Retrospective Analysis of The Distribution of Vancomycin MIC Values Among Clinical Isolates of Methicillin-Resistant Staphylococcus Aureus In A Tertiary Care Hospital, Surat.

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Abstracts: Background: Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as the most common hospital-acquired pathogen and is associated with increased morbidity and mortality compared with other strains. Patients with MRSA infections caused by isolates with a high but 'susceptible' minimum inhibitory concentration (MIC) to vancomycin may suffer poor outcomes. The aim of this study is to determine the distribution of vancomycin MIC values for MRSA isolates in a tertiary care hospital, Surat. Method: Methicillin-Resistant Staphylococcus aureus isolates from different clinical samples of patients admitted in New Civil Hospital, Surat from January 2016 to July 2016 were included in this study. Epsilometer test (E test) method was used to determine vancomycin MIC values for MRSA isolates as per Clinical Laboratory Standards Institute (CLSI) guidelines 2016. Result: A total of 63 Methicillin-Resistant Staphylococcus aureus isolates were studied. The vancomycin MICs were 0.50, 0.75, 1, 1.5 and 2 µg/ml for 1 (2%), 2 (3%), 19 (30%), 38 (60%), 3 (5%) isolates, respectively. No MRSA isolate presented a MIC of 4-8 µg/ml (VISA-Vancomycin Intermediate S. aureus) or MIC of ≥16 µg/ml (VRSA-Vancomycin Resistant S. aureus) in present study.
Conclusion: Vancomycin is the primary treatment for MRSA infection. However, different studies shows that increasing proportions of MRSA isolates with high MICs in the susceptible range (MIC ≤ 2 µg/ml) (vancomycin MIC creep) lead to treatment failure. There is a need to re-establish newer breakpoints of vancomycin MIC for determining sensitivity of MRSA isolates. [Manthan S NJIRM 2017; 8(2):5-8]

Key Words: E test, MRSA, vancomycin, MIC

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Introduction: Staphylococcus aureus is most commonly associated with hospital and community-acquired infections. Now a days, controlling measures for S. aureus infections are being challenged by increasing prevalence of methicillin-resistant S. aureus (MRSA) worldwide. There is also increase in number of S. aureus isolates with reduced susceptibility to vancomycin and other glycopeptides [1].

For MRSA infection, vancomycin remains the treatment of choice. Newer antibiotics, such as linezolid, daptomycin, or quinupristin- dalfopristin have been developed in recent years, but glycopeptides like vancomycin remain the first-line treatment option for MRSA infection [2]. However, due to increasing rate of MRSA infection worldwide and widespread use of Vancomycin to treat MRSA infection has led to an increase in number of MRSA strains with reduced susceptibility to Vancomycin. Although most of these strains have a Vancomycin MIC within the susceptible range as per the Clinical and Laboratory Standards Institute; CLSI 2016, few reports have showed that there is a generalized increase in Vancomycin MIC over a period of time which is also known as "MIC creep" [3].

Data related vancomycin effectiveness against serious MRSA infection suggest that the MIC values at the higher end of the susceptibility range are associated with poor outcome in patients treated with vancomycin. The CLSI susceptibility breakpoint for vancomycin has been reduced to 2 ug/ml, previously which was 4 ug/ml. Increasing failure rate in MRSA isolates having vancomycin MIC at 2 µg/ml and 1.5 µg/ml has led us to rethink about establishment of newer breakpoints of vancomycin MIC for MRSA isolates [1, 4].

The knowledge regarding patients infected with MRSA strains that have elevated MICs to Vancomycin will help in early recognition of patients who are at risk of vancomycin treatment failure. So alternative therapy can be started early in these patients [5]. The present study aims to evaluate Vancomycin susceptibility pattern among Methicillin Resistant S. aureus isolates in a tertiary care hospital, Surat.

Methods: After approval from Institutional Human Research Ethics Committee (HREC), the study was conducted in Department of Microbiology, Government Medical College, Surat from January to July 2016. A total of 63 isolates of Methicillin resistant staphylococcus aureus collected from various clinical specimens like pus, wound swab, urine, blood culture and pleural fluid were included.

The isolates were first identified as S. aureus by colony morphology and standard biochemical techniques like catalase test, slide & tube coagulase test, urease test, mannitol fermentation and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15µg), penicillin (10U), teicoplanin (30µg), ciprofloxacin (5µg), Linezolid (30µg) and other antibiotics of the panel along with cefoxitin (30µg) disc for MRSA detection as per CLSI guidelines 2016 [6].

