Journal of Cardiovascular Disease Research
ISSN: 0975-3583, 0976-2833 VOL 14, ISSUE 10, 2023

"To Study The Prevalence And The Molecular Characterization Of Mec A Gene In MRSA Isolates At A Tertiary Care Centre, Uttar Pradesh, India".

Original Article

Deepak Kumar[1], Sahina Hassan[2], Mohd. Saqib Hasan[3], Deepika Shukla[4], Nashra Afaq[5], Ambika Bhatiani[6], Nilam Nigam[7], Qazi Rais Ahmad[8], Ritika Tiwari[9*]

[1]Assistant Professor and Head, Department of Microbiology, Uma Nath Singh Autonomous State Medical College, Jaunpur, India.

[2]Assistant Professor, Department of Microbiology, Late Baliram Kashyap Government Medical College, Jagdalpur, Chattisgarh, India.

[3]Senior Resident, Department of Microbiology, King George Medical College, Lucknow, India.

[4]Assistant Professor, Department of Microbiology, Maharana Pratap Dental College and Hospital Kothi, Mandhana, Kanpur, Uttar Pradesh, India.

[5]Research Associate, Department of Microbiology and Central Research Laboratory, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India.

[6]Professor and Head, Department of Microbiology, Naraina Medical College and Research Centre, Panki, Kanpur, India.

[7]Professor and Head, Department of Pathology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India.

[8]Professor and Head, Department of Physiology, Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, Uttar Pradesh, India.

[9*]Tutor, Department of Microbiology, Uma Nath Singh Autonomous State Medical College, Jaunpur, India.

***Corresponding author: Ritika Tiwari**

Email: ritikat862@gmail.com

Abstract

Introduction: The emergence of strains resistant to methicillin and other antimicrobials has become a major concern, particularly in the hospital settings as MRSA infections are resistant to the majority of conventional antibiotics, possesing serious risks to hospitals and communities all over the world.

Aim and Objectives: To study the prevalence and the molecular characterization of Mec A gene in MRSA isolates at a tertiary care centre, Uttar Pradesh, India.

Material and Methods: This was a cross sectional study conducted in the Department of Microbiology at a tertiary care centre, for a period of 1 year i.e, August 2022 to August 2023. A

total of 682 clinical isolates was studied out of which 200 isolates of *S. aureus* were identified using the biochemical test from the clinical samples such as pus, swab, blood, wound and urine etc. The different Phenotypic Methods including Cefoxitin and Oxacillin Disc Diffusion test and the genotypic method including MecA gene detection for Methicillin Resistant Staphylococcus aureus isolates was performed. The DNA was extracted using the DNA extraction Qiagen Kit and the Mec A gene was detected by the PCR method.

Results: In the present study a total of 42 MRSA isolates were identified by CX, OX, and E-test test phenotypically. The prevalence of MRSA was found to 21% . The maximum number of isolates were observed from the OPD ward with 38.5%. The ratio of the males 28 (66.6%) was more as compared to the females with 14 (33.3%) with the maximum age of 41-50 years being affected the most followed by 31-40 years and least in the age group of 61years and above. The pus 47.6% was the most common isolate followed by the blood with 23.8% and least for urine and the body fluids 2.3%. All the MRSA isolates were found sensitive to linezolid, Teicoplanin, vancomycin, however all the isolates were recorded resistant with Cefoxitin and Oxacillin. The presence of MecA gene was recorded in all the 42 isolates of MRSA.. The presence of MecA gene was confirmed by the PCR followed by sequencing assay.

Conclusion: This study provides a clear guidance to effectively diagnose and measure MRSA infections in hospitals and communities. Future research could identify MRSA isolates with the help of the data produced in this investigation. For the control of antibiotic resistance, there is a need for ongoing monitoring and the deployment of effective control techniques where continuous surveillance, awareness of the incidence of MRSA, and upkeep of hygienic standards have been needed to reduce MRSA infections.

Keywords: MRSA, Disc Diffusion, Antibiotic Sensitivity, MecA gene, Molecular Profiling

INTRODUCTION

The *Staphylococcus aureus* (*S. aureus*) is one of the pathogens that is well-known to cause infections in human skin, soft tissues, deep-seated tissues, pneumonia, and post-operative sites. Methicillin-susceptible *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) are the two primary strain varieties of the Gram-positive cocci bacteria *Staphylococcus aureus* [1].

The term methicillin-resistance is a classic term that implies resistance to all beta-lactam antibiotics, except for recently introduced anti-MRSA cephalosporin's, such as ceftobiprole. MRSA, which was first reported in the 1960s [2] has become endemic in hospitals and health-care settings worldwide. The frequency of methicillin-resistant *S. aureus* (MRSA) isolates is increasing [3, 4] and this issue can lead to severe therapeutic dilemmas and exacerbate the control of infections in hospitals settings [5]. The mecA gene, which encodes for a modified penicillin-binding protein, PBP2a, with decreased beta-lactams affinity [6] is responsible for methicillin-resistance among bacteria, including MRSA. The gene is located on a mobile genetic element defined as staphylococcal cassette chromosome mec (SCC mec) . Till now, 13 types of SCCmec elements have been characterized (IXIII) that each type has its own specific characteristics.

