Indian Journal of Public Health Research & Development
Volume 13 | Issue 4 | October-December 2022

Comparative evaluation of MIC of Vancomycin among methicillin resistant Staphylococcus aureus (MRSA) isolates in tertiary care hospital

Swarupa Rani[1], Tejashree.A[2], Ranjitha Shankare Gowda[3], Krishna Karthik M.V.S[4], Eeshita Dhar[5], B. Satya Sai[6]

[1]M.Sc Post graduate, Dept of Microbiology, JSSMC, JSSAHER, Mysore, [2]Prof & HOD, Dept of Microbiology, JSSMC, JSSAHER, Mysore
[3]Assistant Professor, Dept of Microbiology, JSSMC, JSSAHER, Mysore, [4]Tutor, Dept of Microbiology, JSSMC, JSSAHER, Mysore
[5]PhD Research Scholar, Dept of Microbiology, JSSMC, JSSAHER, Mysore, [6]Tutor, Dept of Microbiology, JSSMC, JSSAHER, Mysore

How to cite this article: Swarupa Rani, Tejashree.A, Ranjitha Shankare Gowda, Krishna Karthik M.V.S , Eeshita Dhar, B. Satya Sai et al Comparative evaluation of MIC of Vancomycin among methicillin resistant Staphylococcus aureus (MRSA) isolates in tertiary care hospital. Volume 13 | Issue 4 | October-December 2022

Abstract

Vancomycin, a glycopeptide antibiotic with in-vitro activity against all staphylococci and clinical response to methicillin resistant staphylococcus aureus (MRSA) infection, became the backbone of treatment due to MRSA's universal resistance to the antimicrobial agents belonging to beta-lactams and There are no other viable options. In patients with staphylococcal infections, this study demonstrated a significant conflict of minimum inhibitory concentrations (MICs) of vancomycin for MRSA strains between an automated system, Vitek 2, and the method. test E-strip. In the Microbiology laboratory of JSS hospital which is a tertiary care center situated in Mysuru, 90 isolates were acquired from various clinical samples to analyse Vancomycin MIC. Out of 90 isolates, 2.2% MRSA isolates showed highest vancomycin MIC 2µg/ml by Vitek-2 method, where no isolates showed MIC up-to 2µg/ml by E-strip method. But the higher vancomycin MIC 1µg/ml was observed in 82.2% by vancomycin E-strip method compared to 37.7% by Vitek-2 method. Lowest MIC 0.5µg/ml showed by Vitek-2 method in 58.8% compared to 4.4% by E-strip method. The study concludes that all *S. aureus* isolates were resistant to methicillin by both Vitek-2 system and cefoxitin disc diffusion method and also identified as VSSA (vancomycin susceptible *Staphylococcus aureus*) by Vitek-2 and E-test method. Higher vancomycin MIC ≥1µg/ml in 93.3% may be due to using of vancomycin improperly and infrequently in MRSA infection with lowest MIC value. Therefore, this method can also be used as routine laboratory practice or as alternative method where Vitek 2 system or other methods are not available.

Keywords: *Staphylococcus aureus*, MIC (minimum inhibitory concentration), MRSA (methicillin resistant *Staphylococcus aureus*), VSSA (Vancomycin susceptible *Staphylococcus aureus*).

Introduction

Staphylococcus aureus normally found as normal flora in human beings. For more than a centrury, *S. aureus* has been identified as a major source of human diseases. It has been associated with various infections which cover minor skin infections and osteomyelitis to urinary tract infection and severe bloodstream infection. MRSA strains, also known as multidrug-resistant *Staphylococcus aureus* (earlier it is mentioned in 1960s) appeared in the previous decade as a cause infection linked to healthcare accountable for mortal diseases as well as serious life-threatening pneumonia, osteomyelitis, severe sepsis, necrotizing fasciitis, endocarditis, and toxicoses such as toxic shock syndrome. [1]

In 1960, MRSA emerged and spread throughout the world after the implementation of methicillin for the treatment of S. aureus diseases that produce

Corresponding Author:
Badveti. Satya sai
Tutor, Dept of Microbiology, JSSMC, JSSAHER, Mysor
Email ID: badvetisatya@gmail.com

penicillinase. *S. aureus* strains which are resistant to antibiotics, specially to methicillin, are uniformly adapted to hospitals and external territory, developed as a universal pathogen of communal health concern. [2]

MRSA strains are mainly mediated by mecA gene, and resistant to other higher β-lactam group of antibiotics including cephalosporins. To treat MRSA infection other antibiotics also used such as, Cotrimoxazole, aminoglycosides, erythromycin, clindamycin etc. The glycopeptide antibiotic, Vancomycin was once thought to be the best option for treatment. Apart from vancomycin, other efficient medications such as linezolid and teicoplanin are extensively utilised.[3]

