Original Article

"Evaluation Of Several Phenotypic Methods Of Antibiotic Susceptibility Pattern For The Detection Of MRSA With Molecular Profiling RT-PCR With The Detection Of MecA Gene".

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Abstract

INTRODUCTION:- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-recognized public health problem throughout the world and community MRSA strains are now epidemic in India and others countries. The resistance rates of *S. aureus* infection and multidrug resistant strains are increasing, making the clinical anti-infective treatment and diagnosis more difficult.

AIM:- To evaluate several phenotypic methods of the antibiotic susceptibility pattern for the detection of MRSA with Molecular Profiling Rt-PCR with the detection of MecA gene.

MATERIALS AND METHODS:- The present study was a prospective observational study carried out at the Department of Microbiology, Santosh Medical College, Ghaziabad in a tertiary care hospital over a 12-month period from August 1, 2020 to July 31, 2021. Both IPD and OPD clinical sample comprised 385 clinical isolates as *Staphylococcus aureus* from various clinical specimens were included. Antibiotic susceptibility by the Kirby-Baure disc diffusion methods was performed according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. Detection of MRSA by various methods like, Cefoxitin Disk diffusion Method, Oxacillin Disk diffusion method, E-Test method and detection of MecA gene by RT-PCR was carried out.

RESULT:- In the present study out of 384 clinical samples, the majority of isolates from MRSA were found to be resistant to (E-Test) strip Oxacillin 114 (29.7%), followed by cefoxitin 113 (29.4%) and disc diffusion oxacillin 99 (25.8%). However, we observed a high incidence of resistance to other antibiotics such as Erythromycin 265 (69.0%), followed by Clotrimozole 228 (59.4%), Tetracyclin 144 (37.5), Vancomycin 102 (26.6%) and Refampicin 102 (26.6%). We also observed that Linezolid 354 (92.2%) followed by Teicoplanin 325 (84.6%), Gentamycin 272 (70.8%), Clindamycin 249 (64.8%) and Amoxyclave 281 (73.2%). In this study molecular RT-PCR test for the detection of mecA gene observed more in pus with 36 samples out of 113 followed by blood 17, urine 33, Sputum 05, pleural fluid 04, wound swab 16, Vaginal swab 06, CSF 01, Throat swab 2.

CONCLUSION:- The gold standard assay for determining methicillin resistance is the PCR technique for detecting the mecA gene. But Because PCR is still time intensive and expensive, and it is not yet available in the 95% of routine clinical laboratories the phenotypic methods is still the choice where E-test is more reliable method than the disc diffusion method in detecting the drug resistance and can be utilised on a regular basis for better results.

Keywords:- MRSA, *Staphylococccus aureus*, Antibiotic susceptibility pattern, RT-PCR, MecA

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a nosocomial pathogen of major worldwide which mainly cause soft tissue skin infection can be defined as those occurring within 48-72 h of hospital admission, 3 days of discharge or 30 day of an operation. S. aureus is a major pathogen responsible for community-acquired infection.(1)

Staphylococcal infections are a common and significant clinical problem in medical practice. Most strains of Staphylococcus aureus are now resistant to penicillin, and methicillin-resistant *Staphylococcus aureus* (MRSA) are common in hospitals and are emerging in the community. MRSA infections have been reported worldwide and community MRSA strains are now epidemic in the in India and others like United States etc. (2) However, A gene known as mecA gene is responsible for the resistance to methicillin which codes for pencillin-binding protein PBP 2A. The resistance rates of *S. aureus* infection and multidrug resistant strains are increasing, making the clinical anti-infective

treatment and diagnosis is more difficult. (3) . However, certain strains of *S. aureus* developed resistance known as methicillin resistant *Staphylococcus aureus* (MRSA). At present, less than 90% of *S.aureus* strains are resistant to most penicillin derivatives and ordinary antimicrobial agents like drugs from the family of aminoglycoside, macrolides, chloramphenicols, tetracyclines and fluoroquinolones.(4)

A variety of methods have been implemented to optimize MRSA, so as find resistant antibiotic. There are several culture-based methods, as recommended by (CLSI) Clinical and Laboratory Standards Institute, phenotypically, various types of methods for determination of minimum inhibitory concentration (MIC) are available in microbiology laboratories, including broth dilution, agar dilution and E-test. Genotypically, Detection of mec-A gene by PCR-based approaches is universally accepted as the gold standard for MRSA identification. (5,6,7,8)

