DOI: 10.7860/NJLM/2022/53225.2627

Original Article

Microbiology Section

Comparison of Vancomycin MICs by Broth Microdilution Method, E-Test and Vitek 2C among MRSA Isolates in Tertiary Care Centre, Hyderabad, India

BUSHRA[1], KANNE PADMAJA[2], SUKANYA SUDHAHARAN[3], VIJAY DHARMA TEJA[4]

ABSTRACT

Introduction: The most important pathogen isolated from Skin and Soft Tissue Infections (SSTIs) is a gram positive organism, *Staphylococcus aureus (S. aureus).* Wide range of emerging Methicillin Resistant *Staphylococcus aureus* (MRSA) infections is leading to global threat causing Community Acquired-MRSA (CA-MRSA) or Hospital Acquired MRSA (HA-MRSA). Minimum Inhibitory Concentration (MIC) is done to ensure that antibiotics are chosen efficiently in the clinical settings by Vitek 2, Epsilometer-test (E-test) and Broth Microdilution (BMD) method.

Aim: To determine the clinical spectrum of MRSA and comparison of vancomycin MICs obtained by E-test, Vitek 2C and BMD method.

Materials and Methods: This was a cross-sectional study conducted at Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India, during the period of November 1st 2019 to December 31st, 2020 from Inpatient Department (IPD) and Outpatient Department (OPD). Among 464 SSTIs, 132 were *S. aureus,* out of which 38 isolates were MRSA. Identification and sensitivity of the isolates such as pus, wound swabs and tissues were identified through Vitek 2 compact system. Statistical analysis of the demographic and clinical characteristics was represented as frequency and percentages.

Results: Among the total 464 SSTIs, *S. aureus* were 132 of which MRSA were 38 (29%) and Methicillin sensitive *Staphylococcus aureus* (MSSA) were 94 (71%) with male predominance of 29 (76.3%) with MRSA. Most of the patients were in the age group of 21-30 years (26%). Amongst the total 38 patients analysed, 25 were from IPD and 13 were from OPD. Around 23/38 were wound swabs (60%) 12/38 of pus (31.5%) and 3/38 (8%) were tissues. The predominant risk factor observed was Surgical Site Infections (SSI) in 19 cases (50%) followed by prior antibiotic therapy in 17 (44.7%) cases. The median duration of hospitalisation was 31.5 days. Vancomycin susceptibility by all three methods with an MIC range of 0.5-2 µg/m by all three methods, except for one isolate where the MIC was >32 ug/mL by Vitek 2C and 8 ug/mL by E-test, which was sensitive by BMD with an MIC of 0.25 µg/mL.

Conclusion: Implementing infection control practices and controlling the risk factors will help in management of MRSA infections. Drug resistance to glycopeptides can be avoided by regular screening of vancomycin creeps by different susceptibility methods in order to avoid treatment failures.

Keywords: Community acquired *Staphylococcus aureus*, Epsilometer test, Hospital acquired infections, Methicillin resistant *Staphylococcus aureus,* Minimum inhibitory concentration, Skin and soft tissue infections

INTRODUCTION

The most important bacterial pathogen, *S. aureus* results in causing various systemic infections such as bacteremia, endocarditis, osteomyelitis, meningitis, sepsis, SSTIs, pneumonia and septic arthritis. The emergence of MRSA as a nosocomial pathogen leads to wide range of MRSA infections resulting in CA-MRSA or HA-MRSA [1]. Indiscriminate and inappropriate use of vancomycin results in the emergence and spread of resistant MRSA isolates in the health care settings resulting in poor clinical outcomes. The risk factors for MRSA infections are prolonged hospital stay, indiscriminate use of antibiotics, use of invasive medical devices, contact with healthcare workers, suppressed immune system, long-term use of antibiotics, overcrowding and unhygienic conditions [2].

First drug of choice for treating MRSA infections is vancomycin (Glycopeptide). Due to indiscriminate use of vancomycin to treat MRSA, it was observed that several strains with variable susceptibility have emerged showing discrepancies in-vitro that have resulted in treatment failure leading to morbidity and worsening of clinical outcomes [3]. There is an alarming increase in the prevalence of MRSA infections with 28% in 2017 to 35.1% in 2019 [4].

