To Study the Comparison of Different Phenotypic Methods by E-test, Cefoxitin and Oxacillin Disc Diffusion test for Detection of Methicillin Resistant Staphylococcus aureus Isolates at a Tertiary Care Hospital, Uttar pradesh.

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Abstract:

Introduction: MRSA strains have become a severe clinical and epidemiological problem in recent years, as resistance to this antibiotic suggests resistance to beta-lactam antibiotics. The aim of the present study was to compare the conventional methods against the E-test, Cefoxitin and Oxacillin disc diffusion method to determine the best phenotypic method.

Aim and Objective: To Study the Comparison of Different Phenotypic Methods by E-test, Cefoxitin and Oxacillin Disc Diffusion test for Detection of Methicillin Resistant Staphylococcus aureus Isolates at a Tertiary Care Hospital, Uttar pradesh.

Material and Methods: This was a cross sectional study conducted in the Department of Microbiology at RMCHRC, Mandhana, Uttar Pradesh for a period of 1 year i.e, February 2022 to February 2022. A total of 210 isolates of S. aureus were identified using the biochemical test from the clinical isolates such as pus, swab, blood, wound and urine etc. The Comparison of Different Phenotypic Methods including E-test, Cefoxitin and Oxacillin Disc Diffusion test for Detection of Methicillin Resistant Staphylococcus aureus Isolates was done accoding to the CLSI guidelines 2022.

Results: A total of 210 S.aureus isolates were identified from a total of 965 clinical samples in which 58 isolates were identified as the MRSA isolates in clinical specimens. In this study, different phenotypic methods were used to detect MRSA wherein the best result was found from E-test (oxacillin) 59 (39%) Compared by CDD method 59 (38%), ODD 50 (31%) out of 210 isolate S. aureus growth. The ratio of Males 35 (60%) was more compared to the Female with 23 (39.6%) with the maximum age of 21-40 been affected the most.

The gold standard method was chosen to be the E-test. It was also observed that isolates including MRSA were highly susceptible to teicoplanin and linezolid.

Conclusion: The molecular technique is too expensive for patients to afford. Due to its low cost Compared to PCR, the E-test is more affordable and straightforward to perform, most effective substitute for regular usage in most clinical laboratories, in particular in underdeveloped nations.

Keywords: MRSA, Beta-lactam, Oxacillin, Cefoxitin disc diffusion method

Introduction

Staphylococcus aureus is the most frequent reason for skin and soft tissue infections. The 20–30% of people has S. aureus carriage in their anterior nares or elsewhere [1]. Patients can spread S. aureus to one another. Hospitals make a lot of effort to prevent transmission from one patient to another directly as well as through staff members and the surrounding area [2]. Methicillin-resistant S. aureus (MRSA) has been a growing problem in hospital-acquired as well as community associated MRSA (CA-MRSA) infections [1]

Methicillin Resistant Staphylococcus aureus (MRSA) is an MDR strain of Staphylococcus aureus, resistant to penicillins, cephalosporins, carbapenems and macrolides. Methicillin was first introduced in 1959 to treat S.aureus infections resistant to penicillin.

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A risk factor for future MRSA infection has been identified as asymptomatic MRSA colonisation [3]. Penicillin, a medication that has been used in therapeutic settings since 1960 [4], is the source of the antibiotic known as methicillin. As resistance to this drug predicts resistance to beta-lactam antibiotics, MRSA strains have grown to be a serious clinical and epidemiological concern in recent years [5]. Due to the price of alternative forms MRSA is by definition methicillin-resistant and carries the mecA gene. Penicillin Binding Protein (PBP) 2a, which is different from native PBPs of S. aureus, is produced by the mecA gene. When B-lactam antibiotics are present, PBP 2a permits MRSA to continue constructing its cell wall. Contrary to HA-MRSA, CA-MRSA is susceptible to a variety of antibiotics with the exception of erythromycin and B-lactams [6, 7]. In the hospital, methicillin resistance manifested itself.

MRSA isolates are sensitive to antibiotic class glycopeptides and even decreases susceptibility to them is emerging [5]. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc.

are the possible predisposing factors of MRSA emergence, and important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers is the predominant mode for patient-to-patient transmission [6].