Factors such as inadequate infection control practice, antibiotic misuse, chronic co-morbidities, recurrent hospitalization and repeated exposure to healthcare settings, as well as certain host factors such as immunosuppression, have all been suggested to increase the risk of colonization by MRSA and its spread.(9,10) Prevent of infection, the antimicrobial agents available in the United States for the treatment of complicated and uncomplicated MRSA. (11) Methicillin resistance *staphylococcus aureus* is of special concern in regards to treatment because it is usually multi-drug resistant. In addition to most beta-lactams, MRSA is also commonly resistant to erythromycin, clindamycin, aminoglycosides, fluoroquinolones, and co-trimoxazole.(12, 13,14) .

MATERIAL AND METHODS

The present research was carried out in the Department of Microbiology, Santosh Medical College, Ghaziabad, where a prospective observational study in a tertiary care hospital over a 12-month period from August 1, 2020 to July 31, 2021 was done. Both IPD and OPD comprising of 385 clinical isolates as *Staphylococcus aureus* from various clinical specimens such as pus, wound, vaginal swabs, blood, pleural fluid, urine, throat swabs etc were collected. The Institutional Ethical committee of Santosh Medical College, Ghaziabad, provided their ethical approval to this study.

The Kirby-Baure disc diffusion methods were used to evaluate all *S.aureus* isolates for antibiotic susceptibility testing. According to the Clinical and Laboratory Standard Institute (CLSI) guideline:Linezolid (30µg), Linezolid (30µg), Gentamycin (30µg), Tetracyclin (10µg), Erythromycin (15µg), Clindamycin (2µg), Ciprofloxcin (5µg), clotrimozole (30µg), amoxyclave (20µg), vancomycin (30µg), Rifampicin (5µg) disc diffusion and E strips quantitative determination were bought from Hi-Media diagnostic laboratory pvt.Ltd LBS Marg. Mumbai- 400086, India.

IDENTIFICATION OF MRSA BY VARIOUS PHENOTYPIC METHODS

Phenotypic identification of MRSA

Clinical samples were inoculated on 5% sheep blood agar, MacConkey's agar, and CLED agar (Only for Urine), incubated at 37°C for 24 hours, and bacterial growth was studied. Standard methods for identifying *Staphylococcus aureus* include colony morphology, Gram's stain, catalase test, and coagulate test.

Cefoxitin Disk diffusion Method

On Mueller Hinton agar plates, all *S.aureus* strains were evaluated with a 30 mg cefoxitin disc. A bacterial suspension calibrated to 0.5 McFarland will be used for each strain. After 16-18 hours of incubation at 37°C, the zone of inhibition was assessed. The CLSI (2017) criteria were used to interpret zone size: susceptible zone greater than 22 mm and resistant zone less than 21 mm. (15)

Oxacillin Disk diffusion method

On Mueller-Hinton agar with a 4% NaCl addition, all strains of S. aureus were tested with a 1 mg oxacillin disc. A bacterial suspension calibrated to 0.5 McFarland was utilized for each strain. After 16-29 hours of incubation at 35-37°C, the zone of inhibition was measured. The CLSI (2017) criteria were used to interpret zone size: susceptible greater than 13 mm, intermediate 11-12 mm, and resistant less than 10 mm.(16)

(Epsilometer) E-Test method

These are automated systems for measuring the minimum inhibitory concentration (MIC) of bacteria. The inoculum performed was standardized to 0.5 McFarland turbidity and plated on MHA supplemented with (2%) NaCl. MIC strips for oxacillin were applied on the MHA surface with the MIC scale facing downwards. Plates are incubated at 37°C for 24 hours before being examined.MIC is read from the scale at the intersection of the zone with the strip. The MIC of less than 2µg was considered as sensitive and more than 4µg was considered as resistant. (17)