The MIC is done to ensure that antibiotics are chosen efficiently in the clinical settings [5]. The MIC breakpoint of vancomycin used by the Clinical and Laboratory Standards Institute (CLSI) is <2 ug/mL. Creep of vancomycin MIC from 1-2 µg/mL is widely reported leading to suboptimal clinical outcomes [6]. Due to indiscriminate use of vancomycin to treat MRSA several strains with variable susceptibility have emerged and are reported worldwide with suboptimal clinical outcomes and creeping MIC values [6]. By introducing BMD method testing, it allows the option of providing quantitative (MIC) results. It is necessary to perform standard susceptibility testing methods to detect higher MICs.

Automated systems do not accurately detect susceptibility to vancomycin. Henceforth, many laboratories should assess exact MICs by reference method such as BMD method or alternative methods, such as E-test for which this study was undertaken. As per our knowledge, BMD method is a reference method as suggested by CLSI for detection of MICs in comparison with the MICs of Vitek 2 and E-test which was done only in few Indian studies [7]. Therefore, the present study was done to analyse the clinical spectrum of MRSA infections and to compare vancomycin susceptibility by three methods.

MATERIALS AND METHODS

This was a cross-sectional study conducted at Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India, during the period of November 1st 2019 till December 31st of 2020 from IPD and OPD

patients. Among 464 SSTIs, 132 were *S. aureus*, out of which 38 isolates were MRSA. Identification and sensitivity of the isolates such as pus, wound swabs and tissues were identified through Vitek 2 compact system.

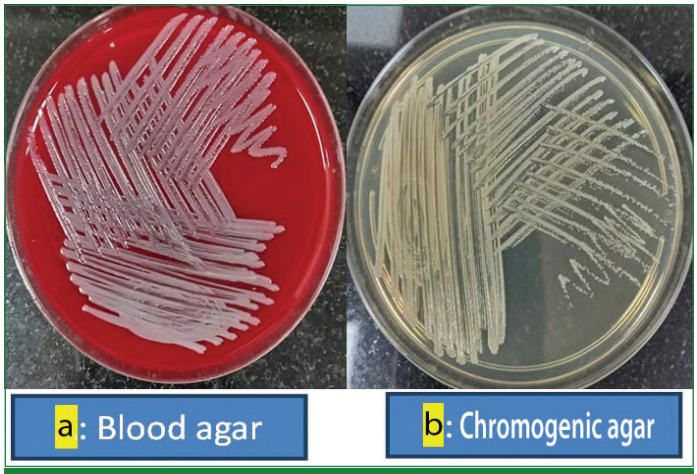
Inclusion criteria: *S. aureus* (Methicillin resistant) isolated from different purulent samples were included.

Exclusion criteria: *S. aureus* (Methicillin sensitive) isolated from different purulent samples were excluded.

All the samples received over a period of one year were included in the study for this reason the sample size was not calculated.

Procedure

Preliminary gram staining from was done for all 464 samples and they were inoculated on chromogenic agar and 5% sheep blood agar (biomeriux, Marcy'l Etoile, France) and Mannitol Salt Agar (MSA) and incubated in aerobic conditions at 37°C for 18-24 hours. Culture showing growth of gram positive cocci were subjected to routine biochemical testing such as catalase test, slide and tube coagulase test [Table/Fig-1]. Identification and antimicrobial susceptibility was performed using Vitek 2- compact system. The gram positive panels used were identification of gram positive organism and its sensitivity panel P628. Vancomycin susceptibilty was compared by three methods Vitek 2C, E-test and BMD method.



[Table/Fig-1]: (a) Showing beta haemolytic colonies on blood agar plate; (b) Showing golden yellow colonies on chromogenic agar plate.

Epsilometer-strip test (E-test): Lawn culture of the bacterial colony was done on Mueller Hinton Agar (MHA) media with density of 0.5 McFarland. Place the vancomycin E-strip and incubate for 24 hours at 37°C. The test was read by a zone of inhibition intersecting the vancomycin MIC strip at graded concentrations of antibiotic [7].

Broth microdilution test: The BMD method was used for determination of the MICs of vancomycin and vancomycin potency of 950 µg/mg was used to prepare 1 mg/mL of stock solution. Further steps are followed according to the CLSI [7].

