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Original Research Article

Vancomycin Resistance among Methicillin Resistant *Staphylococcus Aureus* Isolates From Tertiary Care Hospital

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ABSTRACT

Background: *Staphylococcus aureus* is one of the most common causes of nosocomial infections. Vancomycin is the drug of choice to treat infections caused by MRSA.
Objectives: The study determines Vancomycin MIC of MRSA strains by both agar dilution and Vitek methods. Presence of VAN a gene was also determined.
Materials and Methods: Study was conducted from April 2015-March 2016.A total 70 *Staphylococcus aureus* strains were included. Methicillin resistance was determined by using Cefoxitin disc (30ug) as per CLSI guidelines. Vancomycin resistance was detected by both agar dilution and Vitek methods. PCR was done to determine presence of VAN A gene among VISA strains.
Results: Among 70 *S.aureus* strains, 40 (57%) strains were methicillin resistant. Maximum MRSA strains (55%) were isolated from pus samples. 6 VISA (Vancomycin intermediate S. aureus strains) strains were detected by agar dilution method. Vitek was able to detect 3 VISA strains. All VISA strains were sensitive to Rifampicin and Linezolid by disc diffusion method. All VISA strains were negative for VAN A gene by PCR.
Conclusion: Disc diffusion method misclassifies vancomycin intermediate isolates as fully susceptible strains. Clinical laboratories must perform MIC method to correctly identify VISA strains and avoid treatment failure.
Key words: MRSA, VISA, MIC.

INTRODUCTION

Staphylococcus aureus is one of the most common causes of nosocomial infections. Methicillin resistant *S.aureus* (MRSA) was first detected approximately 40 years ago. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. [(1,2)] Recent guidelines from the Infectious Diseases Society of America state that the patient infected with MRSA strain that have vancomycin MIC >2 ug/ml have a higher probability of treatment failure and recommend changing to alternate therapy. It is essential that clinical microbiological laboratories accurately determine vancomycin MIC to guide appropriate therapy. [(3)]

Trends towards increasing Vancomycin MIC have been noted over the past 5-10 years. Hypotheses for the increase in vancomycin MIC include “MIC creep”, clonal shift and variation in vancomycin MICs demonstrated by various testing methods. [(4)]

Vancomycin resistance in *S.aureus* is difficult to define mainly because of methodological problems in their detection.

In absence of vancomycin pressure, vancomycin resistance is unstable and is expressed at low level. This low level expression of vancomycin resistance in *S.aureus* may be the reason why these strains are difficult to detect clinically. [(5)]

The CLSI disc diffusion method of sensitivity by standard 30ug vancomycin frequently misclassifies intermediate susceptible isolates as fully susceptible. Both conventional and automated methods have problems in distinguishing VISA isolates from Vancomycin susceptible strains. Vancomycin resistance not only has enormous therapeutic implications, but is also important from epidemiological and infection control standpoints. [(6)] Therefore we have undertaken this study to calculate MIC of vancomycin by both agar dilution method recommended by CLSI and VITEK system.

Objectives

1. To detect Methicillin resistance among *S.aureus* isolates.
2. To determine Vancomycin MIC by agar dilution method.
3. To determine Vancomycin MIC by Vitek 2 method.
4. To detect Van A gene among resistant strains.

MATERIALS & METHODS

- **Type of study:** Cross sectional.
- **Place of study:** Tertiary care hospital.
- **Period of study:** April 2015- March 2016.
- **Sample size:** 70.
- ***Inclusion criteria:*** All samples routinely sent for culture yielding growth of *Staph.aureus*.
- ***Exclusion criteria:*** Samples yielding growth of other organisms.

1) **Detection of MRSA:** Antibiotic sensitivity testing was done by Kirby-Baur's method according to CLSI guidelines. MRSA strains were detected by using 30ug Cefoxitin disk as recommended by CLSI. [(7)]
2) **Detection of vancomycin MIC by agar dilution method:** MIC of vancomycin was determined by agar dilution method using CLSI guidelines. Briefly, gradient plates of Muller Hinton agar were prepared with vancomycin (Himedia).Concentrations used were 2ug/ml, 4ug/ml, 8ug/ml, 16ug/ml and 32ug/ml. 0.5 McFarland equivalent inoculum was prepared using 18-24hr old culture. This was spotted on gradient plates. Plates were incubated overnight at 35ºc for 24 hrs before assessing visible growth. [(7)]

Control strains used- Negative control ATCC *S.aureus* 25923

Positive control- *E. faecalis* ATCC 51299

3) **Determination of vancomycin MIC by Vitek System:** This was done by using AST-P 628 cards following manufacturer's instruction.
4) **Detection of van A gene by PCR:** Presence of van A gene was detected by using-

