DOI: 10.7860/JCDR/2015/10072.6625

Original Article

Microbiology Section

Methicillin Resistant *Staphylococcus aureus*: Inconsistencies in Vancomycin Susceptibility Testing Methods, Limitations and Advantages of each Method

HIMANI[1], CHARU AGRAWAL[2], MOLLY MADAN[3], ANITA PANDEY[4], BHASKAR THAKURIA[5]

ABSTRACT

Background: Vancomycin may be ineffective against an increasing proportion of methicillin resistant *Staphylococcus aureus* (MRSA) with minimum inhibitory concentrations (MICs) well within the susceptible range. On the other hand it is common knowledge that determination of vancomycin MICs is method dependent. Therefore, given the apparent variability in vancomycin MIC results obtained with the different methods, the use of the vancomycin MIC to predict the outcome of serious *S. aureus* infections needs to take into account the method used and the results of studies using that particular method.

Aim: Comparative study was carried out to evaluate the MICs obtained by BMD method, E-test, and Vitek 2 method and to detect inconsistencies in these vancomycin for 66 MRSA isolates obtained from various samples of patients attending the OPDs & IPDs within a period of one year.

Materials and Methods: A comparative study was carried out to evaluate the MICs obtained by BMD method, E-test, and Vitek 2 method to detect vancomycin susceptibility in 66 clinical isolates of MRSA obtained from various samples of patients attending the OPDs & IPDs within a period of one year. The study was conducted in Department of Microbiology, Subharti Medical College, Meerut from January to December 2012.

Results: On determination of MICs for vancomycin for the MRSA isolates, all were identified as VSSA by BMD, E-Test & Vitek 2 methods. However, the vancomycin MIC values obtained by E-test correlated better with BMD method (correlation factor= 0.6727) than Vitek 2 (correlation factor=0.5316), indicating E-Test to be a better method for determination of vancomycin MICs as compared to Vitek 2.

Conclusion: MRSA isolates with higher vancomycin MICs, even within the susceptibility range, are being observed more frequently which result in treatment failures with vancomycin. Because of the discrepancy that exists in vancomycin MIC results from different methods, the prediction of outcome of serious *S.aureus* infections should take into account the method used & results of studies using that particular method.

Keywords: BMD, E-Test, Minimum inhibitory concentration, MRSA, Vitek 2

INTRODUCTION

Staphylococcus aureus (*S.aureus*) has been recognized as an important cause of human disease for more than 100 years [1]. Sir Alexander Ogston introduced the name *Staphylococcus* (from Greek staphylé, a bunch of grapes) and first isolated *S.aureus* from a surgical abscess in 1880 [2,3]. *S. aureus* is responsible for many infections but it may also occur as a commensal. The presence of *S. aureus* does not always indicate infection. *S. aureus* can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain [4]. *S. aureus* has been documented in a variety of infections ranging from minor skin infections & chronic bone infections to urinary tract infections and severe septicemias. *S. aureus* is one such bacterium which has been constantly evolving over time with regards to acquisition of complicated mechanisms of antimicrobial resistance and changing disease profiles. One of the significant events in the evolution of antimicrobial resistance in *S.aureus* has been the development of methicillin resistance which has become a notorious problem in many hospitals around the world.

Traditionally, because of the universal resistance of MRSA to β-lactams and because of the lack of other effective alternatives, the glycopeptide vancomycin became the mainstay of treatment, as it provides invitro activity against all staphylococci and demonstrates clinical response against MRSA infection. However, invitro susceptibility of MRSA to vancomycin is no longer universal. A 1997 report of clinical strains of *S. aureus* with intermediate (minimum inhibitory concentration {MIC}, 8–16 mg/mL) susceptibility to vancomycin in Japan was soon followed by descriptions of several frankly vancomycin-resistant *S. aureus* isolates (MIC, ≥32 mg/mL) in the United States [5].

Since that time there has been uncertainty regarding optimal laboratory detection and the clinical relevance of reduced vancomycin susceptibility in *S. aureus*, changes in Clinical and Laboratory Standards Institute (CLSI) breakpoints for vancomycin against *S. aureus*, and increasing concern regarding the efficacy of vancomycin for the treatment of *S. aureus* infections. Recent studies suggests that some vancomycin sensitive MRSA isolates with indicated vancomycin MIC of 2 µg/mL may still result in treatment failure [1].