Vancomycin has been used in clinical practice for more than 50 years and is remain the gold standard for treating MRSA infections. [4] In MRSA infection with lowest MIC value, indiscriminate and sporadic administration of vancomycin has led in the establishment of isolates with higher vancomycin MIC values. Only VISA (Vancomycin intermediate S. aureus) strains were known in the early 1920s, however S. aureus strains with higher vancomycin MIC are now appearing in India. [3] Vancomycin acts especially by suppressing the cell-wall synthesis. It functions against gram positive cell walls by preventing N-acetyle glucosamine from being incorporated into the peptidoglycan matrix. [5] The isolate was designated as VISA because of slightly higher vancomycin MIC, in the range of 4-8µg/ml. [4]

Recently, vancomycin-resistant S. aureus (VRSA) has been discovered. This larger MIC of vancomycin in VRSA is associated with mutations due to excessive peptidoglycan accumulation and cell wall thickening. The vancomycin MIC test is the gold standard for determining whether a strain is susceptible, intermediate, or resistant to vancomycin. [3] According to CLSI criteria, S. aureus is sensitive to vancomycin if its MIC is ≤2µg/ml, resistant strains have MIC ≥16µg/ml while VISA strain has MIC of 4-8µg/ml. [6]

Materials and Method

The study was performed in the Department of Microbiology at a tertiary care hospital, Mysuru, in a duration of 1 year. A complete 90 MRSA clinical isolates were obtained from various clinical samples like pus, blood, endotracheal swabs, blood and other body fluids received in the laboratory. All the isolates were subjected to standard procedure for identification. MRSA was detected by modified Kirby-Bauer disc diffusion method. Isolate suspension of 0.5 McFarland turbidity was applied on Muller-Hinton agar followed by application of Cefoxitin disc (30µg) incubated at 37°C for 18-24 hours. Zone of inhibition around Cefoxitin disc <21mm were considered as methicillin resistant.

For detection of vancomycin MIC by E-test method, isolates were inoculated on Mueller Hinton agar (MHA) media. Vancomycin E-strip (paper strip from Hi-media) impregnated with vancomycin drug, comprises of predefined antibiotic gradient was placed on the MHA plate and incubated for overnight at 35°C to determine the MIC, in µg/ml (Figure 1).

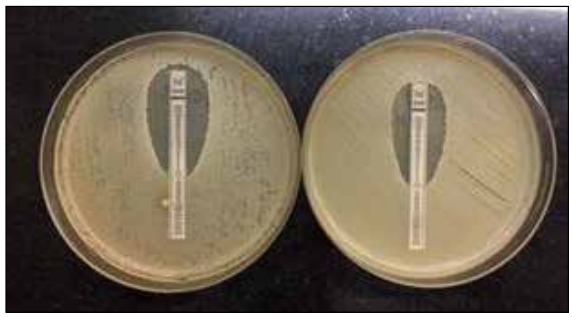

Figure 1: Quality control of Staphylococcus aureus

Figure 2: vancomycin E-strip test result of MIC 1µg/ml.

The test organisms were suspended in sterile physiological saline to 0.5 McFarland standards for the Vitek2 method to obtain the vancomycin MIC values. The bacterial suspension was auto-filled into an antimicrobial susceptibility test card, which was then inserted into the the Vitek 2 system incubator reader and results are expressed as MIC values in micrograms / ml.

Results

A total of 90 MRSA strains were collected from various clinical specimens. Pus was the predominant sample 81(90%) followed by 2(2.2%) were blood samples, 5(5.5%) from Ear swabs and ET (2.2%) samples. **(Chart 1)**

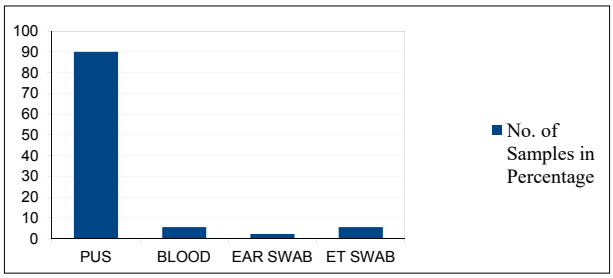

Chart 1: Bar diagram showing the various clinical isolates.

All the MRSA isolates were sensitive (<4 µg/ml) by vancomycin E-strip method and Vitek-2 method also. (**Table 1**)

Table 1: Comparison of vancomycin MIC values by E-test with Vitek-2 system.