Additionally, MRSA has been identified as the source of community- and hospital-acquired (CA-MRSA) infections [7]. Numerous severe infections, including nosocomial, necrotizing fasciitis, potentially deadly illnesses, pneumonia, osteomyelitis, endocarditis, severe sepsis, and toxic shock syndrome, have been linked to MRSA in recent years [8,9]. MRSA have created a big challenges about to cure an infected person due to resistance to multiple classes of antibiotics including penicillin, and methicillin. Furthermore, MRSA has been found co-resistance with vancomycin, linezolid (oxazolidinone), and tigecycline [10]. Only new classes of antibiotic such as Rifampicin

etc have been used to cure MRSA and biofilm infections. However, vancomycin-resistant *S. aureus* and rifampicin-resistant *S. aureus* strains have been recorded in China [11] may be due to mutations in rpoB gene. So, it is important to monitor continuously the prevalence, virulence factors, and antibiotics resistance patterns of MRSA for the control of its infections and to reduce extra in-hospital costs [12].
Drug of choice to treat these multidrug resistant MRSA are glycopeptide antibiotics such as vancomycin [13]. The increase in the resistance to MRSA with the decreases susceptibility of the glycopeptides antibiotics is a worrisome problem observed worldwide.
Therefore, the present study was undertaken to study the prevalence and the molecular characterization of MecA gene in MRSA isolates at a tertiary care centre, Uttar pradesh, India.

MATERIAL AND METHODS

The Present study was a cross sectional study carried out in the Department of Microbiology at a Tertiary care Centre, Uttar Pradesh ,India for a period of 1 years i.e., August 2022 to August 2023. A total of 682 clinical isolates were studied out of which 200 isolates of *S. aureus* were identified using the biochemical test from the clinical samples such as pus, swab, blood, wound and urine etc.

The different Phenotypic methods including cefoxitin , oxacillin disc diffusion test, E test were carried out. The samples were processed immediately to the laboratory and tested for their biochemical test for the identification according to the CLSI guidelines 2022 [14]. In case of delay the samples were kept at 4^0C. The patients demographic profile along with the written consents were obtained from all the participants involved in this study. Ethical Clearance was duly obtained from the Ethical committee before the start of the study.

Screening of the MRSA:

Phenotypic screening:

On the basis of colony morphology, mannitol fermentation, Gram staining, catalase test, coagulase test and DNase activity MRSA isolates were identified. The phenotypic MRSA was performed using the cefoxitin, oxacillin disk diffusion test, E-test as per the protocol of the Clinical and Laboratory Standard Institute guidelines (CLSI) [14].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed by modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (HiMedia laboratories private limited, India) .Antibiotic discs used were ciprofloxacin (5µg), clindamycin (2µg), chloramphenicol (30µg), erythromycin (15µg), gentamicin (10µg), tetracycline (30µg), cotrimoxazole (25µg), rifampin (5µg), mupirocin (200µg), and penicillin G (10 units) cefoxitin, oxacillin as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. [14].
All the isolates were tested by making a lawn culture of 0.5 Mc Farland suspensions of isolates on Muller Hinton Agar (MHA) plate. Plates were analysed after incubation at 37°C for 18 h. The zone diameter of ≤19 mm was considered as antibiotic-resistant for MRSA as per the CLSI guidelines [14].

Genotypic screening

The molecular characterization for the detection of MecA gene of the clinical isolates was performed. The DNA was extracted using the DNA extraction Qiagen Kit (Germany. The MecA gene was identified as gold standard test for the identification of MRSA by using polymerase chain reaction (PCR) [15]. Cefoxitin was considered as an inducer of MecA gene expression.
The primers for MecA gene was synthesized by Chromous Biotech. Pvt. Ltd. (Bangaluru).

Figure No.1: The DNA Extraction kit **Figure No.2:** The Reagents used for the DNA Extraction

Gene	Primer sequence	Length bp)	Reference
MecA gene	5'- GTTGTAGTTGTCGGGTTTGG-3' 5'- CTTCCACATACCATCTTCTTTAAC -3'	335	[16]

Table No. 1 : The Primers used for the MecA gene fragment

Figure No. 3: The MecA gene primers synthesized by Chromous Biotech

Polymerase Chain Reaction (PCR)

The obtained DNA fragment were amplified in PCR (BIO-RAD T100 Thermal Cycler, Singapore) (volume 20 µl) by mixing 10µl master mix (Takara), 5µl nuclease free water, 1 µl forward and reveres primer each and 3µl DNA as a template for PCR conditions with initial denaturation at 94oC for 5 min, then for 34 cycle at 94oC for 30 sec for cycle denaturation, 50oC for 45 sec for annealing for MecA gene.

