Original Article

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Comparison of In-vitro Activities of Linezolid and Vancomycin against *Staphylococcus aureus* Isolated from A Tertiary Care Hospital

Microbiology Section

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ABSTRACT

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as one of the commonest cause of hospital acquired infections worldwide. Vancomycin is the antibiotic of choice for treatment of MRSA, but due to slow increase in vancomycin minimum inhibitory concentration (MIC) (vancomycin creep),Vancomycin has become a suboptimal therapeutic option in critically ill patients. Linezolid has emerged as an alternative drug in the treatment of such cases.

Aim: To compare in vitro activities of linezolid and vancomycin against *Staphylococcus aureus*, in order to help in formulating a better treatment.

Method: 200 strains of *Staphylococcus aureus* were isolated from different clinical specimens between April 2010 to March 2011. Antibiotic sensitivity testing was performed by Kirby Bauer disc diffusion method and MICs of vancomycin and linezolid were determined for all 200 strains by agar dilution method by following CLSI guidelines.

Results: Among 200 strains, MIC for linezolid was 4 µg/ml for 3 strains, MIC was 2 µg/ml for 71 strains, and MIC was 1 µg/ml for 126 strains, while for the same 200 strains of *Staphylococcus aureus*, MIC of vancomycin was 4 µg/ml for 8 strains, it was 2 µg/ml for 103 strains and it was 1 µg/ml for 89 strains.

Conclusion: Linezolid and vancomycin had similar in-vitro efficacies for *Staphylococcus aureus* in disc diffusion method, but the number of strains with higher ranges of MICs of vancomycin (1-4 µg/ml) were more as compared to those which had higher ranges of MICs for linezolid. So, we suggest that linezolid can be a good alternative for the treatment of multidrug resistant *Staphylococcus aureus* as compared to vancomycin.

Keywords: VISA, MRSA, MIC

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as one of the commonest causes of hospital acquired infections worldwide. The infection caused by MRSA increases the length of hospital stay and it is also responsible for raising health care expenses and morbidity. Resistance to all antibiotics which are available for use against *Staphylococcus aureus* has been reported. In a study done by K. Rajaduraipandi, 63.2% MRSA were found to be resistant to gentamycin, cotrimoxazole, cephalexin, erythromycin and cephotaxim [1]. Ciprofloxacin usage has already been known to be associated with selection of MRSA [2]. In past few decades, vancomycin has been established as treatment of choice for MRSA infection. But due to excessive use of this drug, emergence of MRSA strains with reduced vancomycin susceptibilities (2-4 µg/ml) has been reported in past few years.

Currently, measures which are being taken to control *Staphylococcus aureus* infections are being challenged by a large and continuing increase in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), the spread of highly virulent community-associated MRSA, and the emergence of *Staphylococcus aureus* with reduced susceptibilities to vancomycin and other glycopeptides [3]. The condition has been further worsened by the emergence of vancomycin intermediate sensitive *Staphylococcus aureus* (VISA)(MIC 4-8 µg/ml) and vancomycin resistant *Staphylococcus aureus* (VRSA)(MIC ≥16 µg/ml) [4]. Among MRSA strains for which vancomycin MICs are elevated (1-2 µg/ml or 2-4 µg/ml), failure of vancomycin therapy or reduction in its efficacy has been widely reported [5].

The recently developed antimicrobial drug, linezolid, is probably one of the few choices for treatment of vancomycin resistant MRSA. Linezolid is the first drug among a new class of antibiotics, the oxazolidiones. This drug, unlike other protein synthesis inhibitors, acts early in translocation by disrupting the interaction of formyl methionine t- RNA with the 50s ribosomal subunit during initiation of pre initiation complex [6].Its spectrum includes medically important gram-positive bacteria such as methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) [7,8].

So, the present study aimed to determine the activities of linezolid and vancomycin against *Staphylococcus aureus* and to find out the different levels of minimum inhibitory concentrations of both the drugs, so as to formulate a better empirical therapy.