American Type Culture Collection (ATCC) standard strains 25923 (MSSA) and 43300 (MRSA) were used as Quality Controls (QC). Antibiotic susceptibility was interpreted as per CLSI guidelines (M100, 37th edition 2020 [7] were as follows:

MIC of <2 ug/mL for Vancomycin susceptible *S. aureus* (VSSA)

MIC of 4-8 µg/mL for Vancomycin intermediate *S. aureus* (VISA)

MIC of ≥16 µg/mL for Vancomycin resistant *S. aureus* (VRSA)

STATISTICAL ANALYSIS

Statistical analysis of the demographic and clinical characteristics was represented as frequency and percentages.

RESULTS

Among 464 SSTIs, 132 were *S. aureus* of which 38 (29%) *S. aureus* found to be MRSA, 94 (71%) were MSSA and majority of patients were in the age group of 21-30 years as shown in [Table/Fig-2] with males:female ratio of 3.2:1. Previous history of surgeries was the major risk factor observed in 19/38 cases (50%) followed by long-term usage of antibiotics in 17/38 (44.7%) cases as shown in [Table/Fig-3] with median duration of hospital stay was observed to be 31.5 days.

Age group (years)	Males (n=29)	Females (n=9)	Number (n=38)	Percentage (%)
<10	1	0	1	2.6%
10-20	5	0	5	13.1%
21-30	8	4	12	31.5%
31-40	4	2	6	15.7%
41-50	6	1	7	18.4%
51-60	4	2	6	15.7%
>60	1	0	1	2.6%

[Table/Fig-2]: Showing age distribution of patients with Methicillin Resistant *Staphylococcus aureus* (MRSA) (N=38).

Risk factors	Number and percentage
Previous history of surgery	19 (50%)
Prior broad spectrum antibiotic therapy	17 (44.7%)
Presence of invasive devices (central line catheters, urinary catheters)	15 (39.4%)
Intensive care unit admission Mean duration of ICU stay 31.5 days	9 (23.6%)

[Table/Fig-3]: Showing MRSA associated risk factors (N=38).

The different sites and wounds from where MRSA were isolated is shown in [Table/Fig-4,5]. Of the clinical samples, MRSA was predominantly isolated from wound swabs shown in [Table/Fig-5]. Vancomycin susceptibility was performed by Vitek 2C, E-test and BMD. Of the 38 MRSA isolates, 26 (68%) were susceptible to vancomycin with 0.5 µg/mL of MIC by Vitek 2C and E-test.

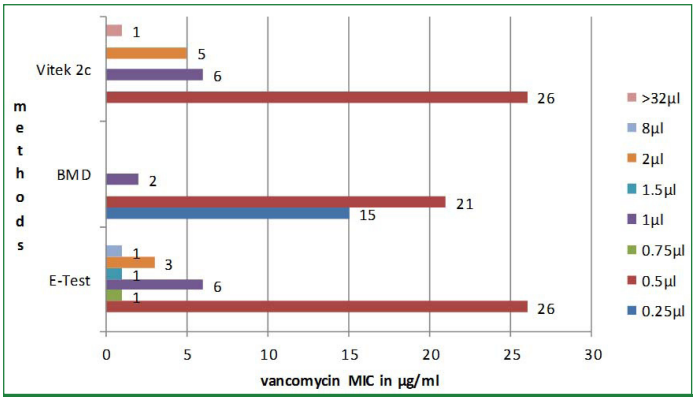
Type of Wound	Diagnosis	No. of patients (n=38)
Surgical site infections	• Postoperative craniotomy wound • Flap surgery wounds • Aneurysmal repair wound • Resection of tumour (GCT) • Crush injury wound • Orthopedic implant fixation • Laminectomy wound	17
Skin and soft tissue infection wound	• Abscess, cellulitis, burn wounds	16
Osteomyelitis wound	Sinus tract wound	2
Continuous Ambulatory Peritoneal Dialysis (CAPD) catheter site	CAPD catheter site wound	3

[Table/Fig-4]: MRSA isolated from different types of wounds (N=38).
CABG: Coronary artery bypass graft surgery; GCT: Giant cell tumour

Clinical Sample	No. of MRSA Isolated (n=38)	Percentage (%)
Wound swab	23	60.5%
Pus	12	31.5%
Tissue	3	7.8%
Total	38	100%

[Table/Fig-5]: Specimens from which MRSA is isolated (N=38).

