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Research Article

Molecular Characterisation and Bacterial Profile of MRSA (Methicillin-Resistant *Staphylococcus aureus*) at a Tertiary Care Center

Pratikshya Mahapatra*

Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, Odisha, India

***Corresponding Author:** Pratikshya Mahapatra, MBBS MD Microbiology, Senior resident, Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, Odisha, India.

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Abstract

Background and Aim: Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with a higher risk of complications, prolonged hospital stays, a longer duration of therapy and higher expenditures than MSSA. The present study aims to show the prevalence of MRSA and its genotypic characterization in various clinical isolates.

Materials and Methods: This was an observational cross-sectional study that included 71 MRSA strains isolated from 3342 unrepeated clinical samples processed in the Department of Microbiology, All India Institute of Medical Sciences (AIIMS), Nagpur, from February 2021 to June 2022. MRSA was identified by standardized phenotypic methods and genotypic characterization done by *mec*A and *mec*C gene detection by real-time polymerase chain reaction (RT-PCR).

Results: Out of 71 MRSA strains, maximum were isolated from pus samples (n = 47,66.20%), followed by 12 (16.90%) from miscellaneous samples. 57 (80.28%) and 54 (76.06%) MRSA isolates were most resistant to erythromycin and clindamycin, respectively. 71 MRSA were subjected to Real-time PCR for molecular characterization. Of these, 67 (94.36%) only showed the *mec*A gene, 1 (1.41%) both the *mec*A and *mec*C genes, and 3 (4.33%) were negative for both the resistant genes.

Conclusion: The current study revealed a high percentage of MRSA in India, highlighting the need for local and country-based investigations to characterize and monitor MRSA and develop strategies to combat it. Mandatory surveillance, both in hospital and community settings, should be performed in a timely manner to monitor antibiotic sensitivity patterns and reduce the prevalence of MRSA.

Keywords: *MRSA; mecA; mecC; Real Time PCR; Skin Soft Tissue Infection*

Abbreviations

MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Sensitive *Staphylococcus aureus*; MRCoNS: Methicillin-Resistant Coagulase Negative *Staphylococcus* Species; MSCoNS: Methicillin-Sensitive Coagulase Negative *Staphylococcus* Species; RT-PCR: Real Time Polymerase Chain Reaction; CSF: Cerebrospinal Fluid; *S. aureus: Staphylococcus aureus*

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have become a serious global threat in recent years. The meta-analysis study conducted by Patil S., *et al.* (2022) reported the pooled prevalence of MRSA in India at 37% (95% CI: 32 - 41) in between 2015-

2020 [1]. MRSA is also associated with a higher risk of complications, a prolonged hospital stay, a longer duration of therapy, and higher expenditures than methicillin-sensitive *S. aureus* (MSSA). In a study from western Maharashtra (Kolhapur region) 2021, out of 184 *S. aureus* strains isolated from 600 nasal swabs, 73 (39.67%) isolates showed MRSA. Of these, healthcare workers showed more MRSA prevalence, i.e. 39 isolates (21.66%) followed by in-patient 22 (12.22%) and out-patient 12 (6.66%) [2].

Methicillin resistance in *S. aureus* is mediated by *mec*A, which codes for an alternative penicillin-binding protein, PBP2 or PBP2a. Only a small interval has passed since the introduction of methicillin. MRSA was first discovered in London in 1961 [3,4]. By 1968, the first case of MRSA in the U.S. was reported [5]. MRSA strains are frequently resistant to many different classes of antibiotics, which is a serious concern. It can be detected using different phenotypic and genotypic methods. Polymerase chain reaction (PCR) for amplifying *mec*A is currently considered the gold standard for detecting methicillin resistance in *S. aureus* [6].

Aim of the Study

The present study aims to show the prevalence of MRSA and its genotypic characterization in various clinical isolates.