MATERIALS AND METHODS

This observational study was conducted in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow, India, between April 2010 to March 2011, after taking permission from the ethical committee of the hospital. Two hundred *Staphylococcus aureus* strains were isolated from various clinical specimens like pus, blood, wound swabs, urine, catheters, sputum or throat swabs, cerebrospinal fluid, high vaginal swabs, and other body fluids which were obtained from patients who were admitted to different wards of our institute. All the specimens were processed in the Bacteriology Lab of Department of Microbiology.

All the specimens were inoculated on Blood agar and MacConkey's agar plates and they were incubated at 37^{0}C for 24-48 hours. A presumptive identification was done on the basis of colony characteristics, Gram's staining, catalase and slide coagulase tests. A confirmation was done by tube coagulase test, growth on mannitol salt agar, DNAse test and modified Hugh's and Leifson's O/F test. In vitro antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. The MICs of vancomycin and linezolid was determined by agar dilution method. An MIC of 50 and an MIC of 90 were calculated statistically for these strains.

Disc diffusion testing was performed by Kirby Bauer method, by overlaying Muller-Hinton agar plates with the inoculum, whose turbidity was equivalent to that of 0.5 McFarland's Standard for *Staphylococcus aureus*. Following antibiotics were tested: Penicillin (10 U), (Cefoxitin 30 Microgram), Gentamycin (10 Microgram), Erythromycin (15 Microgram), Clindamycin (2 Microgram), Doxycycline (15 Microgram), Trimethoprim/Sulfamethaxole (1.25/23.75 Microgram), Pristinomycin (15 Microgram), Linezolid (30 Microgram), Vancomycin (30 Microgram), and teicoplanin (30 Microgram). Antibiotics Norfloxacin (10 Micro gram) and Nitrofurantoin (300 Microgram) were tested in urine specimens only. The zones of inhibition were measured against few inches above a black, nonreflecting background which was illuminated with reflected light, except for linezolid (30 Microgram), and vancomycin(30 Micro gram), which were read in transmitted light (with plates being held up to light source).

As per Clinical and Laboratory Standard Institute 2011 guidelines, Staphylococci for which MIC was ≤ 4 µg/ml were considered to be susceptible to linezolid, while those for which MIC was ≥8 µg/ml were considered to be linezolid resistant *Staphylococcus aureus* (LRSA) [9,10]. Strains of *Staphylococcus aureus* for which vancomycin MICs were ≤ 2 µg/ml were considered to be sensitive, those for which MICs were between 4 µg/ml and 8 µg/ml were considered to be intermediate sensitive (VISA) and those for which MICs were ≥16 µg/ml were considered to be resistant (VRSA) [10].

Detection of MRSA was done by using a cefoxitin disc (30 micro grams) and by doing diffusion test. The strains of *Staphylococcus aureus* with zone diameters of < 21 mm were considered as MRSA and those with zone diameters of >22 mm were considered to be sensitive [11]. *Staphylococcus aureus* ATCC 25923 was taken as control strain for disk diffusion testing.

Determination of MIC was done by Agar Dilution Method. All strains were tested for MICs of vancomycin, and linezolid by agar dilution method, by using Muller Hinton Agar culture medium (MHA). The concentrations of above drugs which were tested, ranged from 0.5 µg/ml to 16 µg/ml. Strains of *Staphylococcus aureus* ATCC 29213 (susceptible) were taken as control strains. Briefly, as per CLSI guidelines, in-house prepared MHA (Hi-Media, India) plates which had concentrations of 0.5, 1, 2, 4, 8, and 16 µg/ml of above mentioned drugs were prepared. Inoculum suspensions were prepared by selecting colonies from overnight growth obtained on nutrient agar plates. The colonies were transferred to sterile saline to produce a suspension that matched the turbidity of 0.5 McFarland's standard. The final inoculum concentration of 10^5 to 10^6 CFU per spot was prepared by adding sterile saline to the bacterial suspension [12]. These suspensions were spot inoculated on MHA plates which had different vancomycin and linezolid concentrations. Any visible growth which was seen, indicated vancomycin and linezolid resistances.