According to recent research [8], MRSA infections are more prevalent among hospitalised patients, necessitating prompt and accurate MRSA identification in order to start the proper antibiotic therapy and stop the spread of MRSA infections. Phenotypic techniques like the Oxacillin Disc Diffusion (ODD) and Cefoxitin Disc Diffusion (CDD), also known as the E-test strip method, are accessible in clinical laboratories, along with the ability to detect the Minimum Inhibitory Concentration (MIC) for phenotypic techniques [9]. The goal of this study was to study the comparison of different phenotypic methods by E-test, Cefoxitin and Oxacillin disc diffusion test for detection of Methicillin Resistant Staphylococcus aureus isolates at a tertiary care hospital, Uttar Pradesh as earlier studies have indicated that there are several methods available to do so.

Material and Methods

This was a cross sectional study conducted in the Department of Microbiology at RMCHRC, Mandhana, Uttar Pradesh for a period of 1 year i.e, February 2022 to February 2023. A total of 210 isolates of S. aureus were identified using the biochemical test from the clinical isolates such as pus, swab, blood, wound and urine, etc. The Comparison of Different Phenotypic Methods including E-test, Cefoxitin and Oxacillin Disc Diffusion test for Detection of Methicillin Resistant Staphylococcus aureus Isolates was done accoding to the CLSI guidelines 2022 [10]. The ethical clearance was received from the ethical committee and each participant's written informed consent was obtained prior to the collection of the sample.

Sample Collection

During the study period, a total of 965 clinical samples were collected out of which 210 clinical isolates of Staphylococcus aureus were obtained and included from various clinical specimens taken from patients admitted in various wards of the hospital. The samples were grown aerobically in MHA and blood. At 37°C, the plates were incubated over night.

Various Methods for MRSA Identification and Antimicrobial Susceptibility Testing

1. E-testing with an epsilometer

These are automated methods for calculating the MIC of bacteria. The inoculum was standardised to 0.5 McFarland turbidity and plated on Mueller Hinton Agar (MHA) supplemented with 2% NaCl. MIC strips for oxacillin were put on the MHA surface with the MIC scale facing down. Before being analysed, plates underwent a 24-hour incubation period at 37°C. At the zone-strip junction, the scale is read to determine the MIC. MICs of less than 2 g and greater than 4 g were classified sensitive and resistant, respectively [10]. The diagnostic kits from Himedia Laboratries Pvt., Ltd., Mumbai, India (EM0065) were bought to perform the E-test [11].

2. Disc diffusion with cefoxitin

All S. aureus strains were tested using a 30 mg cefoxitin disc on MHA plates. For each strain, a bacterial suspension calibrated to 0.5 McFarland was utilised. The zone of inhibition was evaluated following 16–18 hours of incubation at 37°C. Zone size was interpreted using the Clinical and Laboratory Standards Institute (CLSI) (2022) [10] criteria: susceptible zone greater than 22 mm and resistant zone less than 21 mm [10].

3. Method of Oxacillin disc diffusion

To test all S. aureus strains, a 1 mg oxacillin disc on MHA with a 4% NaCl addition was utilised. With a 0.5 McFarland-calibrated bacterial suspension, each strain was assessed. The zone of inhibition was assessed after 16–29 hours of incubation at 35–37°C. The following parameters from CLSI (2022) were used to determine the zone's size: Sensitive to depths of over 13 mm, moderate to depths of between 11 and 12 mm, and resistant to depths of under 10 mm [10].

Results

In the present study a total of 965 clinical isolates were collected out of which 210 were found to be S.aureus and 58 were MRSA isolates.

Table No 1: Total number of Isolates

Type of Isolates	No. of Isolates
Clinical Isolates	965
MSSA	210
MRSA	58

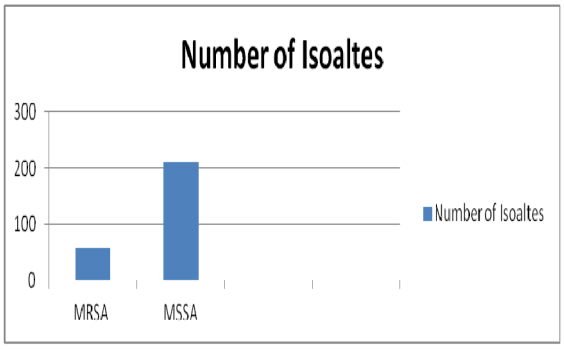
Table 2: Phenotypic Identification with the use of different test

Microscopic observation	Gram's test	Catalase test	Coagulase test	Urease test	Cefoxitin(cx) and Oxacillin(ox)
Cocciform (For all 58 cases)	+	+	+	+	+

It was observed that the maximum number of isolate were of the Males (60.3%) followed by the females (39.6%).