Vancomycin MIC with Vitek 2C showed 0.5 µg/mL (68%) and 1-2 µg/mL (28.9%), E-Test showed 0.5 µg/mL (68%) and 1-2 µg/mL (18.4%) whereas with BMD 0.5 µg/mL (55.2%) and 1-2 µg/mL (5.2%) were observed. One of the isolate was resistant to vancomycin with an MIC >32 µg/mL by Vitek 2C and intermediate susceptibility to vancomycin with MIC of 8 µg/mL by E- test, which was found susceptible by BMD method with an MIC of 0.25 µg/mL as shown in the [Table/Fig-6].



[Table/Fig-6]: Minimum Inhibitory Concentration (MIC) values of vancomycin by Broth Micro Dilution (BMD), Epsilometer (E)-test and Vitek 2 system.

Majority of the outpatients 13/38 (34.2%) were treated with oral linezolid at a dosage of 600 mg for a period of 7 -10 days. Patients who were admitted 25/38 (65.7%) were treated with intravenous vancomycin 1g for a period of 7-10 days. The wound was healthy in 36/38 (94.7%) patients at the time of discharge with an advise to follow-up. Out of two patients 2/38 (5.2%), one with Road Traffic Accident (RTA) with MRSA wound infection and sepsis and other patient with craniotomy site wound infection had a fatal outcome.

DISCUSSION

Staphylococcus aureus, is the most common cause of SSTIs, especially MRSA leading to wide range of infections both at the community and hospital settings [8]. Prevalence of MRSA is alarmingly high and causes a great concern to clinicians in management of infections. Common risk factors for MRSA infection are long term hospitalisation, admission into Intensive Care Units (ICUs), overuse of antibiotics, device associated infections and SSIs. Treating the patients with suboptimal dosage of vancomycin leads to vancomycin creep [8]. Vancomycin is the drug of choice for treating MRSA infection. But with the MIC creep, therapeutic failure remains a challenge in treating the MRSA associated infections in all healthcare institutions. In this study, 132/464 (28.4%) were *S. aureus* isolates from clinical samples such as wound swab, pus, tissues, of which 94 (71%) were MSSA and 38 (29%) were MRSA which is concordant with study done by Abdullahi N and Iregbu KC, 26.9%, Kumari N et al., 26% and Adhikari R et al., 35.50%, Reema HA and Saldanha Dominic RM, 46%, Maina EK et al., 15%, Kshetry AO et al., isolated, 37.6% MRSA [9-14]. A total of 28/47 patients were IPD and 19/47 were OPD, genderwise, showed 30 males and 17 females whereas the present study showed 25/38 IPD and 13/38 OPD with male predominance of 29 males and 9 females [14]. In a study by Adhikari R et al., 13.26% of MRSA isolates were observed among the age group of 18-45 years whereas the present study showed a total of 20 MRSA isolates between 20-40 years (52.6%) [11].

A study by Gupta S et al., showed that previous hospitalisation was found to be an important risk factor associated with MRSA infections, as similar with the present study [15], whereas in a study by Lohan K et al., the maximum number of MRSA was isolated from the patients who underwent surgery (25.9%) but present study showed 50% isolation of MRSA with same risk factor [4]. In a study by Dubert M et al., showed that MRSA was isolated from Sternal Wound Infections (SWI) after sternotomy for cardiac surgery, with an incidence of 0.5-3%, whereas in present study, it was observed to be 15% [16]. In a study by Jiménez-Martínez E et al., it was observed that after craniotomy the incidence of SSI (SSI-CRAN) were 2.2-19.8%, whereas in the present study it is observed to be 10% [17]. In a study by Mehta M et al., observed prolonged hospitalisation had increased risk of infection with MRSA, which is similar to present study [18].

The present study showed vancomycin MICs of all MRSA isolates by E-strip Test and Vitek 2C were susceptible to vancomycin (MIC≤ 2 µg/mL) except one isolate with the above mentioned methods were found resistant whereas BMD showed susceptibility to vancomycin.

In present study, susceptibility of vancomycin MIC by E-strip test were 0.5-2 µg/mL for most of the isolates, which is similar to with studies conducted by Ranjan K et al., and Mouton JW and Jansz AR, [19,20]. Different susceptibility testing methods for vancomycin showed variable results. A study by Sader HS et al., Tandel K et al., Rossatto FC et al., Himani CA et al., and Anitha TK et al., showed vancomycin MICs with different susceptible ranges by different methods as shown in [Table/Fig-7] [21-25].