Materials and Methods

The present study was an observational cross-sectional study that included 71 MRSA strains isolated from 3342 unrepeated clinical samples processed in the Department of Microbiology, All India Institute of Medical Sciences, Nagpur, from February 2021 to June 2022. All clinical specimens, including pus samples, skin lesions, aural swab, throat swab, tracheal aspirate, blood culture, urine samples, high vaginal swab, CSF, joint fluids, and eye discharge, except stool samples, were obtained from the Microbiology Laboratory of AIIMS, Nagpur, and were subjected to bacterial culture and sensitivity testing using CLSI 2021 guidelines. The strains were identified and confirmed as *S. aureus* using the catalase, slide and tube coagulase, DNase, thermonuclease and phosphatase tests.

Antibiotic susceptibility testing

A cefoxitin disk diffusion test was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI) to detect *mec*A-mediated resistance in *S. aureus*. All cefoxitin-resistant strains were subjected to Vancomycin MIC testing using an Epsilometer test. All results were interpreted according to CLSI 2021 guidelines. The results of antimicrobial susceptibility tests by disk diffusion method for penicillin (1 IU), gentamycin (10 µg), clindamycin (2 µg), doxycycline (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), erythromycin (15 µg), linezolid (30 µg), nitrofurantoin (300 µg) were also retrieved from the isolate testing.

Genotyping method

Detection of *the mec*A and *mec*C genes was performed by real-time polymerase chain reaction (RT-PCR) for rapid MRSA identification. Bacterial DNA purification (QIAmp DNA Extraction Mini Kit) was used to extract DNA from purified [on Brain Heart Infusion Broth (BHI) for 24 hours at 37°C] and performed on phenotypically confirmed MRSA and MRCoNS. 400 µl of Lysozyme digestion buffer and 75 µl of Proteinase K were added to 500 µL of the sample and centrifuged. Both wash 1 and wash 2 buffers (500 µL) were added to the spin column and centrifuged. Finally, 40 - 60 µl of elution buffer was added, and the purified DNA was stored at -20°C.The *mec*A and *mec*C genes are the structural genes for a low-affinity penicillin-binding protein (PBP2') that is found in MRSA strains. Antibiotic resistance genes (*mec*A and *mec*C) were amplified using the TRUPCR® MRSA Real-Time PCR Detection Kit primer kit and a real-time PCR thermocycler (QuantStudio™ 5 Real-Time PCR; Applied Biosystems Thermofisher, USA), in which specific primers were used (Table 1). PCR was carried out for both genes (*mec*A and *mec*C) in the following conditions: initial denaturation at 94°C for 10 min, followed by 40 cycles of 94°C for 15 sec, 60°C for 45 sec, and 72°C for 15 sec. Data acquisition and analysis of the real-time PCR assay were performed (threshold value range: 5000 - 40000).

Statistical analysis

In this study, statistical analysis was performed on the data using the chi-squared test. OpenEpi Stat application and Microsoft Excel 2019 were used for statistical analysis, and a p-value ≤ 0.05 was used to illustrate the statistically significant differences.

Results

Clinical characteristics

Out of 144 *Staphylococcus aureus* isolates, 71 (49.30%) MRSA strains were collected between February 2021 and June 2022. The male-to-female ratio was found to be 1.15:1. Most MRSA strains were isolated from patients 41 - 60 years of age (35.21%). Among the MRSA strains, the highest number was observed in pus samples (66.20%), followed by 12 (16.90%) from miscellaneous samples, 7 (9.86%) from respiratory samples, and 3 (4.23%) from blood cultures (Table 1). Most MRSA strains were isolated from patients in the medicine ward (23.94%). The results of antibiotic susceptibility tests were also studied (Table 2). All MRSA isolates were penicillin-resistant. 57 (80.28%) and 54 (76.06%) isolates were resistant to erythromycin and clindamycin, respectively. All the MRSA strains were susceptible to vancomycin and linezolid.