The vancomycin non-susceptible strains, in the form of intermediate-resistant *S. aureus*, (VISA, vancomycin MIC 4-8 µg/mL) remain rare and van A-mediated vancomycin-resistant *S. aureus* (VRSA, vancomycin MIC > 16 µg/mL) are limited to a handful of reported cases. The rising MICs of vancomycin among vancomycin susceptible *S. aureus* (VSSA), referred to as the 'vancomycin MIC creep', has caused a re-evaluation of vancomycin susceptibility criteria in cases of complicated infections like bacteraemia and/or pneumonia [6].

On the other hand, it is common knowledge that determination of the vancomycin MICs is method dependent. In view of this rising

importance of increasing MICs of vancomycin in MRSA isolates and variations in the MICs according to the method employed, the present study was proposed to assess the reduced susceptibility of vancomycin in MRSA isolates in an Indian tertiary care facility and comparison of three methodologies viz. BMD, Vitek 2 & E-Test methods.

MATERIALS AND METHODS

A total of 66 MRSA obtained from different clinical samples received in the Clinical Microbiology laboratory, Department of Microbiology, Subharti Medical College over a period of one year (January to December 2012) were included in this observational study. Permission from the ethical committee was duly obtained. Screening for methicillin resistance was done by cefoxitin 30µg disc as per CLSI guidelines [4]. Vancomycin susceptibility testing was performed on these MRSA isolates by three methods viz. BMD, Vitek 2 & E-Test. Reference strains of *S. aureus* ATCC 25923 and ATCC 43300 were used for quality control for MRSA testing. A clinical isolate of vancomycin resistant *S. aureus* was used as a control to compare the test strains.

CLSI MIC interpretative criteria for Vancomycin in *S.aureus* [7]:

Vancomycin susceptible *S.aureus* (VSSA): ≤2µG/mL

Vancomycin intermediate *S.aureus* (VISA) : 4-8µG/mL

Vancomycin resistant *S.aureus* (VRSA) : ≥16µG/mL

Broth microdilution: The procedure undertaken was precisely as described in the CLSI M07-A9-2012 approved standard [8]. The initial inoculum was prepared by making a direct broth suspension of isolated colonies selected from an overnight growth on blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard (1×10^8 cfu/mL). 1:100 dilution of that broth was made which reduced the colony count to 10^6 cfu/mL. Aliquots of 0.05 mL of this inoculum were added to the wells of the micro titre plate containing 0.05 mL of vancomycin solution (HiMedia, MUMBAI), the 1:2 dilution of the 10^6 cfu/mL inoculum resulted in a final inoculum concentration of 5×10^5cfu/mL which is the recommended final inoculum and also halves the antibiotic concentration in each well. Microtitre plates were incubated in ambient air at 35°C for 24 hours. Inspection of the wells visually against a dark background in broad daylight was done to determine growth which was indicated by turbidity throughout the well. This method was used as the baseline method for further comparisons.

E-Test: The E-Test strips were brought to room temperature. The inoculum was prepared by making a direct broth suspension of isolated colonies selected from an overnight growth on blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland. Lawn culture from this suspension was made using a swab according to standard protocol. E-Test strips (HiMedia) for vancomycin (0.016 to 256 µg/ml) were applied on the plates after being dried for 10 minutes and the plates were incubated at 35°C for 24 hours. MIC was measured where a clear defined zone of inhibition intersected the strip [9].

Vitek 2: A pair of plastic tubes was used for each isolate. Three mililitre of 0.45% NS (normal saline) was taken in each tube. Colonies from an overnight growth were picked up with an inoculating wire and emulsified in the first tube. A 280µl ml from first tube was pipetted into the second tube. The turbidity equivalent to 0.5 Mc Farland was measured using densicheck. Then GP 67 card was put in the second tube. The card with the test tubes was fed into the Vitek 2 machine for antibiotic sensitivity testing where bacterial suspension got vacuum filled in the antimicrobial susceptibility testing card. The card was then inserted in the incubator-reader of the Vitek 2 system and the results were expressed as MIC values in µg/mL. (Vitek 2 Compact Systems Version: 06.01).