Genotypic detection of MRSA with RT-PCR

Extraction of DNA from isolate growth

Each isolated growth sub-culture in peptone water and incubated for 24 hour at 37ºC. Genomic extraction was performed using Qiagen, DNA extraction kit. One mL of QIAzol Lysis Reagent was added into 200 µl of isolate bacterial broth, mixed by brief vortexing and incubated at room temperature (15-25ºC) for 5min. 200µl chloroform was added to sample mixture, capped the tube securely and vortexed vigorously for 15 sec. After 2-3 min incubation at room temperature, each

sample mixture was centrifuged at 12,000 x g for 15min in cold condition (4°C). Upper aqueous phase of each sample was then transferred to new collection tubes while avoiding interphase transfer. Following to this, 1.5 volumes of 100% ethanol was added in each aqueous phase was added and mixed thoroughly by pipetting. Pipetted up to 700 µl sample, including any precipitate, into an RNeasy Elute spin column in a 2ml collection tube. Closed the lid and centrifuged at ≥8000 x g for 15sec at room temperature. Discarded the flow-through and repeated above step using remaining sample. 500µl Buffer RPE was added onto the RNeasy Elute spin column, closed the lid, and centrifuged at ≥8000 x g for 15sec. Discarded the flow-through and added 500µl of 80% ethanol to the RNeasy Elute spin column, closed the lid, and centrifuged at ≥8000 x g for 2min. Discard the flow-through along with the collection tube. Placed RNeasy Elute spin column into a new 2ml collection tube, opened the lid of the spin column and centrifuge at full speed for 5min to dry the membrane. Discarded the flow-through with the collection tube. Placed RNeasy Elute spin column in a new 1.5ml collection tube and added 50µl RNase-free water directly to the center of the spin column membrane, closed the lid gently, and centrifuged at full speed for 1min to elute the *mecA.* Eluted *mecA* of each sample was either immediately converted into DNA or stored at -20ºC for DNA preparation after some time.

Quantitative Real-Time polymerase chain Reaction (RT-qPCR) for detection *MecA* gene

The quantitative expression analysis of *mecA* was performed using SYBR Green-based RT-qPCR in all the patient samples compared to controls relative to endogenous control U6. Primer sequences specific to human Mac-A (Forward: 5'-ACCTGCGTAGGTAGTTTCATGT-3', Reverse: 5'-CGTCAGAAGGAATGATGCACAG-3') AND U6 (Forward: 5'-CGCTTCGGCAGCAGCACATATACTA-3', Reverse: 5'- CGCTTCACGAATTTGCGTGTCA-3' were used to perform RT-qPCR using Real-time PCR system (S1000 BioRad, USA). Two separate reaction mixtures were prepared using 2µL DNA of each sample for MecA and U6 induplicate by adding 0.5 µL of each primer along with 10 µL of SYBR Green mix and 7 µL of dH_2o in 96 well microplates. The reaction plates were incubated in RT-PCR system in a defined program cycles at 95ºCfor 5 min for initial denaturation followed with 40 cycles, melt curve analysis was performed to distinguish primer- dimers with real amplicons of mecA and U6 in each sample. The mean Cq value of each sample for both the targets was exported and calculated for expression of mecA in relation to U6 separately in patients and control samples using. (18, 19)

Statistical Analysis

The IBM SPSS 1.0.0.1406 version was used to analyze the data. All phenotypic methods were compared using mecA PCR as the gold standard technique; sensitivity and specificity were expressed as percentages and MIC was determined using a variety of phenotypic methods. Analysis of age, gender, and risk factors expressed as percentage.

RESULT

Confirmatory 384 *S. aureus* Clinical samples were received during the study period. Among them the strains showing resistance to methicillin (MRSA) were 113 (29%) .Samples were used to isolate growth of *S. aureus* during the study period. Pus, blood, urine, vaginal swab, wounds etc.

Table- 1. Prevalence of MRSA & MSSA

	MRSA	**MSSA**	**TOTAL**
S. aureus	113	271	384

In the present study 384 clinical samples were processed in which 113 MRSA and 271 MSSA were obtained.

Table – 2. Sex wise distribution of MRSA.

Age	**MRSA (N=113)**		**MSSA (N=271)**		**Total (N=384)**
	Male (65)	**Female (48)**	**Male (158)**	**Female (113)**	
≤10	6	4	13	7	30 (7.8)
11- 20 years	2	2	18	17	39 (10.2)
21- 30 years	17	13	27	24	81 (21.1)
31- 40 years	12	10	32	31	85 (22.1)
41- 50 years	14	11	29	17	71 (18.5)
51- 60 years	8	6	21	9	44 (11.5)
>61 years	6	2	18	8	34 (8.9)

In the present study, the majority of the 113 (29.4%) MRSA isolates were from the age groups between 21 to 30 years old and also MRSA in males was 17 and in females was 13.

