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A prospective study to evaluate methods of MRSA detection in patients with soft tissue and bone infection in a tertiary care centre

Serra Saji Moses[1,*], Thomas S Kuruvilla[1], Thressia Thomas[1]

[1] *Dept. of Microbiology, Father Muller Medical College, Mangalore, Karnataka, India*

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ABSTRACT

Introduction: Staphylococcus aureus is a major pathogen causing bacteraemia, pneumonia, skin and soft tissue infections (SSTIs), and osteomyelitis. Over the past 50 years, it has acquired resistance to antimicrobials including the penicillinase-resistant ones like methicillin. Rapid identification and susceptibility testing are mandatory to prevent further dissemination of MRSA and to provide effective antimicrobial treatment. Hence, methods used to detect MRSA should be rapid with high sensitivity and specificity.

Objectives: 1) To compare various phenotypic methods for MRSA detection. 2) To confirm the phenotypic results with Polymerase Chain Reaction. 3) To evaluate the susceptibility of MRSA isolates to other antimicrobial agents.

Methodology: Eighty four MRSA isolates from soft tissue and bone samples identified by the cefoxitin (30μg) disc diffusion method were subjected to Oxacillin Screen Agar (OSA), cefoxitin E-strip, automated identification & sensitivity testing using BD Phoenix system and Polymerase Chain Reaction using the GeneXpert for mecA gene detection.

Results: Although all 84 isolates were resistant by cefoxitin disk diffusion, 83 (95.4%) isolates were positive for the mecA gene. The sensitivities of the OSA, cefoxitin E-strip and BD Phoenix system were 79.5%, 80.7%, and 100%, respectively. All the isolates were sensitive to vancomycin and linezolid. 70% of the isolates were sensitive to cotrimoxazole whereas maximum resistance of 76% was seen to ciprofloxacin.

Conclusion: Automated identification by BD Phoenix system, if available, can be considered as the most sensitive phenotypic method for MRSA detection, while cefoxitin E-strip is the most appropriate test in a resource poor setting.

1. Introduction

Staphylococcus aureus is a major pathogen causing bacteremia, pneumonia, skin and soft tissue infections (SSTIs), and osteomyelitis.[1] Over the past 50 years, it has acquired resistance to antimicrobials including the penicillinase-resistant ones like methicillin.[2] Methicillin-Resistant S. aureus (MRSA) first appeared among nosocomial isolates of S. aureus in 1961.[3] They harbor the mecA gene that encodes a modified penicillin binding protein (PBP2 or PBP2a) with low affinity for methicillin and all ß-lactam antibiotics.[3] There are 3 different strains of MRSA, namely health- care associated MRSA (HA- MRSA), community- associated MRSA (CA- MRSA) and livestock- associated MRSA.[4] MRSA has emerged as a major nosocomial pathogen in the last decade.[5] Today, it has become a serious therapeutic problem worldwide, with a prevalence varying, between <3 and over 70%.[6] In India, MRSA incidence ranges from 30 to 70%.[3] Patients colonized with MRSA act as reservoirs of self-infection as well as dissemination to other patients and to the environment.[7] Failure to report methicillin resistance may lead to treatment failure, poor prognosis, increased cost of treatment, and dissemination of multi-drug resistant strains.[8] Some strains of S. aureus hyper produce beta lactamase, known as borderline oxacillin resistant S.aureus

* *Corresponding author.*
E-mail address: serra.sajimoses@gmail.com (S. S. Moses).

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(BORSA). They appear oxacillin resistant, but do not possess the usual genetic mechanism for resistance. There are also strains of S. aureus which possess a modification of existing penicillin binding proteins rather than the acquisition of a new PBP, known as modified S. aureus (MODSA). Neither of them possess the mecA gene and reporting them as MRSA is an overcall of resistance.[3]