Vancomycin MIC values (µg/ml)	No. of isolates by Vitek-2 method (n=)	No. of isolates by E-strip Method (n=)
0.5	54 (60%)	4 (4.4%)
1	34 (37.7%)	74 (82.2%)
1.5	0	12 (13.3%)
2	2 (2.2%)	0
≥ 4 to 16	0	0

Highest vancomycin MIC determined was 2µg/ml by Vitek-2 method for 2.2% MRSA isolates, where no isolates showed MIC upto 2µg/ml by E-strip method. Higher vancomycin MIC 1µg/ml was observed in 82.2% by vancomycin E-strip method compared to 37.7% by Vitek-2 method. Vancomycin MIC 1.5µg/ml was observed in 13.3% by E-strip test method but no isolate had an MIC at this level by Vitek2 method. Lowest MIC 0.5µg/ml showed by Vitek-2 method in 60% compared to 4.4% by E-strip method.

Discussion

MRSA is becoming a global problem, producing a wide spectrum of infection compared to methicillin-sensitive *S. aureus* (MSSA). Antibiotic choice usually begins with trimethoprim- sulfamethoxazole or, doxycycline or minocycline for the sulfa allergic patient. MRSA infections that do not respond to standard treatment may require a combination of medicines, such as vancomycin and one or more additional antibiotics. When choosing an antibiotic to treat a serious infection, the MIC is the key predictor. Many investigations in recent years have found a link between vancomycin therapy failure and MIC values of 1.5 or 2µg/ml which are within the range of clinical laboratory standards institutes (CLSI).[6]

In the current study maximum numbers of MRSA were isolated from pus sample 80(90%) followed by 2(2.2%) from blood, 5(5.5%) from Ear swabs, and ET (2.2%) samples. Similar findings has been reported by Dimple Raina et al., where 78% of isolates from the pus sample.[7] The frequency of isolating maximum rate of MRSA from pus also reported by Tiwari et al.(68%) in Varanasi, Mallick SK and Basak et al., in Maharashtra (51.8%), which were resistant to penicillin and sensitive to vancomycin and linezolid.[8], [9] Rao B. N et al., in Andhra Pradesh (64%) and Dar JA et al. in Aligarh reported highest (n151;35.5%) percentage of MRSA found in pus specimens followed by sputum and throat swabs.[10], [11]

The incidence of MRSA strains in clinical samples varies in each area of India. This variance could be related to the selective use of antibiotics to battle the infection, or it could be related to MRSA screening of patients and health care personnel.[3]

Vancomycin MICs of MRSA strains were determined using the Estrip test and the Vitek 2 method (MIC measured in µg/ml) in this study. Using both approaches, all MRSA strains were determined to be susceptible to vancomycin (MIC ≤ 2 µg/ml). In our review, the MIC of vancomycin by the Estrip test was in the range of 0.5-2 µg/ml, similar to and correlating with the studies of Anitha T. K et al. (2019), Himani et al. the (2018) and Eeshita (2016) are very good. In their studies, the MIC range of vancomycin was 0.52 g/ml. [2],[12],[3]

The present study with lowest MIC 0.5µg/ml showed by Vitek-2 method in 58.8% compared to 4.4% by E-strip method followed by 11(12.6%) of the isolates showed the MIC range of 1.5µg/ml by E-strip test, which is comparable to the study of Brandon J. et al, where 35 isolates with an MIC of 0.5µg/ml via vitek 2 while only 1 isolate (1.3%) having an MIC at this level according to E-test. Further, 50 isolates with mics of 1.5µg/ml via E-test method, where no isolates with MIC at this level by vitek 2 system in the study

of Brandon j. et al., which is similar to the present study.[13] In the study of Daiana C. S. Rodrigues et al, vancomycin MIC with 0.5µg/ml showed by 42 MRSA isolates out of 51 via vitek 2, where E-test detected only 1 isolate with MIC at 0.5µg/ml. Moreover, in their study, 18% MRSA with vancomycin MIC 1µg/ml by E-strip method and 27% by Vitek-2 method, which is comparable to our study with higher vancomycin MIC 1µg/ml observed in 82.2% isolates by vancomycin E-strip method compared to 37.7% by Vitek-2 method.[14]

In current study, highest vancomycin MIC was 2µg/ml by Vitek-2 method for 2.2% where no isolates showed MIC upto 2µg/ml by E-strip method which can be compare to the study of behara et al., where all the MRSA strains with MIC lower than 2µg/ml by E-test method including those isolates having vancomycin MIC ≥2µg/ml by Vitek 2 method.[15]

To summarize the findings of present investigation, which illustrate that there was substantial conflict amongst vancomycin MICs when correlating E-test results to Vitek 2 results, but all isolates were vancomycin sensitive by both the methods. According to earlier studies, E-test frequently reports MICs higher than 1µg/ml, even among isolates with MICs as low as 0.5µg/ml by Vitek2 system. It is very rare getting vancomycin MIC at ≥2µg/ml by Vitek while no isolates having MIC at this level with E-strip test method.