STATISTICAL ANALYSIS

Comparisons between groups were made with chi square (χ^2) test. Correlation between different methods was made using Karl Pearson's correlation coefficient equation. A p-value of <0.05 was considered significant. Statistical analysis was performed on the data using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 66 MRSA isolates were obtained from different clinical samples of patients attending various OPDs and IPDs of the hospital. Pus was the predominant sample followed by endotracheal secretions, urine, sputum, endocervical swab and cerebrospinal fluid. The distribution of MRSA isolates in relation to various samples is provided in [Table/Fig-1].

A total of 26 MRSA isolates had MIC=2µg/ml by BMD method, which indicates reduced susceptibility (i.e. higher MIC) for vancomycin, although being categorized as VSSA according to CLSI. Out of these 21(80.75%) isolates were obtained from IPD patients and 5(19.2%) isolates obtained from OPDs demonstrating that the reduced vancomycin susceptibility is being associated more frequently with IPD isolates than OPD isolates. Comparison of vancomycin susceptibility by BMD, Vitek 2 & E-Test.

1. BMD vs. Vitek 2

A total of 40/ 66 MRSA isolates, had MIC=1 µg/ml by BMD method. In these isolates when MIC was determined by Vitek 2, 20 isolates showed MIC= 1.5µg/ml and 20 showed MIC =2µg/ml. The remaining 26 MRSA isolates had MIC=2µg/ml both by BMD as well as by Vitek 2 method. Agreement between the two methods is 54.55% as against the expected agreement of 42.61%. Kappa statistics is 0.2080 which shows that it is statistically significant (p<0.05) [Table/Fig-2].

2. BMD vs. E-Test

In a total of 40/66 MRSA isolates, which had MIC 1 µg/ml by BMD method, when MIC was determined by E-Test, all had MIC= 1.5µg/ml. Out of the remaining 26 MRSA isolates which had MIC=2µg/ml by BMD method, 11 had MIC=1.5µg/ml and rest 15 had MIC= 2µg/ml. Agreement between the two methods is 61.36% as against the expected agreement of 47.59%. Kappa statistics is 0.2628 which shows that it is statistically significant (p<0.05) [Table/Fig-3].

CLINICAL SAMPLE	MRSA	PERCENTAGE
Pus	45	68.18
E.T SECR	16	24.24
Urine	4	6.06
Others*	1	1.52

[Table/Fig-1]: Frequency of MRSA (n=66) in samples
(Others*: 2 sputum samples, 1 endocervical swab & 1 CSF)

Vitek 2 method	Broth Micro Dilution method				
	0.5	1.0	1.5	2.0	Total
0.5	0	0	0	0	0
1.0	0	0	0	0	0
1.5	0	20	0	0	20
2.0	0	20	0	26	46
Total	0	40	0	26	66
Agreement	Expected Agreement	Kappa statistic	Std. Err.	Z-value	p-value
54.55%	42.61%	0.2080	0.0482	4.3200	<0.00001*

[Table/Fig-2]: Agreement between BMD & Vitek 2 methods for MRSA isolates (n=66)
*p<0.05

3. Vitek 2 vs. E-Test

Out of 66 MRSA isolates, 51 isolates had MIC=1.5µg/mL & 15 isolates had MIC = 2 µg/mL by E-Test method. When these isolates were tested with Vitek 2, only 20 had MIC=1.5µg/mL while 46 had MIC=2µg/mL. Agreement between the two methods is 53.03% as against the expected agreement of 39.26%. Kappa statistics is 0.2268 which shows that it is statistically significant (p<0.05) [Table/Fig-4].

Correlation between the three methods: Out of 66 MRSA isolates, 40 (60%) isolates had an MIC of 1µg/ml with the BMD method, while none of the isolates showed such MIC with Vitek 2 & E-Test methods. With the Vitek 2 method, 20 (30%) isolates had MIC=1.5µg/ml while with the E-Test 51(77%) isolates showed similar MIC and none by BMD method. By BMD method, 26(40%) isolates had an MIC=2µg/ml while 46 (70%) isolates had similar MICs by Vitek 2 method and only 15 (23%) by E-Test method [Table/Fig-5].