The phenotypic methods available for detection of MRSA include using cefoxitin, a cephamycin, which is a potent inducer of the mecA regulatory system.[3] It is superior to oxacillin particularly in low-level methicillin-resistant strains.[6] Oxacillin screen agar is another method to detect methicillin resistance that can confirm indeterminate results although BORSA and MODSA strains will also grow on this medium.[6] The Phoenix Automated Microbiology System (BD Biosciences, USA) is a new, fully automated system for the rapid identification and antimicrobial susceptibility testing of gram-positive as well as gram-negative bacteria, and is used to detect resistance to antimicrobial agents. It also detects the presence of mecA gene in MRSA isolates.[7] The genotypic method used is mecA polymerase chain reaction (PCR) analysis, and is the gold standard to detect methicillin resistance, with a sensitivity of 100%.[6] Cefoxitin disk diffusion test results are in concordance with the PCR for mecA gene. Thus, the test can be used as an alternative to PCR for detection of MRSA in resource constraint settings.[3]

Rapid identification and susceptibility testing are mandatory to prevent further dissemination of MRSA and to provide effective antimicrobial treatment.[9] In addition, their ability to develop resistance to several classes of antimicrobials poses therapeutic problems.[6] Hence, methods used to detect MRSA should be rapid with high sensitivity and specificity.[3]

2. Implications of the Study

The incidence of nosocomial infections caused by MRSA continues to increase, thus the need for an early detection, especially for therapeutic and epidemiological purposes arises. Employing rapid and sensitive screening assays for MRSA detection helps to further improve infection control, as well as prevent indiscriminate use of antimicrobial agents. The phenotypic and genotypic tests included in this study will identify BORSA, and clearly differentiates it from MRSA isolates.

3. Objectives

1. To compare various phenotypic methods for MRSA detection.
2. To confirm the phenotypic results with Polymerase Chain Reaction.
3. To evaluate the susceptibility of MRSA isolates to other antimicrobial agents.

4. Methodology

A laboratory based cross sectional study was conducted in the Department of Microbiology, Father Muller Medical College Hospital, Mangalore, for a period of 10 months from June 2019 to March 2020.

4.1. Inclusion criteria

MRSA isolates from patients with soft tissue and bone infections.

4.2. Exclusion criteria

Patients with infections other than soft tissue and bone.

Isolates from patients with soft tissue and bone infections other than MRSA.

84 MRSA isolates from pus samples of patients diagnosed with soft tissue and bone infections, that were send to the microbiology laboratory for routine culture and sensitivity testing were included in the study. The sample was processed in the laboratory using standard microbiological procedures.[10] The phenotypic methods used to detect MRSA were confirmed by genotypic method. The phenotypic methods included Cefoxitin (30μg) disc diffusion method (Figure 1), Oxacillin screen agar (Figure 2), Cefoxitin E strip (Figure 3) and automated identification & sensitivity testing using BD Phoenix (Figure 4). The genotypic method used to detect MRSA was the GeneXpert PCR method to detect mecA gene (Figures 5 and 6).

The MRSA isolates were first identified by the Cefoxitin (30μg) disc diffusion method. According to CLSI guidelines, a zone diameter of $<$22mm was considered as an MRSA isolate.[11]

The isolates resistant to Cefoxitin (30μg) were tested on Oxacillin screen agar (OSA). Growth on OSA indicated MRSA. The isolates were also further tested for Cefoxitin E-test and a MIC of $\geq$8μg/mL were considered as MRSA. Automated identification & sensitivity testing of MIC using BD Phoenix system was also used to substantiate the E test method. PCR being the gold standard for detection of MRSA, was performed on the isolates using the GeneXpert as a confirmatory test in this study.

The confidentiality of the collected data is maintained. The details of the patients from which the samples are collected is not published.

Demographic and clinical details of the patients were collected from the case records.

5. Data analysis

Sample size is calculated using the formula:

$n = \frac{z \propto^2 p(1-p)}{d^2}$

$z\alpha = 1.96$

$p = 31.8\%$

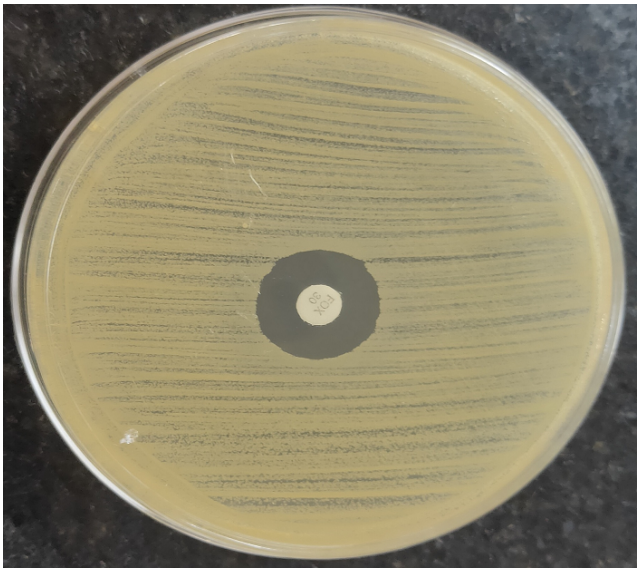


Fig. 1: Cefoxitin disc

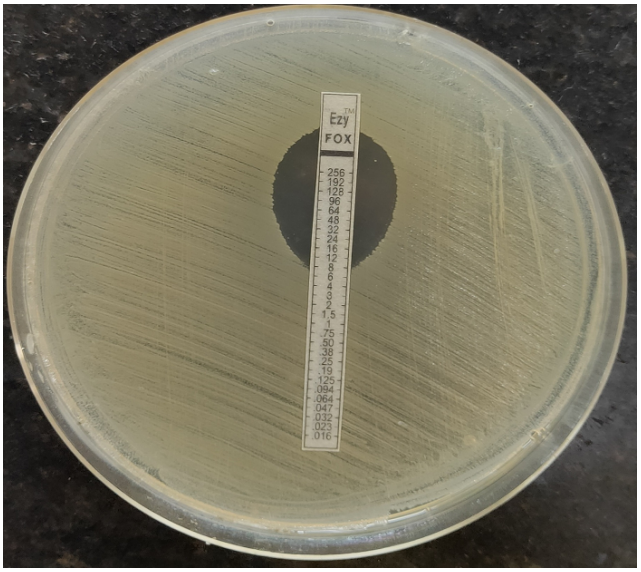


Fig. 3: Cefoxitin E strip

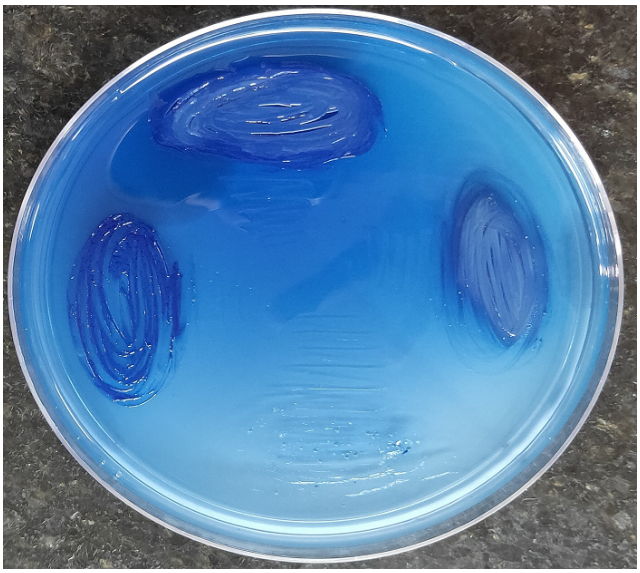


Fig. 2: Oxacillin Screen Agar

Fig. 4: BD Phoenix

d = 10%

Thus, n = 84

Data was analyzed for frequency percentage, sensitivity, specificity, positive predictive value and negative predictive value, using the Statistical Package for Social Sciences (SPSS IBM; version 25.0; Chicago, USA).

6. Results

Eighty four cefoxitin resistant S. aureus isolates from various clinical samples identified by cefoxitin disk diffusion were included in this study. Majority of these were isolated from male patients (65.5%) belonging to 41 to 60 years age group (37.9%). 60% of the patients were admitted in the hospital and 53% had presented with soft tissue and bone infection of less than 1 month duration whereas the remaining had complaints for more than 1 month. Comorbidities like Diabetes Mellitus, Hypertension and Dyslipidemia were present in 51.7%, 28.7% and 18.4% patients respectively. 35.6% had a history of surgery and 14.9% patients had a prosthetic implant. Other risk factors for infection like smoking and associated cancer was seen in 18.4% patients. Majority of the patients (23%) were diagnosed to have an abscess while the least common diagnosis (3.4%) was burns (Figure 7).