Table – 3. Distribution of MRSA in various clinical samples				
S. No	**Samples**	**MRSA**	**MSSA**	**S. Aureus**
1.	Blood	18(29.5%)	43(71.4%)	61(15.9)
2.	Urine	30(28.8%)	74(71.1%)	104 (27.1)
3.	Sputum	4(21%)	15(78.9%)	19 (4.9)
4.	Pus	39(31.45%)	85(68%)	124 (32.3)
5.	Pleural fluid	3(33.3%)	6(66.6%)	9 (2.3)
6.	Wound swab	10(26.3%)	28(73.68%)	38 (9.9)
7.	Vaginal swab	4(28.5%)	10(71.4%)	14 (3.6)
8.	CSF	1(100%)	0	1 (0.3)
9.	Throat swab	2(50%)	2(50%)	4 (1.0)

In this study incidence of MRSA from clinical samples *S. aureus* were more in pus 39(31.45%) followed blood 18(29.5%), urine 30 (28.8%), Sputum 4 (21%), pleural fluid 3 (33.3%), wound swab 10 (26.3%), Vaginal swab 4 (28.5%), CSF 1 (100%), Throat swab 2 (50%).

Table – 4. Distribution of isolate according to the wards.				
Wards	**MRSA (N=113)**	**MSSA (N=271)**	**Total**	**P Value**
Surgery	32(27%)	84(72.4%)	116	0.298
ICU	28(40%)	42(60%)	70	
Medicine	26(24%)	81(75.7%)	107	
ENT	8(28%)	20(71.4%)	28	
Gynae	9(25%)	26(74.2%)	35	
Paediatrics	10(35.7%)	18(64.2%)	28	

Chi Square = 6.087, Significance Level = 0.05, Not Significant

More samples were isolated from surgery 32 (27%), out of a total of 116, while the Intensive care unit (ICU)28 (40%) had the most MRSA isolates out of 70. Followed by medicine 26 (24%) out of 107, ENT 8 (28%), Gynae 9 (25%) and Paediatrics 10 (35.7%).

Table- 5. Comparison of Different types of phenotypic methods with PCR Test.			
Methods	**MRSA**	**MSSA**	**Total**
Cefoxitin	113 (29.4)	271 (70.6)	**384**
Oxacillin	99 (25.8)	285 (74.2)	
Oxacillin (E-test)	114 (29.7)	270(70.3)	
PCR	124 (32.2)	260(67.7)	

0- No Value Less than 36-MRSA, More than 36- MSSA

In this study Comparison of different type of phenotypic and genotypic methods was performed- phenotypically, Oxacillin (E-test) was found to be more sensitive with MRSA 114 (29.7) out of 384. Followed by Cefoxitin 113 (29.4), oxacillin 99 (25.8) and More accuracy sensitive result was observed by Genotypically Polymerase chain reaction (PCR) 124 (32.2) out of 384.

Table- 6: Sensitivity and specificity of phenotypic and genotypic (MecA) gene methods for detection of MRSA				
Methods	**Sensitivity n (%)**	**Specificity n (%)**	**PPV n (%)**	**NPV n (%)**
Cefoxitin	75.8	92.7	83.2	88.9
Oxacillin	60.5	90.8	75.8	82.8
Oxacillin (E-test)	79.8	94.2	86.8	90.7
PCR Gold standard	100	100	100	100

(PPV) Positive predictive value, (NPV) Negative predictive value

In this study, we detected sensitivity and specificity from phenotypic and genotypic methods. PCR gold standard genotypic method was fined 100% sensitivity, specificity or PPV and NPV value compare to phenotypic methods. The oxacillin (E-test) strip had high sensitivity of 79.8%, specificity 94.2% while PPV 86.8% or NPV 90.7% followed by the cefoxitin disc diffusion method, which occured sensitivity of 75.8%, specificity 92.7% while PPV 83.2% or NPV 88.9%. In last oxacillin disc diffusion method sensitivity was 60.5%, specificity 90.8% while PPV 75.8% or NPV 82.8%