Other studies	Year	Vitek 2C vancomycin MIC (µg/mL)	Broth Microdilution (BMD) vancomycin MIC (µg/mL)	Epsilometer-test (E-test) vancomycin MIC (µg/mL)
Anitha TK et al., [25]	2019	1-2 (84.2%)	-	1-2 (51.81%)
Himani CA et al., [24]	2015	1-2 (100%)	1-2 (100%)	1-2 (100%)
Rossatto FC et al., [23]	2014	-	1-2 (54.6%)	1-2 (30.6%)
Tandel K et al., [22]	2012	-	-	1-2 (24.5%)
Sader HS et al., [21]	2009	-	<1 (96.9%)	1.5 (58.3%) 2 (32.1%)
Present study		0.5 (68%) 1-2 (28.9%)	0.5 (55.2%) 1-2 (5.2%)	0.5 (68%) 1-2 (18.4%)

[Table/Fig-7]: Showing comparision of vancomycin MICs in different studies by different methods [21-25].
MICs: Minimum inhibitory concentrations

The treatment of choice of MRSA infections is vancomycin [26]. Majority of the outpatients 46.4% were treated with oral linezolid at a dosage of 600 mg for a period of 7-10 days and patients who were admitted (65.7%) were treated with intravenous vancomycin 1g for a period of 7-10 days. The wound was healthy in 94.7% patients at the time of discharge with an advice to follow-up. The mortality of MRSA infections were 5% [27]. In the present study, all patients were discharged except two patients (2/38) who had other risk factors also, one with RTA with MRSA wound infection and sepsis and other patient with craniotomy site wound infection had a fatal outcome.

Limitation(s)

Molecular detection mecA gene for MRSA isolates was not done in the present study, as credibility of isolating MRSA from clinical samples increases with methicillin (mec) A gene detection.

CONCLUSION(S)

Vancomycin is the drug of choice in treating MRSA infections. MRSA isolates with higher and susceptible range, are to be identified which has treatment failures with vancomycin. Automated systems such as Vitek 2C and E-test do not provide precise MIC values. BMD is the preferred method which has given MIC values in a susceptible range when compared to other methods. The outcome of MRSA *S. aureus* infections should take into account the method used for determining the vancomycin susceptibility.

Declaration

The authors have presented the study in MICROCON 2020 https://linkinghub.elsevier.com/retrieve/pii/S0255085721043085

REFERENCES

[1] Kala Yadhav ML, Panicker GJ. Prevalence and antibiogram of methicillin resistant staphylococcus aureus isolates at a tertiary care hospital in bangalore, south india. Int J Cur Res Rev. 2014;6(17):37-40.
[2] Siddiqui AH, Koirala J. Methicillin Resistant *Staphylococcus aureus*. Stat Pearls Publishing; 2021.