Characteristics n = 144	Parameters	MRSA isolates n = 71 (%)
Age in years	0 - 20	12 (16.90)
	21 - 40	16 (22.54)
	41 - 60	25 (35.21)
	> 60	18 (25.35)
Specialty	Medicine	17 (23.94)
	Surgery	14 (19.71)
	Obstetrics & Gynaecology	7 (9.86)
	Paediatrics	2 (2.82)
	Pulmonary	3 (4.22)
	Dermatology	5 (7.04)
	Orthopaedics	2 (2.81)
	ENT	1 (1.41)
	ICU	7 (9.85)
Specimen	Pus	47 (66.20)
	Blood	15 (21.13)
	Respiratory	5 (7.04)
	Miscellaneous	4 (5.63)
Infection	Skin and soft tissue infection	31 (43.66)
	Bacteraemia	13 (18.31)
	Respiratory	8 (11.26)
	Musculoskeletal	7 (9.86)
	Others	11 (15.49)
Total		71 (100)

Table 1: *Demographic profile and other characteristics amongst MRSA strains (n = 71).*

Name of Antibiotic	S. aureus Isolates (n = 144) (%)	MRSA (n = 71) (%)
Penicillin (10 units)	142 (98.61)	71 (100)
Cefoxitin (30 µg)	71 (49.30)	71 (100)
Erythromycin (15 µg)	106 (73.61)	57 (80.28)
Clindamycin (2 µg)	98 (68.06)	54 (76.06)
Cotrimoxazole (5 µg)	69 (47.92)	28 (39.44)
Gentamycin (10 µg)	18 (12.50)	5 (7.04)
Tetracycline (30 µg)	40 (27.78)	27 (38.03)
Doxycycline (30 µg)	20 (13.89)	12 (16.90)
Ciprofloxacin (5 µg)	87 (60.42)	52 (73.24)
Vancomycin (E strip)	0(0)	0 (0)
Linezolid (30 µg)	0(0)	0 (0)

Table 2: *Antibiotic resistance pattern of the Staphylococcus aureus and MRSA strains isolated from different clinical specimen.*

Genotypic characteristics

Genotyping was done with the following primer probe (*mec*A and *mec*C) Gene oligopeptide Sequence (TruPCR MRSA detection kit).

Gene Name	Nucleotides	Base pair
mecA	5'-GTAGAAATGACTGAACGTCCGATAA-3' 5'-CCAATTCCACATTGTTTCGGTCTAA-3'	162 bp
mecC	5'-CTTGGTTATTCAAAGATGACGA-3' 5'-ACGTCTTTAACATTAATCGCCA-3'	138 bp

Table

Methicillin resistance genes (*mec*A and *mec*C) were tested by multiplex real-time PCR for 71 MRSA and 9 Methicillin-resistant coagulase-negative Staphylococcus species (MRCoNS). Out of the 71 MRSA isolates, 67 (94.36%) were positive for the *mec*A gene, 1 (1.41%) was positive for both *mec*A and *mec*C, and 3 (4.23%) were negative for both genes. Out of the 49 MRSA isolates from hospitalized patients, 45 (91.84%) were positive for *mec*A only, 1 (2.04%) was positive for both *mec*A and *mec*C, and 3 (6.12%) were negative for both genes. All isolates were positive for *mec*A in the community MRSA isolates (n = 22).

Figure 1 and 2 illustrate the positive control and *mec*A gene-positive amplification plots, respectively. Nine MRCoNs were also tested: 6 (66.67%) carried the *mec*A gene only, and 3 (33.34%) were found to be both *mec*A- and *mecC*-positive. Figure 3 illustrates a positive amplification plot for both *mec*A and *mec*C genes in MRCoNs isolates.