Fig. 5: Cepheid GeneXpert PCR - Shortcut

Fig. 6: MRSA cartridge with buffer

69 isolates (79.3%) showed growth in Oxacillin Screen Agar, 67 isolates (77%) had an MIC $\geq 8\mu$g/mL with cefoxitin E test and 85 isolates (97.7%) were detected as MRSA using BD phoenix system. The PCR assay for the mecA gene detected 80 (95.2%) mecA positive and 4 (4.8%) mecA negative isolates. Out of the 80 mecA positive isolates, 66, 67 and 80 isolates were correctly detected as MRSA using OSA, cefoxitin E strip and BD phoenix system respectively. The sensitivity of each of these tests were 79.5%, 80.7% and 100%. Among the 4 mecA negative

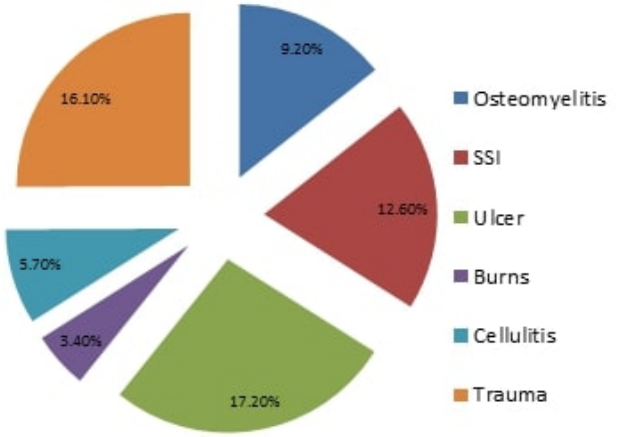


Fig. 7: Diagnosis

isolates, 3 were incorrectly identified as MRSA by OSA and 2 by BD phoenix, but none of them were incorrectly identified by cefoxitin E strip. So, the specificity of each of these tests were 25%, 100% and 50% (Table 1).

Table 1:

	OSA	Cn E Strip	BD Phoenix
True positive	66	67	80
False positive	3	0	2
True negative	3	4	2
False negative	12	13	0
Sensitivity	79.5	80.7	100
Specificity	25	100	50
Positive predictive value	95.7	100	97.6
Negative predictive value	6	20	100

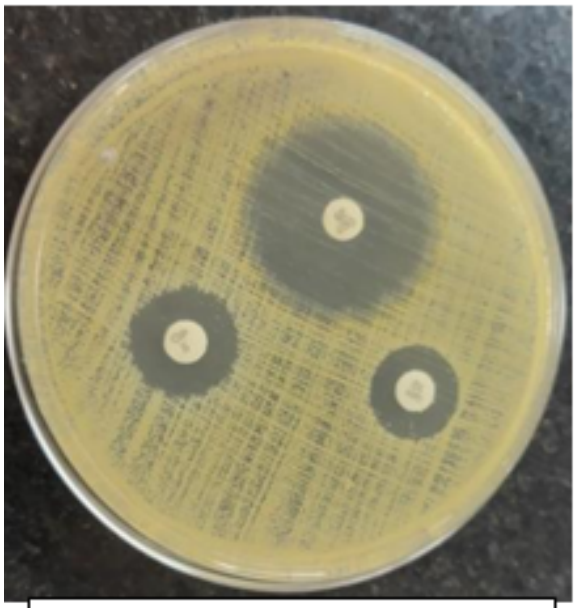
5 isolates (5.7%) showed growth in OSA only after 48 hours of incubation and showed and intermediate MIC of 6μg/mL. This indicates that it could be BORSA.