E-test for Vancomycin MIC [7]: With a loop, the top of 3 or 4 individual colonies of Staphylococcus aureus were touched and transferred to a tube of sterile saline. Turbidity was compared to that of the 0.5 McFarland standard & adjusted to match that standard. Sterile cotton swab was dipped into the inoculum and streaked over the entire surface of the Muller hinton agar plate. The plate was rotated three times approximately 60^{o} to ensure even distribution. Lid of the plate was left open for 5 minutes (no more than 15 minutes) to allow any excess moisture to be absorbed before applying strips.

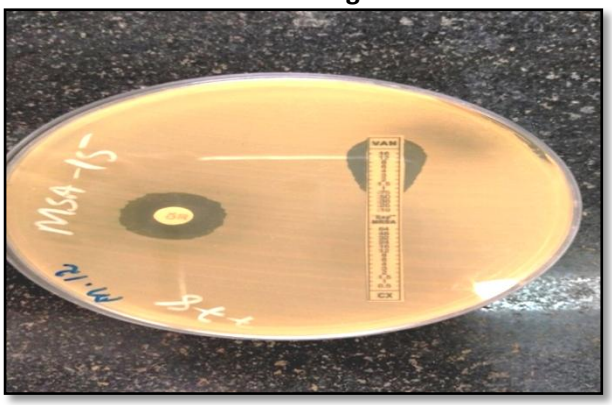
The MIC testing was performed using the E test method, following manufacturer's guidelines. The antibiotic E test strips were applied to the agar surface using an E test applicator and were not moved following application. All plates were incubated aerobically at 37^{0}C for 18-24 hrs.

E test method & the MIC distribution for vancomycin are displayed in Fig. 1 & Fig. 4, respectively.

Result: A total of sixty three (63) Methicillin resistant staphylococcus aureus isolates were tested for susceptibility to different antibiotics of the panel by Kirby Bauer's disc diffusion testing including E test for Vancomycin MIC as per CLSI guidelines 2016.[6]

In this study, out of 63 patients, age & sex wise distribution showed that majority of patients were of the age 21-30 years (17). Among these 63 patients, 36 (57%) were male and 27 (43%) were female as shown in Fig 2 & Fig. 3, respectively. In the present study, the MIC of the vancomycin was determined using E- Test method. Out of which, 3 (5%) isolates of MRSA had MIC values of 2µg/ml followed by MIC of 1.5µg/ml in 38 (60%) isolates, MIC of 1 µg/ml in 19 (30%) isolates, MIC of 0.75 µg/ml in 2 (3%) isolates and MIC of 0.50 µg/ml in 1 (2%) isolate as shown in Fig. 4.

Fig: 1 Vancomycin MIC by E Test on Muller Hinton Agar



Discussion: Since 1958, Vancomycin is known as the cornerstone treatment for MRSA infection, but recently widespread use of this drug has led to resistance among these bacteria. In the past 20 years, usage of vancomycin has been increased due to increased prevalence of MRSA infection worldwide [8].

Fig. 2 Age wise distribution of MRSA isolates

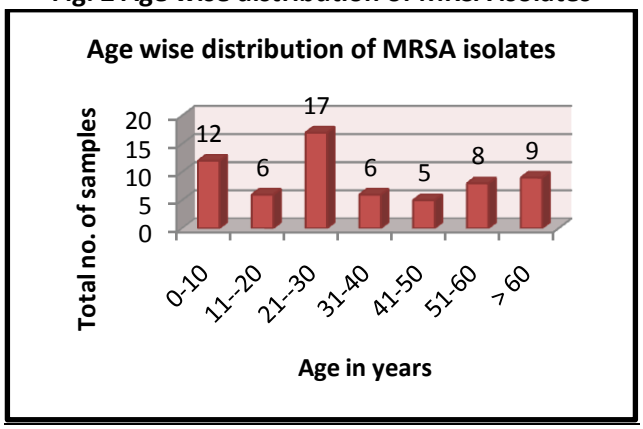
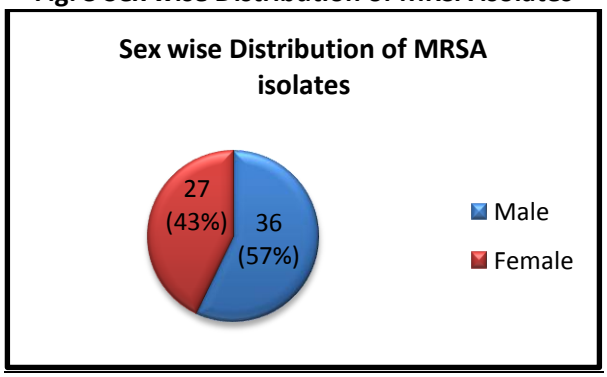