[3] Hsu DI, Hidayat LK, Quist R, Hindler J, Karlsson A, Yusof A, et al. Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Int J Antimicrob Agents. 2008;32(5):378-85.
[4] Lohan K, Sangwan J, Mane P, Lathwal S. Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern. J Family Med Prim Care. 2021;13(10):752-57.
[5] Tarai B, Das P, Kumar D. Recurrent challenges for clinicians: Emergence of methicillin-resistant *Staphylococcus aureus*, vancomycin resistance, and current treatment options. J Lab Physicians. 2013;5(2):71-78.
[6] Soriano A, Marco F, Martinez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis. 2008;46(2):193-200.
[7] CLSI ref. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2021.
[8] Sathish JV, Wadekar MD. Prevalence of MRSA in clinical samples and their antibiotic sensitivity pattern. Int J Curr Microbiol Appl Sci. 2017;6(7):2338-43.
[9] Abdullahi N, Iregbu KC. Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. Ann Trop Pathol. 2018;9(1):6-10.
[10] Kumari N, Mohapatra TM, Singh Y. Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a tertiary-care hospital in eastern Nepal. J Nepal Med Assoc. 2008;47(170):53-56.
[11] Adhikari R, Pant ND, Neupane S, Neupane M, Bhattarai R, Bhatta S, et al. Detection of Methicillin Resistant *Staphylococcus aureus* and Determination of minimum inhibitory concentration of vancomycin for *Staphylococcus aureus* isolated from pus/wound swab samples of the patients attending a tertiary care hospital in Kathmandu, Nepal. Can J Infect Dis Med Microbiol. 2017;2017:219153.
[12] Reema HA, Saldanha Dominic RM. Prevalence and antimicrobial susceptibility pattern of clinical isolates of Methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital in Mangalore. J Int Med Dent. 2016;3(3):134-39.
[13] Maina EK, Kiiyukia C, Njeri Wamae C, Waiyaki PG, Kariuki S. Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi. Kenya. International Journal of Infectious Diseases. 2013;17(2)115-19.
[14] Kshetry AO, Pant ND, Bhandari R, Khatri S, Shrestha KL, Upadhaya SK, et al. Minimum inhibitory concentration of vancomycin to methicillin resistant *Staphylococcus aureus* isolated from different clinical samples at a tertiary care hospital in Nepal. Antimicrob Resist Infect Control. 2016;21(5):27.
[15] Gupta S, Mishra B, Thakur A, Dogra V, Loomba PS, Jain M, et al. Risk factors associated with MRSA. Southern African Journal of Infectious Diseases. 2018;33(3):76-79.
[16] Dubert M, Pourbaix A, Alkhoder S, Mabileau G, Lescure F-X, Ghodhbane W, et al. Sternal Wound Infection after Cardiac Surgery: Management and Outcome. PLoS ONE. 2015;10(9):0139122.
[17] Jiménez-Martínez E, Cuervo G, Hornero A, Ciercoles P, Gabarrós A, Cabellos C, et al. Risk factors for surgical site infection after craniotomy: a prospective cohort study. Antimicrob Resist Infect Control. 2019;8:69. DOI https://doi.org/10.1186/s13756-019-0525-3
[18] Mehta M, Dutta P, Gupta V. Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in north India. Jpn J Infect Dis. 2005;58(3):174-76.
[19] Ranjan K, Arora D, Ranjan N. An Approach to Linezolid and Vancomycin against Methicillin Resistant *Staphylococcus aureus*. Webmed Central MICROBIOLOGY. 2010;1(9):WMC00590.
[20] Mouton JW, Jansz AR. The DUEL study: A multi-center in vitro evaluation of linezolid compared with other antibiotics in the Netherlands. Clin Microbiol Infect. 2001;7(9):486-91.
[21] Sader HS, Rhomberg PR, Jones RN. Nine-hospital study comparing broth microdilution and Etest method results for vancomycin and daptomycin against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2009;53(7):3162-65.
[22] Tandel K, Praharaj AK, Kumar S. Differences in vancomycin MIC among MRSA isolates by agar dilution and E test method. Indian J Med Microbiol. 2012;30(4):453-55.
[23] Rossatto FC, Proença LA, Becker AP, Silveira AC, Caierão J, D'Azevedo PA. Evaluation of methods in detecting vancomycin MIC among MRSA isolates and the changes in accuracy related to different MIC values. Rev Inst Med Trop Sao Paulo. 2014;56(6):469-72.
[24] Himani CA, Madan M, Pandey A, Thakuria B. Detection of Vancomycin Susceptibility in MRSA isolates by micro broth dilution, E Test and Vitek 2 methods. J Clin Diagn Res. 2015;9(10):01-04.
[25] Anitha TK, Rao MR, Shankaregowda R, Mahale RP. Evaluation of vancomycin minimum inhibitory concentration in the clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA). J Pure Appl Microbiol. 2019;13(3):1797-801.
[26] Choo EJ, Chambers HF. Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia. Infect Chemother. 2016;48(4):267-73.
[27] Delaney JA, Schneider-Lindner V, Brassard P, Suissa S. Mortality after infection with methicillin-resistant *Staphylococcus aureus* (MRSA) diagnosed in the community. BMC Med. 2008;31(6):2.

PARTICULARS OF CONTRIBUTORS:
1. Junior Resident, Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.
2. Associate Professor, Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.
3. Associate Professor, Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.
4. Head, Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kanne Padmaja,
Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India.
E-mail: kannepadmaja@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 11, 2021
- Manual Googling: Feb 15, 2022
- iThenticate Software: Feb 19, 2022 (4%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Nov 10, 2021**
Date of Peer Review: **Jan 14, 2022**
Date of Acceptance: **Feb 19, 2022**
Date of Publishing: **Jul 01, 2022**