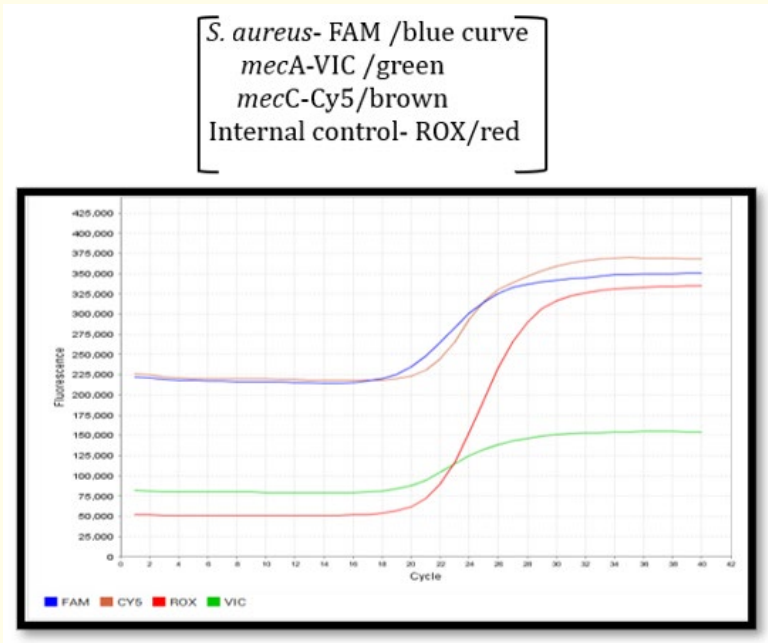

Figure 1: *Positive control multiplex PCR plot of mecA and mecC.*

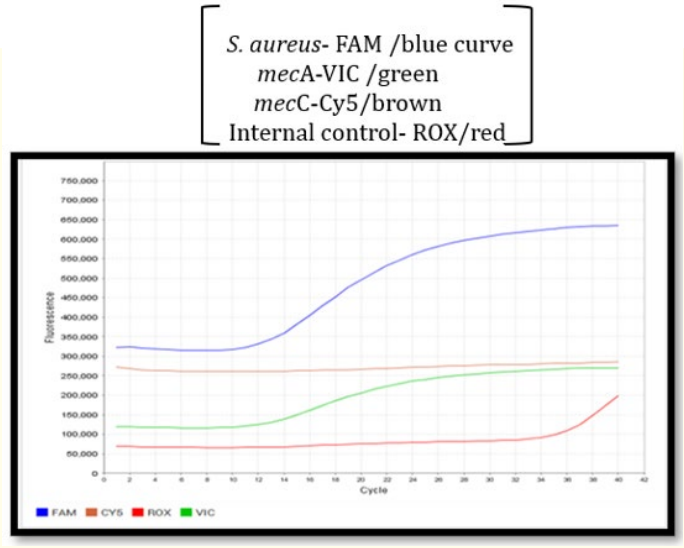

Figure 2: *Sample multiplex PCR plot positive for mecA.*

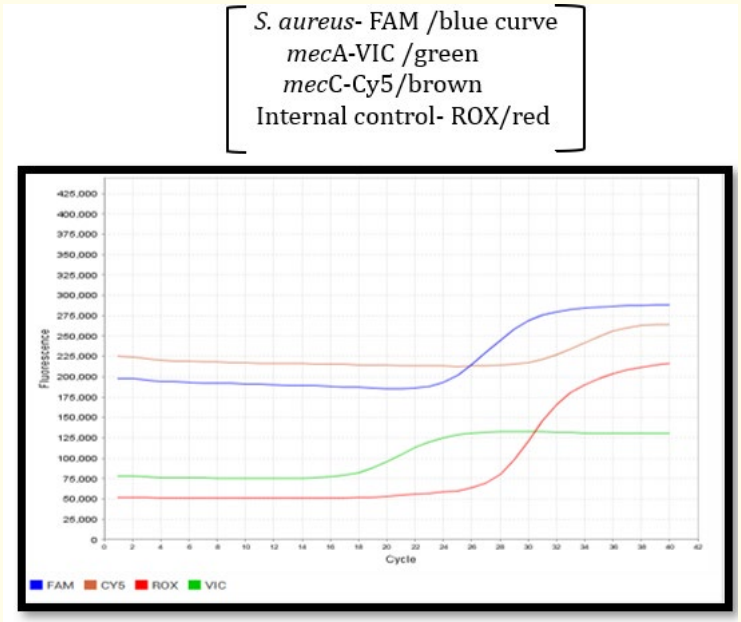

Figure 3: *Sample multiplex PCR plot positive for mecA and mecC.*

Discussion

Normal populations can be colonized by *S. aureus*, commonly on outer skin surfaces (humid areas) and the upper respiratory tract, particularly the anterior nares. MRSA is categorized as one of the major causes that leads to both healthcare- and community-acquired infections [7]. In the present study, among the 159 staphylococcal isolates, 144 (90.56%) were *S. aureus* and 15 (9.43%) were coagulase-negative *Staphylococcus* species (CoNS). Similarly, Mohammad Ali., *et al.* (2019) and Saravanan Murugesan., *et al.* (2021) reported 83.61% of *S. aureus* isolates, followed by 16.37% of CoNS among 183 *Staphylococcus* species isolates, and 66.70% of *S. aureus*, followed by 30.25% CoNS among 1176 *Staphylococci* isolates from cancer patients in Kerala, respectively [8,9]. MRCoNS are one of the most common problem among the *Staphylococcus* species (except *S. aureus*) causing human infections, and their multidrug resistance is more noticeable than that of Methicillin-sensitive coagulase-negative *Staphylococcus* species (MSCoNS). Among CoNS, *S. epidermidis* and *S. haemolyticus* are the predominant species reported in the present study. According to antibiotic surveillance annual report of ICMR (2021-22), CoNS were the predominant isolates in blood (21.8%) and CSF (8.6%) reflecting the high incidence rate of shunt infections and intra vascular device associated infections respectively [10]. Out of 71 MRSA-infected patients, 12 (16.96%) were from age group: 0 - 20 years, 16 (22.50%) from 21 - 40 years age group, 25 (35.21%) from 41 - 60 years age group and 18 (25.35%) from aged > 60 years. Similar results were found in the Lohan., *et al.