RESULTS

In our study, we found that a majority of *Staphylococcus aureus* were isolated from pus specimens which were received in bacteriology lab and that most of them had been sent from surgery and orthopaedics wards [Table/Fig-1].

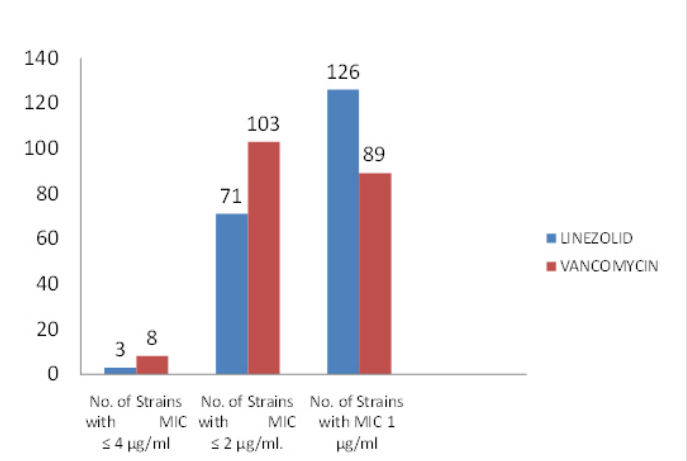
After identifying two hundred *Staphylococcus aureus* strains by standard biochemical tests, 79 isolates were found to be methicillin resistant *Staphylococcus aureus* by using 30 µg cefoxitin discs. The remaining 121 isolates were found to be methicillin sensitive. All 200 isolates were found to be sensitive to vancomycin (30 µg) as well as linezolid (30 µg) discs by Kirby Bauer disc diffusion method. No vancomycin or linezolid resistant *Staphylococcus aureus* strain was detected by determination of MICs by agar dilution method. MICs between 01- 4µg/ml for linezolid and vancomycin were obtained for all 200 strains which were isolated from various clinical specimens, by agar dilution method [Table/Fig-2]. Among 200 *Staphylococcus aureus* strains, MIC of 4 microgram/ml for vancomycin was obtained for 8 strains,, MIC of 2 microgram/ml for vancomycin was obtained for 103 strains and MIC of 1 µg/ml for vancomycin was obtained for 89 strains. MIC of 4 microgram/ml for linezolid was obtained for three strains,, MIC of 2 µg/ml for linezolid was obtained for 71 strains and MIC of 1 µg/ml for linezolid was obtained for 126 strains [Table/Fig-2,3]. MIC 50 for vancomycin and linezolid were 2 µg/ml and 1 µg/ml respectively [Table/Fig-4]. MIC 90 for both vancomycin and linezolid were 2 µg/ml [Table/Fig-4]. The eight strains for which MIC for vancomycin was 4 µg/ml were multiple drug resistant. [Table/Fig-5]. The total number of MRSA (n=79) strains for which MIC levels of vancomycin were 4 µg/ml, 2 µg/ml and 1 µg/ml were 8, 64 and 7 respectively. Similarly, the total number of MRSA strains for which MIC levels of linezolid were 4 µg/ml, 2 µg/ml and 1 µg/ml were 3, 53 and 23 respectively [Table/Fig-6].