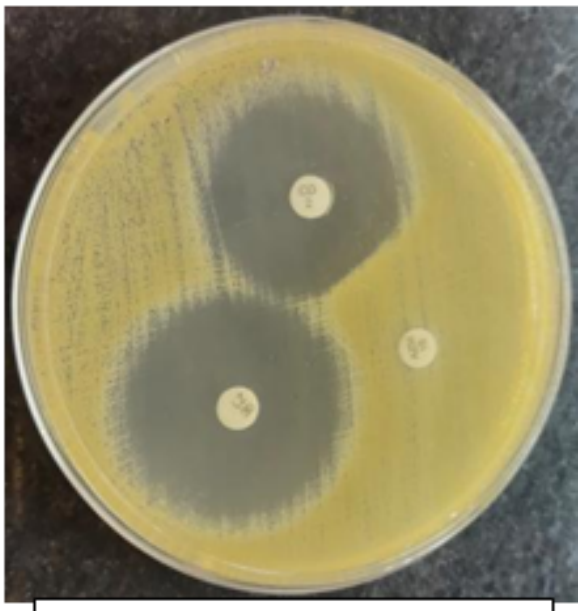
All the 84 MRSA isolates (100%) were sensitive to vancomycin and linezolid. 70% of the isolates were sensitive to cotrimoxazole, 69% to clindamycin and 66% to low level gentamycin. Maximum resistance of 76% was seen to ciprofloxacin, followed by 61% resistance to azithromycin (Table 2, Figure 8).

7. Discussion

MRSA has emerged as a major causative agent of nosocomial infection in the last decade.

Patients serve as reservoirs of self-infection as well as dissemination to other patients and to the hospital environment. So, rapid detection of MRSA is crucial for effective hospital infection control. According to CLSI guidelines, mecA gene PCR analysis is the gold standard for MRSA diagnosis, but it is not affordable for small





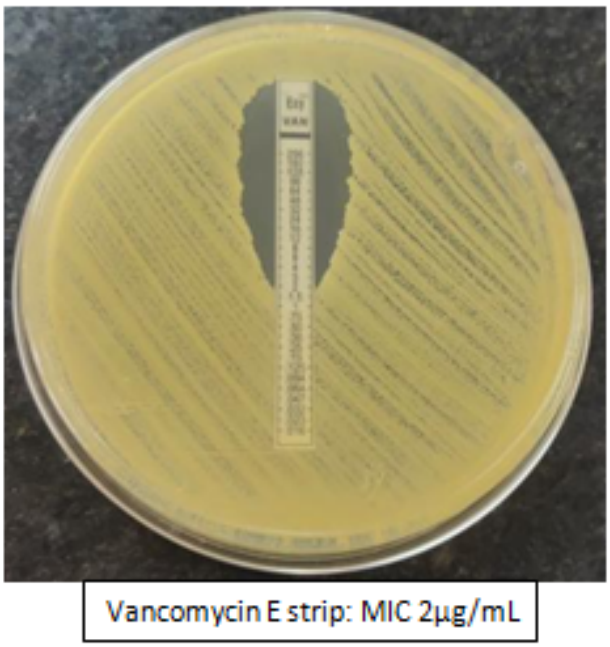


Fig. 8: Antimicrobial Sensitivity Testing with zone size

Table 2:

Antimicrobial agent	Sensitive (%)	Resistant(%)
Cotrimoxazole	70	30
Gentamycin	66	34
Ciprofloxacin	24	76
Azithromycin	39	61
Clindamycin	69	31
Vancomycin	100	0
Linezolid	100	0

laboratories with resource constraint settings. Phenotypic methods like cefoxitin and oxacillin disc diffusion methods give inconsistent results, but are more affordable, hence are being used widely in most of the laboratories for MRSA detection.

In this study, the results of oxacillin screen agar, cefoxitin E strip and BD phoenix has been with mecA gene PCR analysis in 84 MRSA strains isolated from soft tissue and bone infections, mainly associated with trauma (16.10%).

80 (95.2%) isolates were mecA gene positive. BD phoenix showed maximum sensitivity (100%), consistent with reports published by Stefaniuk et al.[7] Specificity was higher for cefoxitin E strip (100%),similar to results quoted by Swenson et al.[12] BD phoenix had a sensitivity of 100% and specificity of 75% in this study, and hence can be used as an alternative to PCR, as also suggested by other studies.[6]

The use of oxacillin screen agar with 6μg of Oxacillin per ml, is useful for identifying MRSA indicated by growth within 24 hours of incubation, although many borderline resistant strains (BORSA) will also grow on this medium. According to several reports, even though oxacillin helps in identification of BORSA, often failed to detect low level heterogeneous MRSA populations[13] and due to lower specificity (25% in this study) should not be used in methicillin resistance detection.