Table-7. Antibiotics resistance pattern from clinical specimens		
Antibiotics	**MRSA n (%)**	**MSSA n (%)**
Linezolid	30 (7.8)	354 (92.2)
Tiecoplan	59 (15.4)	325 (84.6)
Gentamycin	112 (29.2)	272 (70.8)
Tetracyclin	144 (37.5)	240 (62.5)
Erythromycin	265 (69.0)	119 (31.0)
Clindamycin	135 (35.2)	249 (64.8)
Ciprofloxcin	141 (36.7)	243 (63.3)
Cefoxitin	113 (29.4)	271 (70.6)
Oxacillin	99 (25.8)	285 (74.2)
Clotrimozole	228 (59.4)	156 (40.6)
Amoxyclave	103 (26.8)	281 (73.2)
Vancomycin	102 (26.6)	282 (73.4)
Refampicin	102 (26.6)	282 (73.4)
E-Test (oxacillin)	114 (29.7)	270(70.3)

This table shows the antibiotic sensitivity pattern of *S. aureus*. The majority of isolates from MRSA were found to be resistant to (E-Test) strip Oxacillin 114 (29.7%), followed by cefoxitin 113 (29.4%) and disc diffusion oxacillin 99 (25.8%). However, we observed a high incidence of resistance to other antibiotics such as Erythromycin 265 (69.0%), followed by Clotrimozole 228 (59.4%), Tetracyclin 144 (37.5), Vancomycin 102 (26.6%) and Refampicin 102 (26.6%). We also observed that Linezolid 354 (92.2%) followed by Teicoplanin 325 (84.6%), Gentamycin 272 (70.8%), Clindamycin 249 (64.8%) and Amoxyclave 281 (73.2%).

Table 8. Distribution of mecA genes among MRSA isolated from different Specimens

Specimen	Blood	Urine	Pus	Wound Swab	Sputum	Vaginal Swab	Pleural fluid	Throat Swab	CSF
mecA Gene	17	33	36	16	05	06	04	02	01

In this study molecular RT-PCR test mecA gene observed more in pus 36 samples, out of 113 MRSA clinical samples followed blood 17, urine 33, Sputum 05, pleural fluid 04, wound swab 16, Vaginal swab 06, CSF 01 and Throat swab 2.

Amplification of mecA gene

Run Information

Run Date: 23/08/2021 11:41 AM

- Run User instrument: Bio rad
- Run Type: User-defined
- Sample Volume: 150
- Temperature Control Mode: Calculated Lid Temperature: 105
- Base Serial Number: CC014519
- Optical Head Serial Number: 785BR5204

Protocol (Temperature)

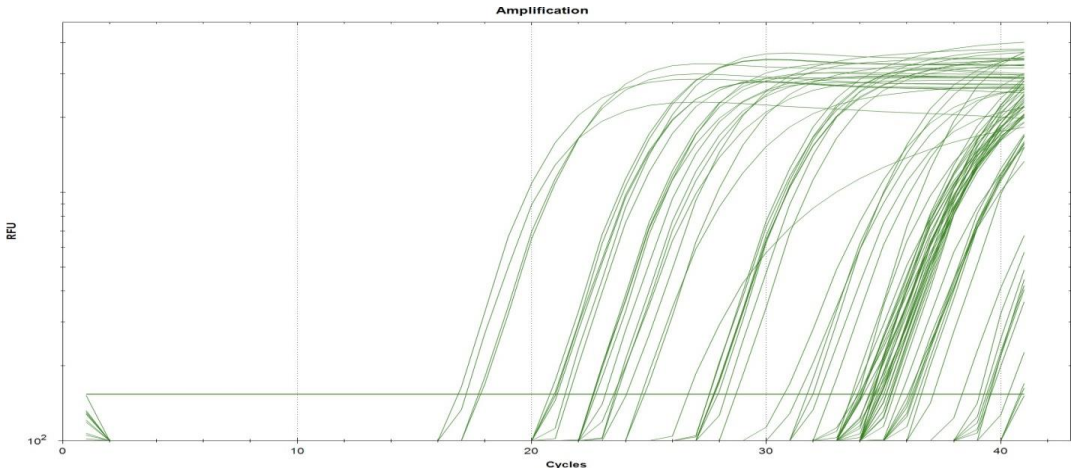
- 1: 95.0°C for 5:00
- 2: 95.0°C for 0:15
- 3: 58.0°C for 0:30