Step	**Program** **MecA gene** **Time** **Temperature**	**Cycles**
Initial denaturation	15 min 95 ºC	
Denaturation	30 s 94 ºC	
Annealing	1 min30 s 59 ºC	30
Extension	1 min 30 s 72º C	
Final extension	10 min 72º C	

Table No. 2 : The PCR cycling conditions to amplify Mec A gene fragments.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System
The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1% agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [17,18].

RESULTS

A total of 682 clinical isolates were studied out of which 200 isolates of *S. aureus* were identified using the biochemical test from the clinical samples such as pus, swab, blood, wound and urine etc. The different Phenotypic Methods including Cefoxitin ,Oxacillin Disc Diffusion test E test and the genotypic method including MecA gene detection for Methicillin Resistant *Staphylococcus aureus* isolates for the MecA gene was performed. Out of which a total of 42 MRSA isolates were identified by CX, OX, and E-test. The DNA was extracted using the DNA extraction Qiagen Kit and the Mec A gene was detected by the PCR.

Microscopic observation	Gram's test	Catalase test	Coagulase test		Urease test	Cefoxitin(cx) and Oxacillin(ox)	DNAase Test
Cocci form (For all 220 cases)	+	+	Slide+	Tube+	+	+	+

Table No.3: Phenotypic Identification of *S.aureus* with the use of different test

Type of Clinical Isolates	Number of Ioslates	Percentage
S.aureus	200	29.3%
Others clinical isolates	482	80.3%

Table No. 4: The type and the total number of clinical isolates

From the Table no. 4 it was observed that the *S.aureus* isolates were 29.3%, and the other clinical isolates were 80.3%.

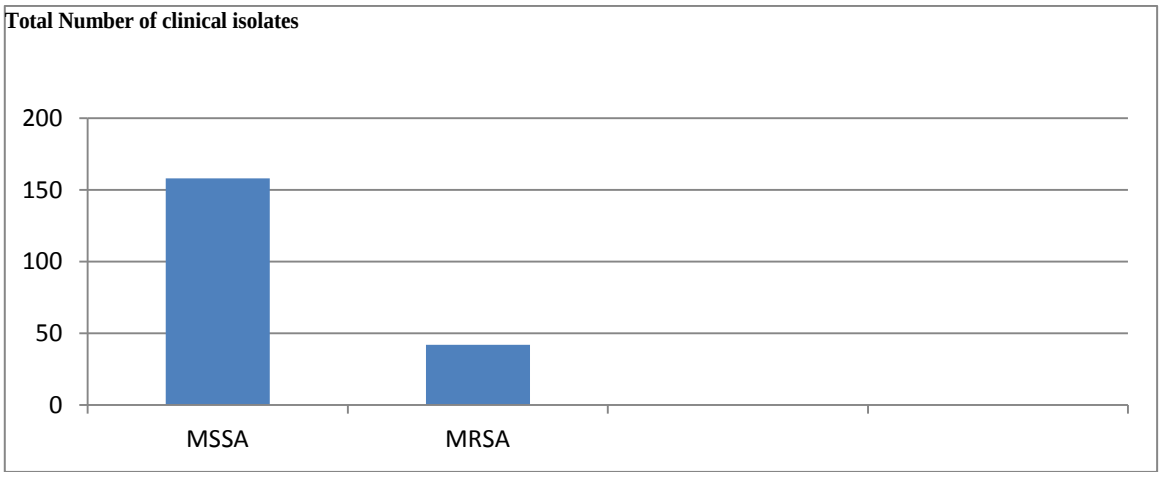
S.N.	Location	Isolates N=200	Percentage
1.	Surgery ward	58	29%
2.	NICU	17	8.5%
3.	Medicine ward	48	24%
4.	OPD	77	38.5%

Table No. 5: The ward wise distribution of *S. aureus*

The maximum number of isolates was obtained from OPD of the hospitals followed by the surgery ward 29%.
For the Table no. 6 it was observed that out of the 200 isolates of *S.aureus* there were 42 isolates of the MRSA with the prevalence of 21%.