Forward primer-
ATGAATAGAATAAAAGTTGC
Reverse Primer-
TCACCCCTTTAACGCTAATA
Amplification condition-Initial
denaturation 98ºc for 10sec
Annealing 50ºc for 1 min
Polymerization at 72ºc for 1 min
Final extension at 72ºc for 5 min. [(1)]

RESULT

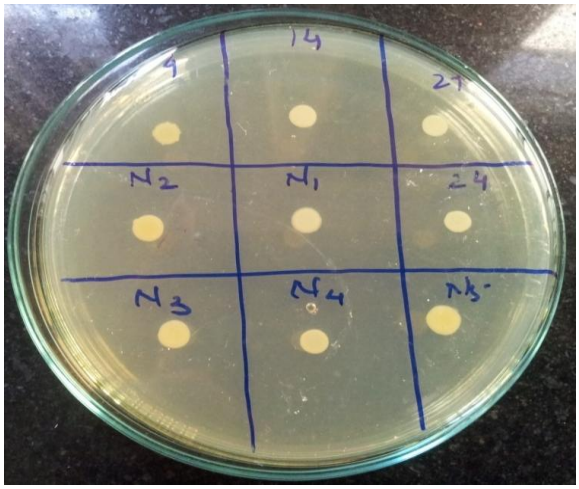


Fig 1: Muller Hinton Agar without vancomycin

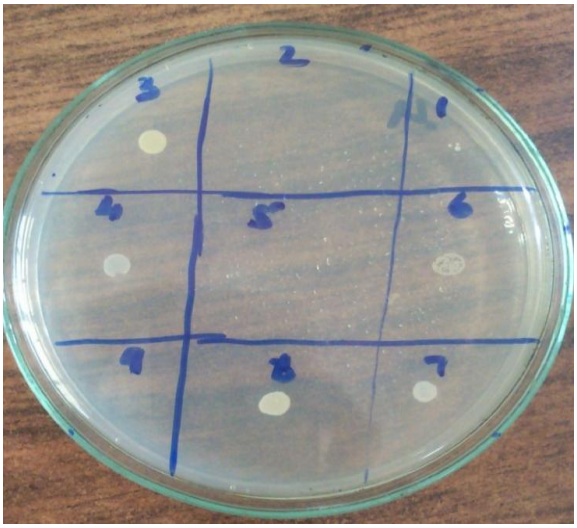


Figure2: MHA with vancomycin (>2ug/ml)

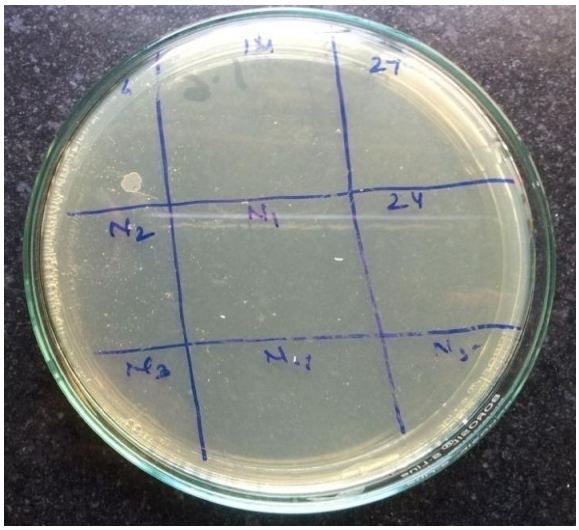


Figure3: MHA with vancomycin (>8ug/ml)