In this study, 76% of all MRSA strains were resistant to ciprofloxacin. Vancomycin and linezolid resistance was not detected. Although resistance to azithromycin and clindamycin is mediated by a similar mechanism, resistance rates were different for both; 61% and 31%, respectively. The low resistance rates for clindamycin could be because of rare prescription of this drug. According to other reports, MRSA strains recovered from inpatients are often resistant to a wide range of antimicrobial agents including macrolide, and aminoglycoside.[14] In this study, overall among the antimicrobials tested, MRSA strains were more resistant to the majority of available antimicrobials tested, leaving a limited choice for treatment.

8. Conclusion

PCR is the gold standard for the diagnosis of MRSA, and automated identification by BD phoenix system, if available, can be considered as the most sensitive phenotypic method for MRSA detection, while cefoxitin E-strip is the most appropriate test in a resource constraint setting. Drug of choice for treatment of MRSA is vancomycin, but they can show resistance to other antimicrobial agents, mainly to ciprofloxacin. The possibility of a resistant strain to be BORSA or MODSA should be considered while reporting MRSA from clinical samples.

9. Source of Funding

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10. Conflict of Interest

None.

References

1. Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. Characterization of methicillin-resistant Staphylococcus aureus from skin and soft tissue infections in patients in Nairobi, Kenya. *Int J Infect Dis*. 2013;17(2):e115–9.
2. Diekema D, Pfaller M, Schmitz F, Smayevsky J, Bell J, Jones R, et al. Survey of Infections Due to Staphylococcus Species: Frequency of Occurrence and Antimicrobial Susceptibility of Isolates Collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program. *Clin Infect Dis*. 1997;32(2):114–32.
3. Mathews A, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistantS. aureus. *Indian J Pathol Microbiol*. 2010;53(1):79.
4. Turner AN. Methicillin-resistant Staphylococcus areus: an overview of basic and clinical research. *Nat Rev*. 2019;17:203–18.
5. Malhotra-Kumar S, Abrahantes JC, Sabiiti W, Lammens C, Vercauteren G, Ieven M, et al. Evaluation of Chromogenic Media for Detection of Methicillin-Resistant Staphylococcus aureus. *J Clin Microbiol*. 2010;48(4):1040–6.
6. 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.
7. Stefaniuk E, Baraniak A, Gniadkowski M, Hryniewicz W. Evaluation of the BD Phoenix Automated Identification and Susceptibility Testing System in Clinical Microbiology Laboratory Practice. *Eur J Clin Microbiol Infect Dis*. 2003;22(8):479–85.
8. Kali A, Stephen S, Umadevi S. Laboratory evaluation of phenotypic detection methods of methicillin-resistant Staphylococcus aureus. *Biomed J*. 2014;37(6):411.
9. Kriegeskorte A, Idelevich EA, Schlattmann A, Layer F, Strommenger B, Denis O, et al. Comparison of Different Phenotypic Approaches to Screen and Detect mecC-Harboring Methicillin-Resistant Staphylococcus aureus. *J Clin Microbiol*. 2017;56(1). doi:10.1128/jcm.00826-17.
10. Cheesebrough M. Medical laboratory manual for tropical countries. New York: Cambridge university press; 2005.
11. Clinical and Laboratory Standards Institute / NCCLS Performance standards for Antimicrobial disc diffusion tests; Approved standards.29th ed. CLSI Document M100. Wayne Pa: Clinical and Laboratory Standards Institute; 2019.
12. Swenson JM, Tenover FC. Cefoxitin disk study group: Results of Disk diffusion testing with Cefoxitin correlate with presence of mecA in Staphylococcus sp. *J Clin Microbiol*. 2005;43:3818–28.
13. Mathews A, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistantS. aureus. *Indian J Pathol Microbiol*. 2010;53(1):79–82.
14. Roisin S, Nonhoff C, Denis O, Struelens MJ. Evaluation of New Vitek 2 Card and Disk Diffusion Method for Determining

Susceptibility of Staphylococcus aureus to Oxacillin. *J Clin Microbiol*. 2008;46(8):2525–8.

Author biography

Serra Saji Moses Post Graduate Resident

Thomas S Kuruvilla Associate Professor

Thressia Thomas Assistant Professor

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