* (2020) study, which reported that the maximum number of MRSA strains (34.56%) were isolated from patients aged > 60 years, followed by 0 - 15 year-old patients (22.20%), 45 - 60 year-old patients (16%) and 35 - 45 year-old patients (14.80%) [11]. No significant association with the age of patients were found in our study.

Emergence of MRSA remains a constant threat for physicians treating life threatening infections. As per recent 2021-22 ICMR antibiotic surveillance report, *S. aureus* remains predominant cause of sepsis and skin and soft tissue infection after *Enterobacterales* (specifically *E. coli* and *Klebsiella pneumoniae*) [10]. In the present study, among the pus samples, 47 (66.20%) were MRSA isolates, followed by 15 (21.13%) blood isolates, 5(7.04%) respiratory isolates, and 4 (5.63%) miscellaneous samples. In contrast to our findings, Anjholani., *et al*.

(2019) reported that the isolation of MRSA was maximum from respiratory (69%) followed by 7.56% from body fluids [12]. But similar results were reported by Kaur., *et al.* in 2019: 59.2% of MRSA strains were isolated from pus samples, followed by 18.5% in urine, 12.9% in blood, 4.5% in respiratory samples, and 3% in body fluids. *S. aureus was* isolated in maximum numbers from pus samples and MRSA accounts for most of these infections. The factor contributing to the emergence of MRSA in pus samples could be due to carriage of MRSA in the nose, axilla, perineum and hands of patients and healthcare workers, longer hospital stay, irrational use of antibiotics, presence of indwelling devices like catheter and cannulas, immunosuppression, elderly age, insulin-requiring diabetes, decubitus ulcers, etc [13]. Another probable explanation could be *S. aureus* having surface proteins [for example, such as fibronectin-binding protein A (FnBPA), FnBPB, clumping factor A (ClfA), ClfB, and collagen adhesin (Cna)] which bind to extracellular matrix proteins and enable bacteria to attach to and multiply on wounded tissues [14].