Broth Micro Dilution method	E-Test method					
	0.5	0.75	1.0	1.5	2.0	Total
0.5	0	0	0	0	0	0
0.8	0	0	0	0	0	0
1.0	0	0	0	40	0	40
1.5	0	0	0	0	0	0
2.0	0	0	0	11	15	26
Total	0	0	0	51	15	66
Agreement		Expected Agreement	Kappa statistic	Std. Err.	Z-value	p-value
61.36%		47.59%	0.2628	0.0481	5.4600	<0.00001*

[Table/Fig-3]: Agreement between BMD & E-Test methods for MRSA isolates (n=66)
*p<0.05

Vitek 2 method	E-Test method					
	0.5	0.75	1.0	1.5	2.0	Total
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0
1.5	0	0	0	20	0	20
2.0	0	0	0	31	15	46
Total	0	0	0	51	15	66
Agreement		Expected Agreement	Kappa statistic	Std. Err.	Z-value	p-value
53.03%		39.26%	0.2268	0.0781	2.9100	0.0018*

[Table/Fig-4]: Agreement between E-Test & Vitek 2 methods for MRSA isolates (n=66)
*p<0.05

METHOD	MICs (µg/ml)						
	≤ 0.5	0.75	1.0	1.5	2.0	≥2.0	TOTAL
BROTH MICRO DILUTION	-----	-----	40(60%)	-----	26(40%)	-----	66(100%)
VITEK 2	-----	-----	-----	20(30%)	46(70%)	-----	66(100%)
E-TEST	-----	-----	-----	51(77%)	15(23%)	-----	66(100%)

[Table/Fig-5]: A comparison of vancomycin MICs determined by BMD, Vitek 2 & E-Test (n=66)

Clinical Isolates	Methods	Correlation coefficient r- value	t -value	p-value
MRSA	Broth Micro Dilution and Vitek 2 methods	0.5316	5.0212	0.00001*
	Vitek 2 and E-Test methods	0.3576	3.0634	0.0032*
	Broth Micro Dilution and E-Test methods	0.6727	7.2727	0.00001*

[Table/Fig-6]: Correlation between BMD, Vitek 2, and E-Test methods by Karl Pearson's correlation coefficient (n=66)
*p<0.05

The Karl Pearson correlation coefficient between BMD and E-Test method was calculated to be 0.6727 which was more than correlation coefficient between Broth Micro Dilution and Vitek 2 which came out to be 0.5316. This indicates that that E-Test provided values closer to the reference Broth Micro Dilution method (MIC in µg/ml) (MIC in µg/ml) than Vitek 2. The association or difference between all the three methods was found to be statistically significant (p<0.05) [Table/Fig-6].

DISCUSSION

Subtle but potentially important variability in vancomycin MICs of the MRSA isolates is obtained with different methods [1]. BMD is a cumbersome test and is not used routinely in clinical laboratories. Since it is reliant on a twofold dilution, it offers limited quantitative information [6]. Currently most clinical laboratories use E-Test and automated susceptibility tests for measuring the vancomycin MIC. Various automated systems like MicroScan Walk Away, Vitek 2, Phoenix, Sensititre, Vitek Legacy are available having variable sensitivities and specificities.

In the current study we compared the vancomycin MICs of MRSA isolates by BMD, E-Test & Vitek 2 method (MIC in µg/ml). All the MRSA isolates were susceptible to vancomycin (MIC≤2µg/mL) by all of the three methods. But the vancomycin MIC values obtained by E-Test correlated better with BMD method (correlation factor= 0.6727) as compared to when values obtained by Vitek 2 (correlation factor=0.5316) which indicates E-Test to be a better method for determination of vancomycin MICs as compared to Vitek 2 in the current study. Vancomycin MICs generated by E-Test were consistently higher than MICs generated by BMD method i.e. 40 MRSA isolates which had a MIC of 1µg/ml by BMD, when tested with E-Test had MIC of 1.5 µg/ml. Vancomycin MICs generated by Vitek 2 were also higher than MICs generated by BMD method i.e. out of 40 MRSA isolates which had a MIC of 1µg/ml by BMD, when tested with Vitek 2, 20 isolates had MIC of 1.5µg/ml while the rest 20 had MIC of 2µg/ml.