Plate Read

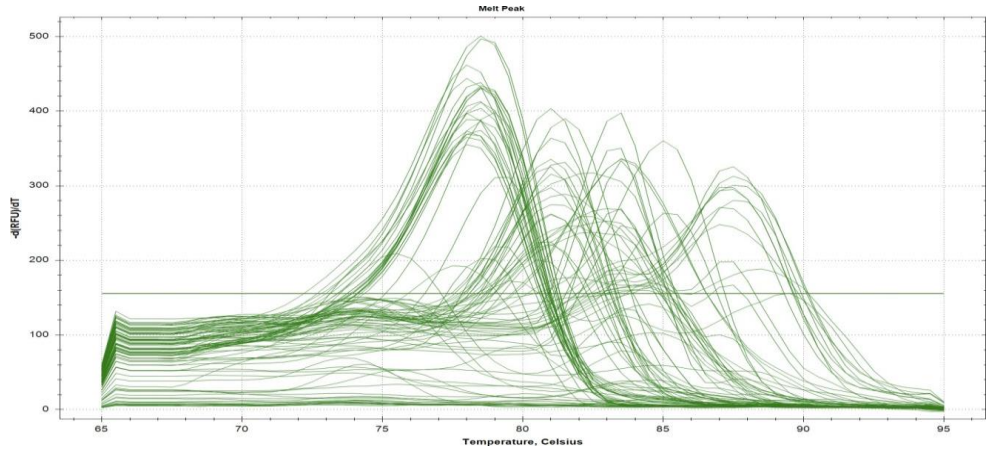
- 4: 72.0°C for 0:40
- 5: GOTO 2, 40 more times
- 6: Melt Curve 65.0°C to 95.0°C: Increment 0.5°C 0:05 Plate Reaction

Primer sequences specific to human mecA gene

Forward	5'-ACCTGCGTAGGTAGTTTCATGT-3
Forward	5'-CGCTTCGGCAGCAGCACATATACTA-3
Reverse	5'-CGTCAGAAGGAATGATGCACAG-3
Reverse	5'- CGCTTCACGAATTTGCGTGTCA-3



Ct cut-off for fluorescent: SYBR ct ≤35
Analysis Mode: Fluorophore Cq
Determination: Single Threshold
Baseline Method: SYBR: Auto Calculate



Melt cure Chart- Highly specific SYBER Green assay where the melt curves exhibits a single melt peak apex around 85- 90°C indicating a single PCR amplicon present.

DISCUSSION

In recent years, MRSA has become a challenge for clinical laboratories. As a result, detecting methicillin resistance accurately and quickly is essential in the prognosis of *S. aureus* infections. To maintain PPV (positive predictive value) or NPV (negative predictive value), which is a proceed by the CLSI guideline for treating infections caused by this organism, several phenotypic disc diffusion or E-test strip methods with high accuracy, sensitivity, and specificity are required.(**20**). In this study, there were 384 *staphylococcus aureus* strains, among which 113 (29%) strains were found to be methicillin resistant. In support to this, a study conducted by Joshi S et al **(21)** in India reported 42% cases of MRSA. Similarly, a study from Choudhary D et al**(22)** also reported slightly higher prevalence (42.96%) compared to the present study. Other investigations have found a significant incidence of MRSA in various regions of the nation, such as 32% in a study by Bilal Ahmad et al(**23)**similar to this study another research conducted by Karem H. Alzoubi in Jordan found that the total prevalence of MRSA was 34%(**24)**. In support to the above findings, Rajaduraipandi et al. also found 31.1% MRSA strains in their investigation,(**25)**. Various studies from different regions of India including Mumbai, Haryana and Greater Noida, presented the prevalence of MRSA similar to the present study(**26,27**,**28)** More than 50% prevalence of MRSA was observed in MRSA from different states of India.(**29, 30, 31)**. Around 5 year's back from low prevalence to high prevalence was observed from different study from different places of India (**32, 33, 34, 35)**. In the inpatient setting, a compromised immune system is one of the major risk factors for MRSA.