The maximum number of MRSA isolates were obtained from IPD (65.28%) from which medicine unit 17 (23.94%) have the highest number of *S. aureus* isolates, followed by surgery unit 14 (19.71%). On contrary to our study, Kaur., *et al.* in 2019 study reported most of the isolates from surgery and orthopedics unit [13]. Possible explanation behind this trend could be the isolation of MRSA from skin and soft tissue infections received from mostly surgery and orthopedics unit. In our study, resistance rate against erythromycin and ciprofloxacin were reported high in MRSA isolates. That was in accordance to ICMR annual report 2021-22 which reported higher resistance pattern against erythromycin (76%) and ciprofloxacin (87%) in all samples across India [10]. These drugs still remain first line of treatment for *S. aureus* associated infections and its resistance in these isolates leads to management dilemma for treating physicians. All MRSA isolates in the present study were susceptible to vancomycin and linezolid. This indicates that vancomycin is the preferred medication for treating multidrug-resistant MRSA infections; nonetheless, routine testing for other, more recent drugs, such as linezolid, and regular monitoring of vancomycin sensitivity should be done [9].

Real-time PCR was performed using two sets of primers for the amplification of the *mec*A and *mec*C genes. Out of the 71 MRSA isolates, 67 (94.36%) showed only the *mec*A gene, and 1(1.41%) showed both *mec*A and *mec*C genes. 3 (4.23%) from 71 MRSA isolates that were characterized as MRSA by all three phenotypic methods used did not show any of the methicillin resistance genes in genotypic identification. These isolates were obtained from IPD patients with a history of prolonged hospital stays and comorbidities. According to Sharma., *et al.'s* (2017) [15] study, a PCR assay for the *mec*A gene identified 84 isolates, of which 80 (95.2%) were *mec*A-positive and 4 (4.8%) were *mec*A-negative. While Other researchers, including Anitha Madhavan., *et al*. (2021) [16] and Pramodhini., *et al.* (2017) [17], reported complete detection of the *mec*A gene in every MRSA isolate that had been identified phenotypically. The above findings of our study of three isolates that were *mec*A- and *mecC-negative* could be due to point mutations or deletions in genes. The lack of *mec*A and *mec*C genes may be due to other methicillin resistance mechanisms, such as *mec*A homologous genes [17]. Additionally, nine MRCoNs were also tested; six (66.67%) carried the *mec*A gene only, and three (33.34%) carried both *mec*A and *mec*C genes. Variations between phenotypic and genotypic methods may be attributable to culture settings, temperature, medium configuration, inoculum size, incubation time, and the manual skills of medical personnel [18]. The PCR method (detection of *mec*A gene) remains the gold standard for the detection of methicillin resistance in *Staphylococcus aureus* and it is not yet available in most routine clinical laboratories in India. Genotyping should be performed in the reference laboratories for epidemiological monitoring of resistance genes and to maintain vigilance on novel MRSA genes.

Conclusion

MRSA is a causative agent of a vast range of serious infections, from skin and soft tissue infections to life-threatening illnesses such as pneumonia, bloodstream infections, and surgical site infections. The current study revealed a high percentage of MRSA among *S. aureus* isolates, highlighting the need for local and country-based rapid diagnostic modalities to characterize and monitor MRSA and develop

the strategies to combat it effectively. All hospital care institutes should strictly follow stringent measures to implement antimicrobial stewardship and hospital infection control policies, particularly strict compliance with hand hygiene practices. Mandatory surveillance, both in hospital and community settings, should be performed in a timely manner to monitor antibiotic sensitivity patterns and reduce its prevalence.