Organism	Disc diffusion test	E-test	MIC test
MSSA	158 (%)	-	-
MRSA	42 (CX, OX) (%)	42	-
Total	200		

Table No. 6: Identification of staphylococcal strains with the use of different microbiological tests

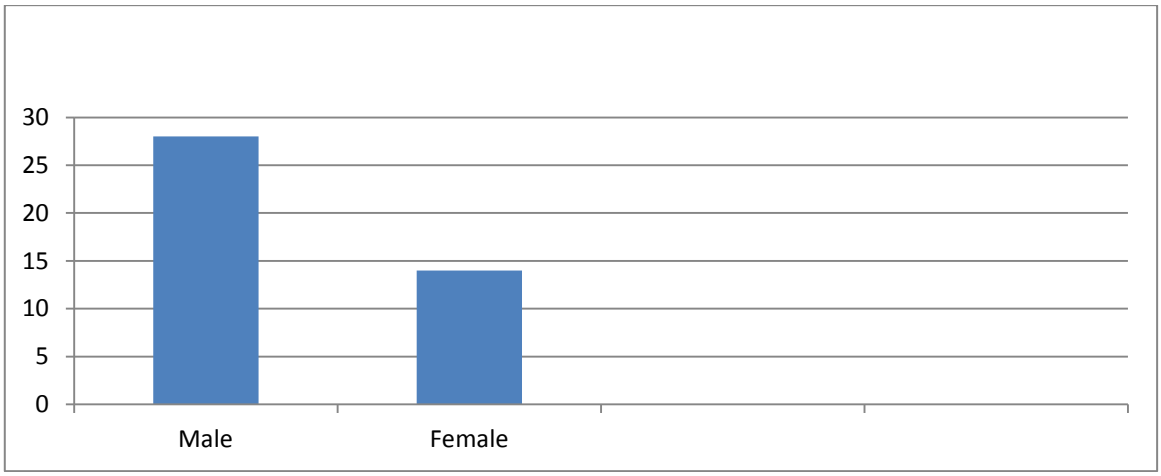


Graph No.1 : The graphical representation of the distribution of MSSA and MRSA isolates

Genderwise	Number of Is0lates	Percentage
Male	28	66.6%
Female	14	33.3%

Table No. 7: Total Number of clinical isolates

From the Table no.7, it was observed that the ratio of the males 28 (66.6%) was more as compared to the females with 14 (33.3%) with the maximum age of 41-50 years being affected the most followed by 31-40 years and least in the age group of 61years and above.



Graph No. 2: Graphical representation of Gender wise distribution of isolates

S.No.	Sample Site	Number of Isolates	Percentage
1.	Pus	20	47.6%
2.	Blood	10	23.8%
3..	Urine	1	2.3%
4.	Sputum	5	11.9%
5.	Throat swab	5	11.9%
6.	Body fluids	1	2.3%

Table No. 8: The distribution of *S. aureus* from different sample site

The pus 47.6% was the most common isolate followed by the blood with 23.8% and least for urine and the body fluids with 2.3%.

S.N.	Age group (Years)	Male N=130	Percentage	Female N=70	Percentage	*p*-value
1.	0-10	13	10%	7	9.98%	0.054
2.	11-20	18	13.84%	9	12.85%	
3.	21-30	14	10.76%	13	18.57%	
4.	31-40	18	13.84%	11	15.71%	
5.	41-50	34	26.15%	18	25.71%	
6.	51-60	24	18.46%	4	5.71%	
7.	61-70	9	4.61%	8	11.42%	

Table No. 9: Age wise distribution of the *S. aureus* infected patients

Phenotypic Identification of MRSA

Antibiotic sensitivity pattern was obtained with disc diffusion test. The obtained resistance and sensitivity zone are recorded in the following Table no. 10. All methicillin-resistant *staphylococci* were analysed for their susceptibility against commonly used antibiotics. All MRSA isolates were found sensitive to linezolid, Teicoplanin, vancomycin, and were found resistance to Cefoxitin and Oxacillin.

S.N.	Antibiotic	Disc potency	Resistance (mm)	Sensitive (mm)
1.	Deoxycycline (D)	30µg	30 (15%)	170 (85%)
2.	Erythromycin (ER)	15µg	75 (37.5%)	125 (62.5%)
3.	Gentamycin (GM)	10µg	20 (10%)	180 (90%)
4.	Linezolid	30µg	-	200 (100%)
5.	Oxacillin (OX)	1µg	42 (21%)	158 (79%)
6.	Penicillin (P)	10µg	180 (90%)	20 (10%)
7.	Teicoplanin (TEI)	30µg	-	200 (100%)
8.	Tetracyclin (TE)	30µg	30 (15%)	170 (65%)
9.	Vancomicin (VAN)	30µg	-	200 (100%)
10.	Ampicillin (AMP)	10µg	40 (20%)	160 (80%)
11.	Amoxicillin Clavunic acid (AMC)	20/10µg	25 (12.5%)	175 (84.09%)
12.	Cefoxitin (CX)	30µg	42 (21%)	158 (79%)
13.	Chloramphenicol (C)	30µg	40 (20%)	160 (80%)
14.	Ciprofloxacin (CIP)	5µg	25 (12.5%)	175 (87.5%)
15.	Clindamycin (CD)	2µg	85 (42.5%)	115 (57.5%)
16.	Co-Trimoxozole(COT)	25µg	35 (17.5%)	165 (82.5%)

Table No. 10: Antibiotic sensitivity pattern of *staphylococcus aureus* (N=200)

Molecular analysis:

The authentic confirmation of MRSA was decided by the molecular analysis. The presence of MecA gene was detected in all the 42 isolates of MRSA The gene sequences of MecA gene was obtained and it was confirmed by homology of sequences.