Type of specimen	Number of specimens	*S. Aureus* isolated (%)
Pus	436	114
Blood	99	18
Urine	710	27
Vaginal swab	81	6
Sputum, Tracheal aspirate	87	23
Throat swab, Pleural fluid, Peritoneal fluid, CSF, Tissue aspirate	154	12

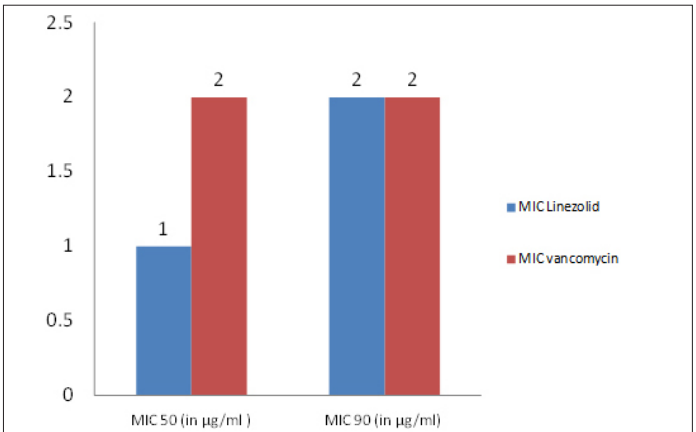
[Table/Fig-1]: Distribution of *Staphylococcus aureus* strains isolated from the various clinical specimens

Minimum inhibitory concentration	Linezolid	Vancomycin
No. of Strains with MIC ≤ 4 µg/ml	3	8
No. of Strains with MIC ≤ 2 µg/ml	71	103
No. of Strains with MIC ≤ 1 µg/ml	126	89

[Table/Fig-2]: Comparison of MIC of Linezolid and Vancomycin



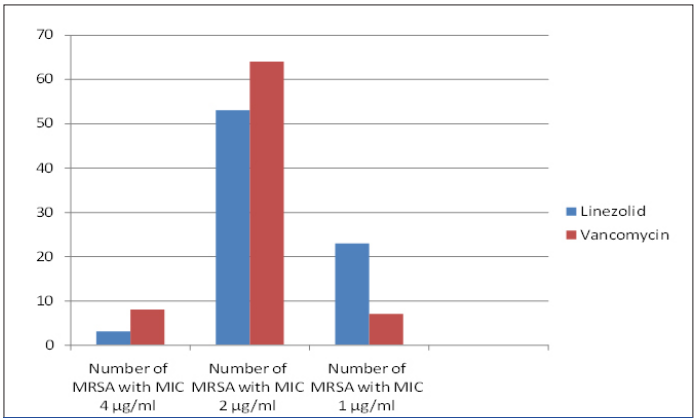
[Table/Fig-3]: Comparison of MIC of vancomycin and linezolid



[Table/Fig-4]: Comparison of MIC 50 and MIC 90 of vancomycin and linezolid

VAN MIC µg/ml	Lz MIC µg/ml	Van 30 µg	Lz 30 µg	Cn 30 µg	T 15 µg	Cp 5 µg	G 10 µg	E 15 µg	Cd 2 µg	P 10 U	Pm 15 µg	Clo 30 µg	PIT 100/10 µg
4	2	S	S	R	R	R	S	R	S	R	S	R	S
4	4	S	S	R	R	R	R	R	R	R	R	R	R
4	4	S	S	R	R	R	R	R	R	R	R	R	R
4	4	S	S	R	R	S	R	R	R	R	R	S	S
4	2	S	S	R	R	R	R	R	R	R	S	S	S
4	2	S	S	R	I	R	R	R	R	R	R	S	R
4	2	S	S	R	R	R	S	R	S	R	S	R	R
4	1	S	S	R	R	R	R	-	S	R	S	R	S

[Table/Fig-5]: Comparison of Staphylococcus aureus Strains having Vancomycin MIC 4 µg/ml with MIC of and Linezolid
(Cn-Cefoxitin, Van-Vancomycin, T-Tetracycline, Cp- Ciprofloxacin, E-Erythromycin, G- Gentamycin, Cd-Clindamycin, Lz-Linezolid, Pm-Pristinomycin, Clo-Cloremphenicol, PIT- Piperacillin Tazobactum



[Table/Fig-6]: Comparison of MIC of vancomycin and linezolid for MRSA strains

DISCUSSION

In current study, we found that MICs of linezolid and vancomycin for all strains were within the range of 01- 4µg/ml by agar dilution method [Table/Fig-2]. All strains, including the strains which had MICs of 4µg/ml for vancomycin and linezolid, were found to be sensitive to vancomycin and linezolid on measuring the sizes of zones of inhibition by Kirby Bauer Disk Diffusion method by following CLSI guidelines. Few other studies have also described that strains for which MIC of vancomycin (≥ 2 µg/ml) was even higher, could appear to be sensitive in disk diffusion test on measuring diameters of zones of inhibition [4].