The majority of the MRSA isolates in this study (29.4%) came from people between the ages of 21 and 30 years old. MRSA was found to be 17 in males and 13 in females.Which is similarly studied by Giri N et al**(36)** 25-35 age find MRSA 6 (5.4%)while many study Soe PE et al**(37)** reported MRSA increase find 45 to 64 old age which was greater than the our study and also reported by Dilnessa T et al**(38)**In this investigation, the pus sample had the greatest number of MRSA cases 113 (29%), followed by blood 18(29%), urine 30(28%), would swab 10(26%), and vaginal swab 4(28.5%). According to Dr. Uma devi et al,**(39)** the highest prevalence was detected in pus samples (28.8%). It is a well-documented fact that a lot of risk factors associated with MRSA infections exist in the ICU like potential environment reservoirs, opportunities for cross transmission, sick, immune-compromised patients who are colonized, patients with multiple wounds and indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands

of health care workers while patient care**(40,41)** .This fact was supported by our results, where in our study more samples were isolated MRSA (29.4), from surgery (27%), out of a total of 116, while the Intensive care unit (ICU)28 (40%) had the most MRSA isolates out of 70. Followed by medicine Ward 26 (24%) out of 107, ENT Ward 8 (28%), Gynae Ward 9 (25%) and Paediatrics 10 (35.7%) which is similar to the study conducted by Chada CKR et al**(42)** also reported. In this study, different types of phenotypic and genotypic approaches were compared to Phenotypically, oxacillin (E-test) was found to be more sensitive to MRSA 114 (29.7) out of 384. While Cefoxitin 113 (29.4), oxacillin 99 (25.8), and Genotypically Polymerase Chain Reaction (PCR) 124 (32.2%) out of 384 was found more accurate and sensitive results. This study correlates with that by Panda RK et al similarly specificity of cefoxitin and oxacillin discs were 100% & 98.5% respectively. And also similar by Girgis S.A et al**(43 .)**In this investigation, sensitivity and specificity were determined using phenotypic and genotypic approaches. The PCR gold standard genotypic method was found to have 100% sensitivity, specificity, or PPV and NPV values when compared to phenotypic methods. The oxacillin (E-test) strip had a high sensitivity of 79.8%, specificity of 94.2%, and a PPV of 86.8% or NPV of 90.7%, followed by the cefoxitin disc diffusion method, which had a sensitivity of 75.8%, specificity of 92.7%, and a PPV of 83.2% or NPV of 88.9%. The sensitivity of the oxacillin disc diffusion method was 60.5%, and the specificity was 90.8%, with a PPV of 75.8% and an NPV of 82.8%.The same result was found when all MRSA isolates were 100% susceptible by the oxacillin E-test, according to Karami S et al.**(44)**In the antibiotic sensitivity pattern of *S.aureus*, Demir T et al. found a significant rate of MRSA antibiotic resistance to cefoxitin 113 (29.4%) and oxacillin 99 (25.8%) in the antibiotic sensitivity pattern of S.aureus, as confirmed by Demir T et al., who concluded that oxacillin (1 g) resistance was 29 percent and cefoxitin (30 g) resistance was 31 percent out of 100 isolates of pure S.aureus growth, followed**(45).** In terms of determining antibiotic sensitivity/resistant patterns, Dhuria N et al. and Anand KB et al. obtained similar results **(46,47).** MSSA was found to be extremely antibiotic sensitive to Linezolid 354 (92.2%), Tiecoplanin 325 (84.6%), and Gentamycin 272 in this investigation (70.8%). In their study, Shanthi M et al. found Linezolid, Tiecoplanin, and many other drugs to be 100 % sensitive **(48,49).** Because of the variable resistance showed by many clinical isolates, the currently available phenotypic techniques for screening MRSA are challenging (50). The setups that cannot afford PCR testing for mecA as a confirmatory test should go for phenotypic method E-test MIC which is highly recommended to be used as a surrogate marker.

CONCLUSION

The present study indicates the E-test to be more reliable than the disc diffusion method in detecting drug resistance, therefore it can be utilised on a regular basis for better results.

The gold standard assay for determining methicillin resistance is the PCR technique for detecting the mecA gene is the PCR but PCR is still time intensive and expensive, and it is not yet available in the 95% of routine clinical laboratories. As a result, in laboratories with limited resources, the phenotypic methods is still the choice.

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