Detection of Mec A gene: In this study, 42 MRSA isolates were subjected for the molecular analysis. We extracted a good quality fragment of the DNA of all the isolates.The Gel photographs of the DNA samples are mentioned below observed by Gel documentation system.

Figure No. 4: The DNA isolated from *S. aureus* isolates

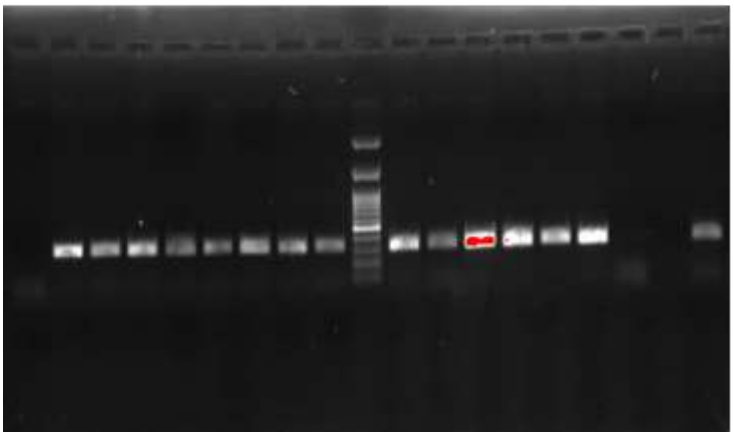


Figure No. 5: The Photograph of the amplified Mec A gene in *S. aureus*, the amplified DNA band size was obtained 336 bp, L corresponding to 100bp ladder used, where Lane 15 is the positive control , Lane 16 - Lane 17a Negative control, and Lane 1-9, L10-L14 and Lane 18 is the sample positive for the MecA gene detection.

GTTGTAGTTGTCGGGTTTGGTATATATTTTTATGCTTCAAAAGATAAAGAAATTAATAA
TACTATTGATGCAATTGAAGATAAAAATTTCAAACAAGTTTATAAAGATAGCAGTTAT
ATTTCTAAAAGCGATAATGGTGAAGTAGAAATGACTGAACGTCCGATAAAAATATATA
ATAGTTTAGGCGTTAAAGATATAAACATTCAGGATCGTAAAATAAAAAAAGTATCTAA
AAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGTAACATTGAT
CGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAG

Figure No. 6: Obtained gene sequences of MecA gene in *S. aureus*

‘

In the molecular study, we have obtained 100% prevalence of MecA gene recorded.

DISCUSSION

Nowadays, MRSA strains have become an important health problem due to the limitations of treatment options, the cost of antibiotics used in treatment and infection control measures. A wide range of resistance mechanisms have been described for *S. aureus* including PBP alterations (β-lactam agents), cell wall structure modifications (glycopeptides), point mutations in the quinolone resistance-determining regions of GyrA and GrlA (quinolones), inactivating enzymes (aminoglycosides) ribosome alterations (macrolides, lincosamides, oxazolidones and tetracyclines), efflux pumps (tetracyclines, macrolides, quinolones) or spontaneus mutations in the gene) .Recently, innovation of different and precise molecular techniques has played a big role in the

detection of resistance genes , including DNA hybridization and polymerase chain reaction (PCR) [19].

In the present study the prevalence of MRSA was observed to be 21%. This study was in support with the study performed by the other author where the prevalence rate was observed to be 20% [20] but in contrast with the study by the other authors Gadepalli R *et al*.,in 2009 and Gopalakrishnan R, *et al*.,in 2010 where the prevalence was found to be 41% and 40% respectively [21,22]. In the present study the maximum number of isolates of *S.aureus* was observed from the OPD ward 77 with 38.5% followed by Surgery ward with 58 (29%) and least for NICU with 17 (8.5%). This study was parallel to the study performed by the other investigator Zaw Myo Tun *et al* in 2021 where the maximum isolates were observed in the Surgery ward [23]. In the present study the ratio of Males 28 (66.6%) was more as compared to that of the female 14 (33.3%). This study was in accordance to the study performed by the other author where the ratio of males (64%) was observed to be more as compared to the females with (36%) with the data recorded by Rao *et al*., 2012 [24] but in contrast with the study by Srivani vijaya subhashini bonangi et al in 2023 where the rate of females was higher than males with 112 (60%) were females and 73 (40%) were males [25].