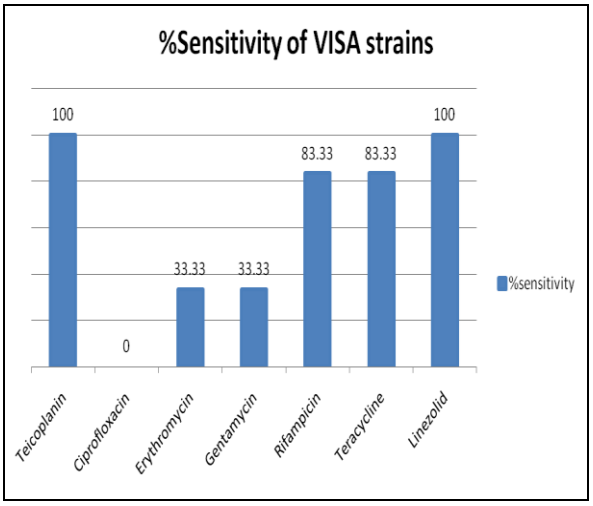


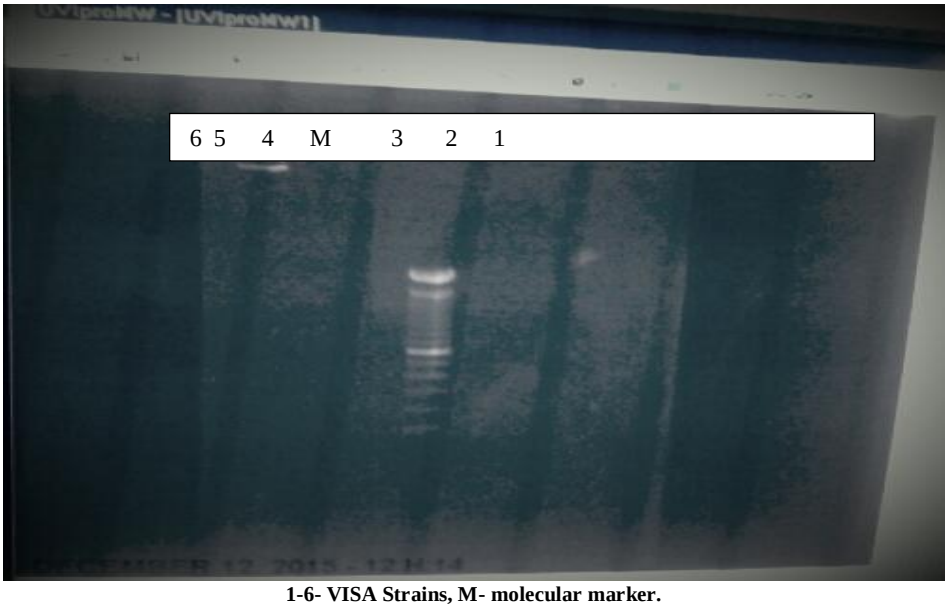
Fig.4. Antibiotic sensitivity pattern of VISA isolates

- Out of 70 *S. aureus* strains 40 (57%) were MRSA.
  Maximum [22(55%)] MRSA were isolated from pus samples. Table 1.shows percentage of MRSA isolates.
- Amongst the 40 MRSA strains, 6 strains showed resistance to vancomycin by the agar dilution test (table 2).
- MIC by agar dilution and by Vitek 2system of the 06 isolates is shown in table 3.

Fig 1, 2 and 3 shows agar dilution results

- **Antibiotic sensitivity patterns of VISA isolates**: All 6 VISA strains were sensitive to linezolid and rifampicin (fig.4).

PCR Results-All 6 strains were negative for VAN A gene by PCR (fig 5).



1-6- VISA Strains, M- molecular marker.
Fig.5: PCR results.

Outcome of the patients

- One VISA strains with Vancomycin MIC of >8µg/ml was isolated from a patient with perianal abscess. Patient was treated with Teicoplanin and Cotrimoxazole for prolonged period.
- Among other 5 VISA strains (>2µg/ml)-3strains responded to linezolid (600 mg IV twice daily) and remaining 2 strains responded to high-dose of Injection vancomycin along with linezolid (600 mg P/O twice daily)

Table 1: Percentage of MRSA isolated

Sample	Percentage of MRSA isolated
Pus	(22) 55%
Blood	(07) 18%
Sputum	(06) 15%
Urine	(5) 12%
Total	40

Table 2: MICs by agar dilution method

MIC	Vancomycin Resistant strains by agar dilution N=6
>8ug/ml	1
>2ug/ml	5

Table3: MICs of VISA strains

Strain	Agar Dilution	Vitek
1	>8ug/ml	>4ug/ml
2	>2ug/ml	>2ug/ml
3	>2ug/ml	>2ug/ml
4	>2ug/ml	>1ug/ml
5	>2ug/ml	>1ug/ml
6	>2ug/ml	< 0.5ug/ml

DISCUSSION

Infections caused by methicillin resistant *S.aureus* have been associated with high morbidity and mortality rates. In Indian hospitals, MRSA is one of the common causes of hospital acquired infections. This study reported 57% of MRSA. Similar findings are reported by *Maqsood ali et al* [(5)] Vancomycin is one of the main antibiotics used to treat serious infections with MRSA. However overuse of this antibiotic in oral form for conditions like pseudomembranous colitis has inevitably changed this situation. [(1,2)]

In our study, only 6(15%) strains were intermediate sensitive to vancomycin. Tiwari et al [(6)] in 2006 and Thati et al [(1)] in 2008 reported 0.76% and 1.9% VISA incidence respectively. This suggests increase in incidence of VISA in recent years. We have considered strains with vancomycin MIC >2ug/ml as VISA according to recent guidelines of CLSI. [(7)] This may be the reason for isolating higher number of VISA. Clinically also these patients responded to either higher dose of vancomycin or linezolid. A reduction in the efficacy of vancomycin against MRSA strains for which vancomycin MICs are elevated (>2ug/ml) has been widely reported, suggesting that modest elevations in MICs may explain some suboptimal clinical outcomes. [(8)]