Fig. 3 Sex wise Distribution of MRSA isolates



In a present study majority MRSA isolates were having MIC of 1.5 µg/ml (60%), similar results were observed in Lodise et al.[9] & Joana et al.[7] studies. Sreenivasulu Reddy P et al.[8] & Ullah et al.[11] had observed MIC of 2 µg/ml in 42% & 53.1% MRSA isolates, respectively. The Vancomycin MIC distribution in MRSA isolates in present study is compared with other studies as shown in Table 1.

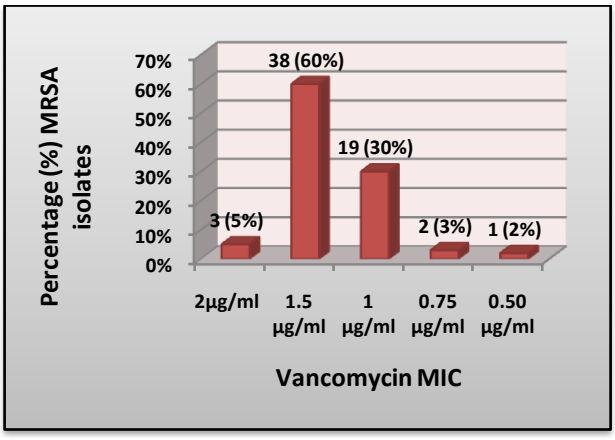
Table: 1 Comparison of Vancomycin MIC distribution in MRSA isolates (Percentage %)

Vanco-mycin MIC	Lodise et al. [9]	Asha Pai K.B et al.[10]	Joana et al.[7]	Sreeni-vasulu Reddy P et al.[8]	Ullah et al.[11]	Present study (n=63)*
2 mg/L	9.8	21.42	37.6	42	53.1	5
1.5 mg/L	**62**	--	**40.9**	--	--	**60**
1 mg/L	18.5	44.64	20.4	25	15.2	30
0.75 mg/L	9.8	--	1.1	--	--	3
0.50 mg/L	--	32.14	--	15	15.2	2

*** Total no. of MRSA isolates**

These data revealed that there is a emerging threat of wide spread vancomycin resistance which possesses a serious health concern. Proper screening for MRSA and other staphylococci with reduced susceptibility to vancomycin is a major key component of successful infection control strategies to prevent MRSA infection at early stage [8].

Fig: 4 Distribution of Vancomycin MIC among MRSA isolates



Despite its sustained in vitro microbiologic inhibitory activity, there is a question on the continued utility of vancomycin for methicillin-resistant Staphylococcus aureus (MRSA) infections. There is a constant need to monitor local status of vancomycin MICs in MRSA isolates to screen for all the possibility of MIC creep in each hospital settings [7].

Conclusion: Vancomycin is the primary treatment for serious MRSA infection. However, different studies shows that increasing proportions of MRSA isolates with vancomycin MICs at higher end of the susceptible range (MIC ≤ 2 µg/ml) lead to treatment failure in patients treated with Vancomycin. We were unable to find the presence of vancomycin MIC creep among MRSA isolates due to unavailability of vancomycin MIC data of previous years. This phenomenon seems not to be generalized; as a result each institution should have systematically monitor vancomycin MIC over time in MRSA infection. There is a need to re-establish newer breakpoints of vancomycin MIC for determining sensitivity of MRSA isolates and proper treatment outcome.

Acknowledgment: Special thanks to all the technical staff for their support and to Dr Tanvi Panwala, Assistant Professor, Department of Microbiology, Government Medical College, Surat for her guidance.

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Conflict of interest: None
Funding: None
Cite this Article as: Manthan S, Geeta V, Mullan S. Retrospective Analysis Of The Distribution Of Vancomycin MIC Values . Natl J Integr Res Med 2017; 8(2):5-8