In the current study, it was also observed that the maximum number of isolates were observed more for the pus sample 20(47.6%) followed by the blood 10(23.8%) and least for the urine and the body fluids with 2.3%. This study was in support with the study performed by the other author where a high prevalence of MRSA was observed from blood and the pus [26]. The presence of more MRSA in pus may be due to direct exposure of wound with environmental microorganisms which makes the wound more prone for infection of *S. aureus*. Similar results were recorded by Mallick *et al.*, 2010 in Maharashtra (61.4%) [27] and Rao *et al*. 2012 in Andhra Pradesh (64%) [28]

It was also found that the maximum number of isolates were observed in the age group of 41-50 years followed by 31-40 years and least was observed in the age group of 61 years and above. Similar finding was recorded by Sharma *et al.,* 2011 where the maximum isolates were in the age group of 41-50 years of age [29].

In recent years, detection of mecA by PCR is considered as the gold standard for identification of MRSA. In this study, we evaluated method as PCR [30], where phenotypic method of Cefoxitin was equally accurate for the detection of MRSA. Cefoxitin disc diffusion test was perceived to be the most sensitive method for detection of mecA mediated resistance. CLSI has also recently substituted the oxacillin disc with cefoxitin disc for detection of MRSA [31]. Numerous studies including the current one have informed that the results of the cefoxitin disc diffusion test correlates better with the presence of mecA compared with those of the oxacillin disc diffusion test. The results about cefoxitin disc diffusion method are consistent with previous report [32]. However, Broekeme *et al.,* reported the sensitivity and specificity of this method 97.3% and 100%, respectively among *S. aureus* isolates [33].

Cefoxitin is taken into consideration as it is a more potent inducer of mec-A gene expression than oxacillin or methicillin and the results obtained are comparable with detection of mec-A gene using PCR and also can be used in the constraint setups that cannot afford PCR testing for mecA as a confirmatory test [32].

In this study the sensitivity of antibiotics were screened (disc diffusion) and found that the maximum resistance was observed for the Linezolid , Teicoplanin and Vancomicin with 100 % to the above antibiotics, while Penicillin with only 20% sensitive . Our finding is strongly supported by Lohan *et al*., 2021 [34] where the linezolid , vancomycin and teicoplanin was observed to be the least sentitive.

Out study also correlated with the study by Perika Sharma et al. 2021 [36], Singh N SS et al. 2018 [37] Radhakrishna M et al. 2013 [38] and Banerji et al. 2018 [39]. MRSA isolates were resistant to Cefoxitin (100%), which correlated with studies of Banerjee et al. 2018 [39], Perika Sharma et al. 2021 [36]. MSSA isolates were sensitive to Teicoplanin (100%), Linezolid (100%), Vancomycin (100%),) which correlated with studies of Singh N SS et al. 2018 [37], Banerjee et al. 2018[39] and Perika Sharma et al. 2021 [36].
MRSA has emerged as an important human pathogen with increasing trend of resistance toward currently used antimicrobial therapy.
Molecular diagnostics have dramatically improved the therapy of MRSA and MSSA infections globally. While culture methods remain important due to the need for extended antimicrobial susceptibility testing, PCR-based methods offer more rapid results, which reduces the time to optimal antimicrobial therapy initiation. Compared to oxacillin, cefoxitin is a better drug to detect *mec*A gene in MRSA and is considered as a substitute marker where cefoxitin is used as a more reliable marker than oxacillin for methicillin resistance. However, resistance to cefoxitin does not mean detection of the *mec*A gene or its PBP2a product. Screening with cefoxitin will determine which isolates will be tested by other methods, phenotypic or genotypic, for the detection of methicillin resistance markers, the *mec*A gene or its product.

Detection of *mec*A gene or its product PBP2a by cefoxitin is considered as the gold standard for MRSA confirmation. The results of molecular methods are also usually available faster than that of phenotypic methods [40]. PCR assay was performed using a single set of primers for the amplification of *mec*A gene where all 42 isolates which were confirmed by cefoxitin , detected the presence of MecA gene which was in support with the study by India and Australia [41,42].
Although PCR- based detection technique outweighs other conventional techniques, combination of these methods can offer diagnostic accuracy. The implementation of strict aseptic techniques in hospitals to prevent the colonization of the hospital environment by resistant strains, the identification and treatment of carriers, and the screening of hospital staff and facilities are some of the key measures that can mitigate the spread of MRSA [43].
All the resistance isolates were confirmed by PCR methodology and gene sequencing which is more powerful technique used recently.

CONCLUSION

For the control of antibiotic resistance, there is a need for ongoing monitoring and the deployment of effective control techniques. Rapid and precise MRSA diagnosis is required to start the proper antibiotic therapy and stop the spread of MRSA infections due to the high prevalence of MRSA infections among hospitalised patients. Due to their high sensitivity and specificity, molecular approaches like the detection of the mecA gene are preferred.

DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

REFERENCES

1. Roberts S, Chambers S. Diagnosis and management of Staphylococcus aureus infections of the skin and soft tissue. Intern Med J. 2005; 35 (2) :S97-105.

2. Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, Naderinasab M. Epidemiology of mecAmethicillin resistant Staphylococcus aureus (MRSA) in Iran: A systematic review and meta-analysis. Iran J Basic Med Sci. 2012; 15:1010-9.

3. Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first communityacquired methicillin-resistant Staphylococcus aureus strains from Central Iran. Int J Infect Dis. 2013; 17:e949- 54.

4. Sekiguchi JI, Fujino T, Saruta K, Konosaki H, Nishimura H, Kawana A, et al. Prevalence of erythromycin-, tetracycline-, and aminoglycosideresistance genes in methicillin-resistant Staphylococcus aureus in hospitals in Tokyo and Kumamoto. Jpn J Infect Dis. 2004; 57:74–7.

5. Naves KSC, Trindade NVD, Filho PPG. Methicillin-resistant Staphylococcus aureus blood stream infection: risk factors and clinical outcome in non-intensive care units. Rev Soc Bras de Med Trop. 2012; 45 (2): 189-193 .

6. Kumar S, Budhani D, Sayal P, Sindwani P, Jain PK. Emphasis on antibiotic optimisation in difficult to treat methicillin resistant staphylococcus aureus infection. J Evolution Med Dent Sci. 2017; 6:5055–8.

7. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010; 23:616–87

8. Maya-Martinez R, Alexander JA, Otten CF, Ayala I, Vollmer D, Gray J, et al. Recognition of peptidoglycan fragments by the transpeptidase PBP4 from Staphylococcus aureus.FrontMicrobiol.2018;9:3223.

9. Ebrahim-Saraie HS, Motamedifar M, Sarvari J, Hoseini Alfatemi SM. Emergence of SCCmec type I obtained from clinical samples in shiraz teaching hospitals, SouthWest of Iran.JundishapurJMicrobiol. 2015;8:e16998.

10. Fasihi Y, Kiaei S, Kalantar-Neyestanaki D. Characterization of SCCmec and spa types of methicillin-resistant Staphylococcus aureus isolates from health-care and community-acquired infections in Kerman, Iran. J Epidemiol Glob Health.2017; 7:263-7.

11. Xiao YH, Giske CG, Wei ZQ, Shen P, Heddini A, Li LJ. Epidemiology and characteristics of antimicrobial resistance in China. Drug Resist Updat. 2011; 14: 236-250.

12. Bongiorno D, Mongelli G, Stefani S, Campanile F. Burden of rifampicin-and methicillinresistant Staphylococcus aureus in Italy. Microb Drug Resist. 2018; 24: 732-738.

13. Ghia CJ, Waghela S, Rambhad G. A Systemic Literature Review and Meta-Analysis Reporting the Prevalence and Impact of Methicillin Resistant Staphylococcus aureus Infection in India. Infect Dis (Auckl). 2020; 13: 1-16.

14. Clinical Laboratory Standards Institute 2017 . Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. Wayne, PA: Laboratory Standard Institute; 2022.

15. Ahmadinejad M, Snyder JW, Perlin MH. A combined molecular approach to screen for mec Gene variants from Methicillin-resistant Staphylococcus aureus. Diagn Microbiol Infect Dis 1998; 30(1): 17-20

16. Anderson DJ, Sexton DJ, Kanafani ZA, Auten G, Kaye KS. Severe surgical site infection in community hospitals: epidemiology, key procedures, and the changing prevalence of methicillin-resistant Staphylococcus aureus. Infect Control Hosp Epidemiol. 2007; 28(9): 1047-1053.

17. Sapkota J, Sharma M, Jha B, Bhatt CP. Prevalence of Staphylococcus aureus Isolated from Clinical Samples in a Tertiary Care Hospital: A Descriptive Cross-sectional Study. J Nepal Med Assoc. 2019; 57(220): 398-402.

18. .Aneta K, Urbanek 1 , Zofia Łapi ´ nska , Daria Derkacz 1 and Anna Krasowska . The Role of ERG11 Point Mutations in the Resistance of Candida albicans to Fluconazole in the Presence of Lactate. Pathogens 2022; 11, 1289.

19. John MA, Burden J, Stuart JI, Reyes RC, Lannigan R, Milburn S, Diagre D, Wilson B, Hussain Z. Comparison of three phenotypic techniques for detection of methicillin resistance in Staphylococcus spp. reveals a species-dependent performance. J Antimicrob Chemother 2009; 63: 493-496.

20. Maj Puneet Bhatt, Gurpreet Singh Bhalla, Kundan Tandel, Prashant Jindamwar, CN Chaudhari, Naveen Grover Ajay Kumar Sahni. Antimicrobial Susceptibility Profile of Methicillin-resistant Staphylococcus aureus at a Tertiary Care centre. Archives of clinical microbiology. 2015; 6 : 3:6.

