contribution:
AR- Concept and design the study, prepared first draft of manuscript; **SWK-** Interpreted the results; reviewed the literature and manuscript preparation; **SMZ-** Concept, coordination, and interpretation, preparation of manuscript and revision of the manuscript; **HMK-** Concept and revision of the manuscript; and **MS-** Drafting, literature review, and final version

Work attributed to:
Jawaharlal Nehru Medical College, AMU, Aligarh - 202002, Uttar Pradesh India

Orcid ID:
Dr. Shariq Wadood Khan - https://orcid.org/0000-0003-3442-105X
Dr. Adil Raza - https://orcid.org/0000-0002-9470-824X
Dr. Shaik Mohammed Zakir - https://orcid.org/0000-0002-5499-0906
Prof. Haris Manzoor Khan - https://orcid.org/0000-0002-5352-310X
Prof. Mohammad Shahid - https://orcid.org/0000-0002-4684-3607

Source of Support: Nil, **Conflicts of Interest:** None declared.