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Conflict of Interest

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The author declare no competing interests.

Bibliography

1. Patil Sharanagouda S., *et al*. "Prevalence of methicillin-resistant *Staphylococcus aureus* in India: a systematic review and meta-analysis". *Oman Medical Journal* 37.4 (2022): e440.

2. Parthasarathy Arun Kumar and Roma A Chougale. "Prevalence of Methicillin Resistant *Staphylococcus aureus* and its Associated SCCmec Types among Healthcare workers and Patient Visitors from Western Maharashtra, India". *Journal of Pure and Applied Microbiology* 16.2 (2022).

3. Saga Tomoo and Keizo Yamaguchi. "History of antimicrobial agents and resistant bacteria". *Japan Medical Association Journal* 52.2 (2009): 103-108.

4. Maeder K., *et al*. "Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in patients with spinal cord injury". *Spinal Cord* 31.10 (1993): 639-644.

5. Kallen Alexander J., *et al*. "Health care-associated invasive MRSA infections, 2005-2008". *Journal of the American Medical Association* 304.6 (2010): 641-647.

6. Bhutia Kunsang O., *et al*. "Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus*". *International Journal of Applied and Basic Medical Research* 2.2 (2012): 84-91.

7. Mehraj Jaishri., *et al*. "Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* nasal carriage in a random sample of non-hospitalized adult population in northern Germany". *PloS one* 9.9 (2014): e107937.

8. Ali M., *et al*. "Prevalence of Staphylococcus Species from Clinical Samples Obtained from Some Hospitals on Kano Metropolis, Nigeria". *American Journal of Biomedical Science and Research* 5.3 (2019).

9. Murugesan Saravanan., *et al*. "Methicillin-resistant staphylococci prevalence in cancer patients at a tertiary care cancer centre-a retrospective study". *Asian Journal of Pharmaceutical and Clinical Research* 14.7 (2021): 82-85.

10. ICMR Annual Report (2021-22) | Indian Council of Medical Research | Government of India.

11. Sangwan Jyoti., *et al*. "Prevalence pattern of MRSA from a rural medical college of North India: a cause of concern". *Journal of Family Medicine and Primary Care* 10.2 (2021): 752-757.

12. Aljohani Sameerah., *et al*. "Occurrence of vancomycin MIC creep in methicillin resistant isolates in Saudi Arabia". *Journal of Infection and Public Health* 13.10 (2020): 1576-1579.

13. Kaur Kiranjeet., *et al*. "Methicillin resistance, vancomycin intermediate and vancomycin resistance *Staphylococcus aureus* prevalence in a tertiary care hospital of Punjab, India". *National Journal of Laboratory Medicine* 8.3 (2019): 01-03.

14. Lee Andie S., *et al*. "Methicillin-resistant *Staphylococcus aureus*". *Nature Reviews Disease Primers* 4.1 (2018): 18033.

15. Sharma Sheetal., *et al*. "Evaluation of different phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus* and antimicrobial susceptibility pattern of MRSA". *International Journal of Community Medicine and Public Health* 4.9 (2017): 3297-3301.

16. Madhavan Anitha., *et al*. "Comparison of PCR and phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus*". *Iranian Journal of Microbiology* 13.1 (2021): 31-36.

17. Pramodhini S., *et al*. "Comparison of various phenotypic methods and mecA based PCR for the detection of MRSA". *Journal of Clinical and Diagnostic Research* 5.7 (2011): 1359-1362.

18. Shahi Kushal., *et al*. "Methicillin resistant *Staphylococcus aureus*: prevalence and antibiogram in various clinical specimens at Alka Hospital". *Tribhuvan University Journal of Microbiology* 5 (2018): 77-82.

19. Rao Sangeeta., *et al*. "Antimicrobial resistance and genetic diversity of *Staphylococcus aureus* collected from livestock, poultry and humans". *One Health* 15 (2022): 100407.

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