Conclusion

In this study we conclude that in the determination of vancomycin MIC by Vitek-2 method and E-test method, all the MRSA isolates were confirmed as VSSA by both methods. The performance of E-test is cheaper, and easiest to determine the vancomycin MIC when compare to other methods. This method can also be used as routine laboratory practice or as alternative method where Vitek 2 system or other methods are not available. Higher vancomycin MIC may be the result of inappropriate and infrequent use of vancomycin in MSSA infection or in MRSA infection with lowest vancomycin MIC values. Vancomycin is still remained the corner stone of treating MRSA infection. MRSA isolates with higher MIC values (even in the susceptible range) appeared more frequently, leading to vancomycin treatment failure. The higher vancomycin MICs in isolates of MRSA have become a matter of concern, and further studies will help detect significant MRSA infections.

Source of Funding: Nil

Ethical Clearance: Institutional Ethics committee approval was obtained from JSS Medical college, Mysore.

Conflict of Interest: No potential conflict of interest relevant to this article reported

References

1. Eyob Yohaness Garoy, Yacob Berhane Gebreab, Oliver Okoth Achila, Daniel Goitom Tekeste, Robel Kesete, Robel Ghirmay, Ruta Kiflay, Thomas Tesfu. Methicillin-resistant *Staphylococcus aureus* (MRSA): prevalence and antimicrobial sensitivity pattern among patients-A multicentre study in asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019 Feb 6;2019:8321834. doi: 10.1155/2019/8321834.
2. Anitha T.K., Morubagal Raghavendra Rao, Ranjitha Shankaregowda, Rashmi P. Mahale, Sowmya G.S. and Vidyavathi B. Chitharagi, Evaluation of Vancomycin Minimum Inhibitory Concentration in the clinical isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA), *J Pure Appl Microbiol*., 2019; **13**(**3**): 1797-1801. https://doi.org/10.22207/JPAM.13.3.56.
3. Eeshita Dhar, Sakshita Agnihotri, B. G. Unni Comparison of Vancomycin MIC against methicillin resistant *Staphylococcus aureus* and methicilin sensitive *Staphylococcus aureus* isolated at a tertiary care centre in Jaipur, India. December 2016: Vol.-6, Issue- 1, P. 76-82.
4. Maureen U. Okwu,1, Mitsan Olley,2 Augustine O. Akpoka,1 and Osazee E. Izevbuwa. Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. AIMS Microbiol. 2019 Apr 15;5(2):117-137. doi: 10.3934/microbiol.2019.2.117.
5. Enu Ju Choo, Henry F. Chambers. Treatment of Methicillin Resistant *Staphylococcus aureus* Bacteremia. Infect Chemother. 2016 Dec;48(4):267-273. doi: 10.3947/ic.2016.48.4.267.
6. Wayne, PA: Clinical and Laboratory Standards Institute(CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100. 2020.
7. Raina D, Sakeena, Chandola I, Roy R. Detection of vancomycin susceptibility among methicillin resistant *Staphylococcus aureus* in a tertiary care hospital. Panacea J Med Sci. 2020;10(3):245-249.
8. Tiwari HR, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance. 2008:1 57–61.

9. Mallick SK and Basak S. MRSA- too many hurdles to overcome: a study from Central India. Tropical Doctor. 2010; 40: 108–110.

10. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, et al. Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. Ann Clin Microbiol Antimicrob. 2006; 5:22.

11. Rao BN, Srinivas B. A prospective study of Methicillin resistant *staphylococcus aureus* (MRSA) in a teaching hospital of rural setup. Journal of Pharmaceutical and Scientific Innovation. 2012; 1(2): 37-40.

12. Himani, Vinay Kumar Srivastava. Prevalence of methicillin resistance & comparison of vancomycin minimum inhibitory concentration by e test & vitek 2 in *staphylococcus aureus* isolates. International Journal of Research & Review (www.ijrrjournal.com). 2018 September. Vol.5; Issue: 9; 2454-2237.

13. Brandon J. Bloomgren and Brad R. Laible. Etest versus Vitek 2 vancomycin minimum inhibitory concentration testing methods for methicillin-resistant *staphylococcus aureus*: an antimicrobial stewardship initiative to evaluate the degree of discordance among methods at a rural tertiary hospital. *Journal of Pharmacy Practice* 2013 26: 415.

14. Daiana C. S. Rodrigues, Ana Paula P. Costa, Paulo Roberto V. Santos, Elizabeth A. Marques, Jose F. Nogueira Neto, Robson S. Leao. Comparative evaluation of the phoenixr, vitekr 2, e-testr and microdilution test for vancomycin susceptibility testing in *staphylococcus aureus* isolated from bloodstream infection. BJHBS, Rio de Janeiro, 2021;20(1):11-18.

15. Behera B, Mathur P. Erroneous reporting of vancomycin susceptibility for staphylococcus spp. by Vitek software version 2.01. Jpn. J. Infect. Dis.2009; 62: 298-9.