Table No 3: Gender wise distribution of the isolates

Type of isolates	Gender	No. of isolates	Percentage
MRSA (N=58)	Male	35	60.3%
	Female	23	39.6%
Total		58	



Graph No 1: Graphical representation of the Isolates

In the current study it was observed that the maximum age of 21-40 were been affected and the least was in the age group above 61 years [Table no. 4].

Table No 4: Age wise distribution of the isolates

S. No.	Age (in years)	No. of Cases	Percentage
1	0- 10	1	1.70%
2	20-Nov	8	13.70%
3	21-30	15	25.80%
4	31-40	19	32.70%
5	41-50	11	18.90%
6	51-60	3	5.70%
7	≥61	1	1.70%

Table No 5: Collection of different samples from different wards.

Ward	Pus	Urine	Sputum	Wound swab	Blood	Vaginal swab	Pleural fluid	Throat swab	Total
Surgery	177	74	17	62	50	0	18	0	**398**
Medicine	81	62	10	05	62	0	3	42	**265**
ICU	90	53	2	2	3	0	0	0	**150**
OBG	8	13	0	11	05	48	0	0	**85**
ENT	10	01	01	40	10	0	0	05	**67**
Total	366	203	30	120	130	48	21	47	**965**

There were 58 (38.1%) strains resistant to MRSA among them. In this study, different phenotypic methods were used to detect MRSA the best result was found from E-test (oxacillin) 59 (39%). Comparison by CDD method 59 (%), ODD 50 (31%) out of 210 isolate S. aureus growth [Table 6].

Table no. 6: Comparison of phenotypic methods for detection of MRSA. MSSA: Methicillin sensitive staphylococcus aureus

Methods	N=210	Susceptibility test	
		MRSA	MSSA
Cefoxitin (disc diffusion method)	Resistance	**58**	**0**
	Susceptible	**0**	**152**
Oxacillin (disc diffusion method)	Resistance	**50**	**0**
	Susceptible	**0**	**160**
E-test (oxacillin)	Resistance	**59**	**0**
	Susceptible	**0**	**151**

Discussion

Recently, MRSA has posed a challenge for clinical laboratories. As a result, determining methicillin resistance accurately is crucial in the prognosis of S. aureus infections.

Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen with a worldwide distribution. Given the increasing rate of MRSA infections, implementing of reliable, accurate and rapid testing for diagnosis of MRSA is necessary.

In the present study 965 clinical samples were studied .Out of 210 S.aureus isolates 58 isolates were identified as the MRSA isolates. This study was in support with the study performed by the other author where a high prevalence of MRSA (35% in ward and 43% in ICU) was observed from blood culture specimens in a study in Delhi [12]. The prevalence of MRSA in the present study was found to be 27.6%. This study was similar to the study in South India where the incidence of MRSA varies from 25 per cent in western part of India to 50 per cent [13]. It was observed that the maximum number of cases of MRSA reported was that of Males being affected with 35 (60.3%) followed by Females with 23 (39.6%). The study conducted by Joshi S et al., in India found that 42% of cases of MRSA were found [14]. In a similar way, Choudhary D and Chakravaty P observed a slightly greater prevalence (42.96%) than the present study [15].

Due to high prevalence of MRSA infections among hospitalized patients, rapid and accurate identification of MRSA is needed to initiate appropriate antimicrobial therapy and prevent the spread of MRSA infections. Usually, molecular methods such as detection of the mecA gene are preferred for this task because of high sensitivity and specificity. The results of molecular methods are also usually available faster than that of phenotypic methods [16].

In recent years, detection of mecA by PCR is considered as the gold standard for identification of MRSA. In this study, we evaluated other methods as alternatives to PCR [17], where phenotypic method was equally accurate for the detection of MRSA. CLSI has also recently substituted the oxacillin disc with cefoxitin disc for detection of MRSA [18].