Vitek2 system could not identify three VISA strains. Also there was difference in MIC reporting by Vitek and agar dilution method. Behera B and Mathur P have also noticed erroneous reporting by Vitek. [(9)]

Vancomycin resistant *S.aureus* tends to be multidrug resistant against a large number of currently available antimicrobial agents, compromising treatment options. [(1)] In present study, all VISA strains were sensitive to linezolid by disc diffusion method. Clinically also patients responded well. So, use of linezolid shall be restricted for only MRSA and VISA/VRSA strains.

The genetic mechanism of vancomycin resistance in S.aureus is not well understood. Fully vancomycin resistant strains of *S.aureus* due to acquisition of VAN A gene from vancomycin resistant Enterococci were first reported from United States in 2002. However, to date, only 9 cases of VRSA have been reported from United States, with two additional cases, one from India and one from Iran. This indicates that although this mechanism of resistance is significant, it is not spreading rapidly. [(9)]

All VISA isolates were negative for VAN a gene in our study. A number of studies have demonstrated absence of vancomycin resistant genes in VISA and hetero VISA strains. [(10,11)] This highlights the importance of other mechanism of drug resistance *i.e.* cell wall thickening. [(11,12)] So, clinical laboratories shall use dilution

method along with PCR for detection of vancomycin resistance.

CONCLUSION

- Our study highlights the importance of using vancomycin as a reserve drug as there is an increase of MIC trend for this drug.
- Vancomycin resistance not only has enormous therapeutic implication, but is important also from epidemiological and infection control standpoints.
- Therefore, every institution must carefully evaluate the screening methods being used for vancomycin resistance in order to correctly report sensitivity against this important antimicrobial to avoid treatment failure.
- Agar dilution is gold standard but difficult to perform as compared to vitek 2 system. But it can be used in critical cases in order to correctly report vancomycin MIC.

REFERENCES

1. Thati V, Channappa T, Shivannavar, Subhaschandra M. Vancomycin resistance among methicillin resistant Staphylococcus *aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res 2011; 134:704-08.
2. Appelbaum P. The emergence of vancomycin- intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2006; 12(1):16-23.
3. Kruzel M ,Lewis C, Kerry w, Lewis E, Dundas N, Mohr N et al. Determination of Vancomycin and Daptomycin MICs by different testing methods for Methicillin resistant *Staphylococcus aureus*. J Clin Microbiol 2011; 49(6): 2272-73.
4. Hsu D. Comparison of method specific vancomycin MIC values and their predictability for treatment outcome of MRSA infections. Int. J. Antimicrob. Agents. 2008; 32:378-85.
5. Maqsood Ali, Abbasi Shahid, Arif Shazia,, Mirza Irfan. Nosocomial infections due to methicillin resistant *Staphylococcus aureus* in hospitalized patients. Pakistan J Med Sci.2007:4:23-25
6. Tiwari H K,Sen M. Emergence of Vancomycin resistant *Staphylococcus aureus* from a tertiary care hospital from northern part of India.BMC Infect Diseases.2006; 6:156-158.
7. Clinical and Laboratory Standards Institute.2006.Performance standard for antimicrobial susceptibility testing, 16th informational supplement, M100-S16.Clinical and Laboratory Standards Institute, Wayne, PA.
8. Sader H, Rhomberg P and Jones R. Nine hospital study comparing Broth Microdilution and E test method results for vancomycin and daptomycin against methicillin resistant *S.aureus*. Antimicrobial agents and chemotherapy.2009; 7:3162-65.
9. Behera B, Mathur P. Erroneous reporting of vancomycin susceptibility for *Staphylococcus spp*. By Vitek software version 2.01.Jpn. J.Infect.Dis.2009; 62:298-99.
10. Cui L, H.Murakami, K.Kuwahara Arai, H.Hanaki and K.Hiramatsu. Contribution of thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. Antimicrob. Agents. Chemother. 2000; 44:2276-85.
11. Howden B, Davies J,Johnson P, Stinear T,Grayson M. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin intermediate and heterogenous vancomycin intermediate strains: Resistance mechanisms, laboratory detection and clinical implications. Clin Microbiol Rev.2010; 99-139.
12. Srinivasan A, Dick J, Perl T. Vancomycin resistance in staphylococci. Clin. M icrobiol. rev. 2002; 15:430-38.

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