21. Gadepalli R, Dhawan B, Kapil A, Sreenivas V, Jais M,Gaind R, et al. Clinical and molecular characteristics of nosocomial methicillin-resistant Staphylococcus aureus skin and soft tissue isolates from three Indian hospitals. JHosp Infect. 2009; 73:253–63.

22. Gopalakrishnan R, Sureshkumar D. Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India. 2010; 58:25–31.

23. Zaw Myo Tun ,Dale A. Fisher,Sharon Salmon, Clarence C. Tam. Ward-level factors associated with methicillin-resistant *Staphylococcus aureus* acquisition–an electronic medical records study in Singapore. 2021; https://doi.org/10.1371/journal.pone.0254852 J.

24. Sharma S, and Mall A. The prevalence, antibiogram and characterisation of methicillin resistant Staphylococcus aureus among the patients from the Doon Valley hospitals. Afr J Microbiol Res. 2011; 5: 3446-51.

25. Srivani vijaya subhashini bonangi, s. s. vijaya sri badampudi. screening of methicillin-resistant staphylococcus aureus among healthcare workers and their antibiotic susceptibility pattern in a tertiary care hospital. International Journal of Current Pharmaceutical Research. 2023; 15(5):109-114.

26. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organismsin tertiary care hospital in Delhi, India. J Assoc. Physicians India. 2010; 58(Suppl):32–6.

27. Kali A, Stephen S, Umadevi S. Laboratory evaluation of phenotypic detection methods of methicillin-resistant Staphylococcus aureus. Biomed J. 2014; 37: 411-4.

28. Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018; 12; 31(4): e0002018.

29. Mallick SK, Basak S. MRSA-Too many hurdles to overcome: A study from Central India. Trop Doct. 2010; 40: 108-10.

30. Bhutia KO, Singh TS, Biswas S, Adhikari L. Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in Staphylococcus aureus. Int J Appl Basic Med Res. 2012; 2:84–91

31. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant Staphylococcus aureus. J Infect Dev Ctries. 2008; 2:46– 50.

32. Ananl K, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for mecA gene for detection of MRSA. Indian J Med Microbiol. 2009; 27:27–29.

33. Broekema NM, Van TT, Monson TA, Marshal SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in Staphylococcus aureus in a large-scale study. J Clin Microbiol. 2009; 47:217–219.

34. Goudarzi M, Seyedjavadi SS, Azad M, Goudarzi H, Azimi H. Distribution of spa Types, Integrons and Associated Gene Cassettes in Staphylococcus aureus Strains Isolated From Intensive Care Units of Hospitals in Tehran, Iran. Arch Clin Infect Dis. 2016; 11(4): doi: 10.5812/ archcid. 38813.

35. Rawani K, Pimpalkar S. Screening of nasal MRSA carriage among health care workers at a tertiary Care Centre in Chhattisgarh at Rajnandgaon District. Int J Curr Microbiol App Sci. 2020; 9(12):1467-74.

36. Sharma P, Tomar R. Screening of methicillin resistant staphylococcus aureus (MRSA) carriage among health care workers and its antibiotic susceptibility pattern in a tertiary care hospital. Int J Res Rev. 2021; 8(4):429-34.

37. Singh N, Mohanty S, Panda SS, Sahoo S, Pattnaik D, Jena J. Methicillin resistant Staphylococcus aureus (MRSA) carriage among health care workers in a tertiary care hospital in Bhubaneswar. Int J Community Med Public Health. 2018; 5(8):3276-82.

38. 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. 2013; 7(2):257-60.

39. Banerjee J, Madhavi K, Kiranmai S, Sureka RK. Screening for nasal carriage of methicillin resistant staphylococcus aureus among healthcare workers at a Rural Teaching Hospital in Medchal District, Telangana. Int J Microbiol Mycol. 2018; 7(2):1-7.

40. Dadashi M, Hajikhani B, Darban Sarokhalil D, Van Belkum A, Goudarzi M. Mupirocin resistance in staphylococcus aureus: a systematic review and meta-analysis. J Glob Antimicrob Resist. 2020; 20:238-47.

41. Mehndiratta PL, Bhalla P, Ahmed A, Sharma YD. Molecular typing of methicillin-resistant *Staphylococcus aureus* strains by PCR-RFLP of SPA gene: a reference laboratory perspective. *Indian J Med Microbiol*. 2009;27:116-122.

42. Cloney L, Marlowe C, Wong A, Chow R, Bryan R. Rapid detection of mecA in methicillin resistant *Staphylococcus aureus* using cycling probe technology. *Mol Cell Probes*. 1999;13:191-197.

43. Sur V.P., Mazumdar A., Kopel P., Mukherjee S., Vitek P., Michalkova H., Vaculovičová M., Moulick A. A Novel Ruthenium Based Coordination Compound Against Pathogenic Bacteria. *Int. J. Mol. Sci.* 2020; 21:2656.