Similarly, Hsu et al., in their study also reported highly variable results of vancomycin MICs using different methods like E-Test, Vitek 1, Micro Scan and comparing them with BMD. In their study, E-Test method (MIC in µg/ml) (MIC in µg/ml) appeared to be relatively more reliable as compared to Vitek 1 [10]. In the study conducted by Sader et al., the E-Test provided vancomycin MIC results that were consistently higher than those provided by the BMD method [11]. In a study by Leonard et al., the MIC results tended to be higher by E-Test than by BMD [12]. These studies had findings similar to our study. In the study by Kruzel et al., all isolates were susceptible by all testing methods. The vancomycin MICs determined by E-Test method were consistently elevated than those determine by BMD. Using frozen Trek panels as the reference method, the essential agreement for in-house broth microdilution was 99.4%, while it was 76.4% for the E-Test method, 96.3% for Vitek 2 in the study done by Kruzel et al., [13]. Behera et al., from All India Institute Of Medical Sciences, New Delhi, in their study observed that out of 49 MRSA isolates, 2 had MIC in the intermediate range (8µg/mL) & 1 isolate had vancomycin MIC of 16 µg/mL by Vitek 2 method, but all the isolates were susceptible (MIC≤2µg/mL) when tested by broth dilution & E-Test methods [14]. These findings were consistent with our study.

On the contrary, Rybak et al., observed that on comparison of Vitek 2 and E-Test methods with BMD, the E-Test & BMD method had 36.7% agreement while Vitek 2 system & BMD method had 54.3% agreement [15]. In another study, Mason et al., observed that out of 117 *S.aureus* isolates, all but one had MIC≤1µg/mL by BMD method. 96% of the same isolates tested by E-Test had MIC of 1, 1.5, 2µg/mL. All isolates were categorized as susceptible by CLSI breakpoints when tested by either method which correlates with our study [16].

Vancomycin treatment failure is not uncommon, even when MRSA strains are fully susceptible to vancomycin (MIC ≤2 mg/mL). A reduction in the efficacy of vancomycin against MRSA strains with a high vancomycin MIC (1–2 mg/ mL) has been described in observational studies with low number of patients, suggesting that subtle changes in the MIC may explain clinical failures. Another possible explanation for vancomycin failure when the MIC is at the limit of the susceptibility range could be the presence of heteroresistance [17]. In our study, we have obtained 39.39% MRSA isolates which had MIC=2µg/mL by BMD method. Although correlation between vancomycin MICs and clinical outcome of MRSA infections could not be made, possibility of ineffective treatment with vancomycin cannot be overruled and is a matter of concern. Presence of resistant subpopulations in seemingly susceptible MRSA strains might also be responsible for failure with vancomycin therapy. These resistant subpopulations are difficult to detect by the BMD method or E-Test or Vitek 2. Alternative treatment for MRSA infections in vancomycin treatment failure cases could be guided with the help of previous vancomycin therapy records.

There has been recent interest in the use of vancomycin MIC results (within the susceptible range) to predict outcomes for patients with serious *S. aureus* infections being treated with vancomycin [17,18]. Generally, these studies demonstrated a higher failure rate for vancomycin treatment of *S. aureus* strains with higher vancomycin MICs within the susceptible range. Because of the discrepancy that exists in vancomycin MIC results from different methods, the prediction of outcome of serious *S.aureus* infections should take into account the method used & results of studies using that particular method.

CONCLUSION

On determination of MICs for vancomycin for the MRSA isolates, all were identified as VSSA by BMD, E-Test & Vitek 2 methods. But the vancomycin MIC values obtained by E-Test correlated better with BMD method (correlation factor= 0.6727) as compared to when values obtained by Vitek 2 were correlated with BMD method (correlation factor=0.5316) which indicated E-Test to be a better, cheaper and easily performed method for determination of vancomycin MICs as compared to Vitek 2 for routine testing in laboratories in our opinion.