The results about cefoxitin disc diffusion method are consistent with previous report [19]. However, Broekeme et al., reported the sensitivity and specificity of this method 97.3% and 100%, respectively among 1,611 S. aureus isolates [20].

In the present study a total of 965 clinical isolates were collected out of which 210 were found to be S.aureus. In the 210 s.aureus isolates, there were 58 isolates of MRSA observed.

There were 58 (38.1%) strains resistant to MRSA among them. In this study, different phenotypic methods were used to detect MRSA the best result was found from E-test (oxacillin) 59 (39%). Comparison by CDD method 58 (38%), ODD 50 (31%) ,out of 210 isolate S. aureus growth .

Different phenotypic approaches were utilised to identify MRSA, with the E-test (oxacillin) yielding the best results 59(39%), followed by the CDD method 58 (238%), and the ODD method 31% In accordance of the findings of this investigation, Sharma S et al., concluded that the E-test can be used as a substitute for the molecular method and is simple to perform in routine [21]. With the E-test MIC, Rahbar M et al., reported 100% sensitivity and 100% specificity, which is identical to present findings [22].

Similar to this work, Kumar VA et al., found that the MICs of oxacillin for isolates were in the susceptible range by E-test [23]. Despite this, Rahbar M and Safadel N reported that the CDD method is a good alternative to the ODD for MRSA detection when compared to the E-test strip method [24]. The E-test, on the other hand, has the advantage of being as easy to set up as a disc diffusion test. In a study comparable to this one, Shariati L et al., showed that the phenotypic E-test oxacillin technique detected MRSA 100% of the time [25]. In the antibiotic sensitivity pattern of S. aureus, a significant rate of MRSA antibiotic resistance was found to cefoxitin 58 (58%) and oxacillin 50 (31%), as confirmed by Demir T et al., who concluded that oxacillin (1 g) resistance was 29% and cefoxitin (30 g) resistance was 31% out of 100 isolates of pure S.aureus growth followed by other antibiotics [26]. Similar results were reported by Dhuria N et al., and Anand KB et al., in terms of determining antibiotic sensitivity/resistant patterns [27,28]. MSSA in present study was found to be highly antibiotic sensitive to linezolid , tiecoplanin gentamycin . While Shanthi M et al., identified linezolid, teicoplanin, and many other medicines to be 100 percent sensitive in their investigation [29]; the pattern is identical to the present findings. In addition to the findings of this research, a study from Iran concluded the E-test accuracy and its superiority to disk diffusion method in detecting multi drug resistance.

MRSA diagnosis is required to begin appropriate antibiotic therapy and prevent MRSA infections from spreading. In clinical laboratories, phenotypic methods such as the Oxacillin Disc Diffusion (ODD) method and Cefoxitin Disc Diffusion (CDD) method, or the E-test strip method, are available, as well as the measurement of the Minimum Inhibitory Concentration (MIC) for phenotypic methods.

Since the results of this study indicate that the E-test is more reliable than the disc diffusion method in identifying drug resistance, it can be utilised regularly for better outcomes.

Conclusion

The E-test and the results of the PCR (Polymerase Chain Reaction) results are consistent. The molecular technique is too expensive for patients to afford. Due to its low cost Compared to PCR, the E-test is more affordable and straightforward to perform, most effective substitute for regular usage in most clinical laboratories, in particular in underdeveloped nations. Additional investigation could be done in the future to validate this assertion.

References

1. Bouvet C, Gjoni S, Zenelaj B, Lipsky BA, Hakko E, Uçkay I. Staphylococcus aureus soft tissue infection may increase the risk of subsequent staphylococcal soft tissue infections. International Journal of Infectious Diseases. 2017; 60:44-48.
2. Lancet Infect Dis. 2005; 5:751-62.
3. Davis KA, Stewart JJ, Crouch HK, Florez CE et al Methicillin-Resistant Staphylococcus aureus (MRSA) Nares Colonization at Hospital Admission and Its Effect on Subsequent MRSA Infection. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Clin. Infect. Dis. 2004; 39:776-82.
4. Lowy FD. Staphylococcus aureus infection. N Eng J Med. 1998; 339:520-32.
5. Ferry T, Perpoint T, Vandenesch F, Etienne J. Virulence determinants in Staphylococcus aureus and their involvement in clinical syndromes. Curr infect Dis Rep. 2005; 7:420-28.
6. [Kurbis CA, Wylie JL. Community-based cluster of methicillin-resistant . Staphylococcus aureus in Manitoba. Can J Infect Dis. 2001; 12(30):149-52
7. Anupurba S, Sen M R, Nath G, Sharma B M, Gulati A K, Mohapatra T M. Prevalence of methicillin-resistant Staphylococcus aureus in a tertiary care Referral Hospital in Eastern Uttar Pradesh. Indian J Med Microbiol 2003; 21:49-51.

8. Kumar P, Gupta G, Gupta GK, Mishra V, Gupta G. Prevalence of MRSA and antimicrobial susceptibility staphylococcus aureus in clinical samples in National Capital Region, India. JPRI. 2021; 33:209-15.
9. Pourmand MR, Hassanzadeh S, Mashhadi R, Askari E. Comparison of four diagnostic methods for detection of methicillin resistant Staphylococcus aureus. Iran J Microbiol. 2014; 6(5):341-44.
10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 15th Informational Supplement, M100-S15. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.
11. Thiruvannamalai R, Charles J, Sundaramurthy R, Ramesh A. Study on prevalence of methicillin resistant staphylococcus aureus; comparison of four diagnostic methods for detecting MRSA– from a tertiary care hospital, Madurai. J Evolution Med Dent Sci. 2018; 7(07):864-68.
12. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in tertiary care hospital in Delhi, India. J Assoc Physicians India. 2010; 58(Suppl):32–6.
13. 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(Suppl):25–31.
14. Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, et al. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res. 2013; 137(2):363-69.
15. Choudhary D, Chakravaty P. Prevalence and antimicrobial susceptibility pattern [16] of methicillin resistant Staphylococcus aureus in Silchar Medical college and Hospital, Assam, India. Int J Basic Clin Pharmacol. 2016; 5(5):2174-77.
16. Barski P, Piechowicz L, Galinski J, Kur J. Rapid assay for detection of methicillin-resistant Staphylococcus aureus using multiplex PCR. Mol Cell Probes. 1996; 10:471–475.
17. 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
18. 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.
19. 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.
20. 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.
21. Sharma S, Srivastava P, Kulshrestha A, Abbas A. Evaluation of different phenotypic methods for the detection of methicillin resistant Staphylococcus aureus and antimicrobial susceptibility pattern of MRSA. Int J Community Med Public Health. 2017; 4(9):3297-3301.
22. Rahbar, M., Karami, S., Vand Yousefi, J. Evaluation of Five Phenotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus (MRSA). Iranian Journal of Pathology. 2011; 6(1):27-31.
23. Kumar VA, Steffy K, Chatterjee M, Sugumar M, Dinesh KR, Manoharan A, et al. Detection of oxacillin-susceptible mecA-positive Staphylococcus aureus isolates by use of chromogenic medium MRSA ID. J Clin Microbiol. 2013; 51(1):318-19.
24. Rahbar M, Safadel N. Evaluation Of Cefoxitin Disk Diffusion Test For Routine Detection Of Methicillin-Resistant Staphylococcus Aureus. Iranian Journal of Pathology. 2006; 1(4):145-48.
25. Shariati L, Validi M, Tabatabaiefar MA, Karimi A, Nafisi MR. Comparison of Real- Time PCR with Disk Diffusion, Agar Screen and E-test Methods for Detection of Methicillin-Resistant Staphylococcus aureus. Curr Microbiol. 2010; 61(6):520-24.
26. Demir T, Coplu N, Esen B. Comparative analysis of phenotypic and genotypic detection of methicillin resistance among Staphylococcus aureus. Indian J Pathol Microbiol. 2016; 59(3):314-17. Doi: 10.4103/0377-4929.188103.
27. Dhuria N, Nagpal N, Arora S. A Comparative Analysis of Different Phenotypic Methods Used in Detection of Methicillin Resistant Staphylococcus Aureus. Int J Med Res Prof. 2019; 5(2);173-75.
28. Anand KB, Agarwal 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(1):27-29.
29. Shanthi M, Sekar U. Antimicrobial susceptibility pattern of methicillin resistant staphylococcus aureus at sri ramachandra medical centre. Sri Ramachandra J Med. 2009; (2):01-04.