Vancomycin has till now remained the cornerstone of treating serious MRSA infections. Reduced susceptibility to vancomycin in MRSA isolates has now therefore become an area of concern and research. MRSA isolates with higher MICs, even within the susceptibility range, are being observed more frequently which result in treatment failures with vancomycin.

Because of the discrepancy that exists in vancomycin MIC results from different methods, the prediction of outcome of serious *S.aureus* infections should take into account the method used & results of studies using that particular method.

ACKNOWLEDGEMENT

We owe our great thanks to Department of Microbiology, Subharti Medical College, Meerut for extending their kind support as a funding source and encouraging us in pursuing this study.

REFERENCES

[1] Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin Microbiol Rev*. 2010;23:99–139.
[2] Ogston A. Micrococcus poisoning. *J Anat*. 1882;17(24):58325.
[3] Smith G. Ogston's coccus: 102 years and still going strong. *South Med J*. 1982;75:1559–62.
[4] http://en.wikipedia.org/wiki/Methicillin-resistant_*staphylococcus_aureus*
[5] Boucher H, Miller LG, Razonable RR. Serious infections caused by methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis*. 2010;51(Suppl 2):S183–97.
[6] Dhand A, Sakoulas G. Reduced vancomycin susceptibility among clinical *Staphylococcus aureus* isolates ("the MIC Creep"): Implications for therapy. *F1000 Medicine Reports*. 2012;4:4.
[7] CLSI Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement. CLSI document M100-S22. Wayne PA: Clinical & Laboratory Standards Institute. 2012.
[8] CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute. 2012.
[9] Koneman EW, Allen SD, Janda WM, Schreckenerger PC, Winn WC. Antimicrobial susceptibility testing. pp. 785-847. In Color Atlas and Text book of Diagnostic Microbiology 5th ed. Philadelphia: Lippincot Williams Wilkins; 1997.
[10] Hsu DI, Hidayat LK, Quist R. 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.
[11] 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.
[12] Leonard SN, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother*. 2009;63(3):489–92.
[13] Kruzel MC, Lewis CT, Welsh KJ, Lewis EM, Dundas NE, Mohr JF, 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.
[14] Behera B, Mathur P. Erroneous Reporting of Vancomycin Susceptibility for *Staphylococcus spp*. by Vitek Software Version 2.01. *Jpn J Infect Dis*. 2009;62(4):298-99.
[15] Rybak MJ, Vidaillac C, Sader HS, Rhomberg PR, Salimnia H, Briski LE, et al. Evaluation of Vancomycin Susceptibility Testing for Methicillin-Resistant *Staphylococcus aureus*: Comparison of Etest and Three Automated Testing Methods. *J Clin Microbiol*. 2013;51(7):2077-81.
[16] Mason EO, Lamberth LB, Hammerman WA, Hulten KG, Versalovic J, Kaplan SL. Vancomycin MICs for *Staphylococcus aureus* Vary by Detection Method and Have Subtly Increased in a Pediatric Population Since 2005. *J Clin Microbiol*. 2009;47(6):1628–30.
[17] 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* bacteraemia. *Clin Infect Dis*. 2008;46(2):193–200.
[18] Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteraemia treated with vancomycin. *Antimicrob Agents Chemother*. 2008;52(9):3315–20.

PARTICULARS OF CONTRIBUTORS:
1. Post Graduate Resident, Department of Microbiology, Subharti Medical College, Meerut, India.
2. Assistant Professor, Department of Microbiology, Subharti Medical College, Meerut, India.
3. Professor and Head of the Department, Department of Microbiology, Subharti Medical College, Meerut, India.
4. Professor, Department of Microbiology, Subharti Medical College, Meerut, India.
5. Associate Professor, Department of Microbiology, SubhartiMedical College, Meerut, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Charu Agrawal,
Professor, Department of Microbiology, Subharti Medical College, Subhartipuram, NH-58, Meerut, India.
E-mail : drcharuagrawal@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 22, 2014**
Date of Peer Review: **Aug 07, 2014**
Date of Acceptance: **Jul 27, 2015**
Date of Publishing: **Oct 01, 2015**