In current study, *Staphylococcus aureus* was found to be the commonest pathogen which was isolated from patients with localized pyogenic and surgical wound infections admitted to surgery ward, which was in accordance with the findings of other workers, who had also reported that *Staphylococcus aureus* was the commonest pathogen which was isolated from surgical site infections [13].

All 200 *Staphylococcus aureus* isolates were found to be sensitive to both the drugs by disc diffusion method, but vancomycin showed comparatively higher MICs than linezolid by agar dilution method [Table/Fig-3]. [Table/Fig-6] demonstrates the MICs of vancomycin and linezolid for MRSA strains. Based on MIC levels of vancomycin and linezolid for MRSA strains (n=79), we can say that MRSA strains were also more susceptible to linezolid. Significant differences were found between MICs of vancomycin and linezolid for MRSA strains (Chi square test, p value ≤0.05). Our study correlated with study of S. Srinivasan, in which MIC for linezolid was 0.25 -2 µg/ml for 100 % MRSA strains, while for the same strains, MIC for vancomycin was 1-4 µg/ml [14]. In a study done by Fatima Kaleem in Rawalpindi, Pakistan, MIC of linezolid was found to be < 1 µg/ml for 100% MRSA, while for the same organisms, MIC for vancomycin was < 4 µg/ml [15]. Few other studies have also described linezolid as a good therapeutic option for MRSA, for reduction of burden on vancomycin for treatment of MRSA strains.

Based on MIC50 values, linezolid was found to be twofold more efficacious in vitro than vancomycin against both MRSA and MSSA. Results have been interpreted in [Table/Fig-2,4]. Similarly, low MIC values for linezolid were also reported by other investigators [13]. Statistically significant correlations were found between MIC 50 of vancomycin and linezolid. Besides, we isolated 8 strains of *Staphylococcus aureus* which had MIC of 4µg/ml for vancomycin, but only 3 of them had MIC of 4µg/ml for linezoild (as shown in [Table/Fig-5]). The eight *Staphylococcus aureus* strains for which MIC for vancomycin was 4 µg/ml, again indicated the emergence of vancomycin resistance, as VISA strains can spread if vancomycin is given to the patient for a prolonged period. These eight strains were also multiple drug resistant (as has been shown in [Table/Fig-5]). So, on the basis of above mentioned data, we can say that *Staphylococcus aureus* strains are still more susceptible to linezolid as compared to vancomycin in this region.

In the current study, it was also found that 6 *Staphylococcus aureus* strains for which MIC for vancomycin was 4 µg/ml were isolated from clinical samples of patients who were admitted to orthopaedics ward. As the duration of stay and antibiotic treatment of patients admitted to this ward were more as compared to those seen for patients admitted to other wards, this may be a cause of higher MICs and multidrug resistance associated with these strains, as chronic exposure of antibiotics may lead to selection of antibiotic resistant strains. Other studies have also reported that multiple drug resistant strains were more prevalent in orthopaedics ward [1].

In current study, no linezolid resistant strain was isolated. This was similar to that which was seen in other studies which had reported that clinical isolates were linezolid sensitive. Rajaduraipandi reported 2.4% linezolid resistant *Staphylococcus aureus* (LRSA) in south India in year 2006, which was found by Kirby-Bauer Disc diffusion method and recently, linezolid resistant strains were isolated in Nagpur from patients who were admitted to orthopaedics ward of a hospital [1,16-18]. But in UP, no linezolid resistant *Staphylococcus aureus* strain has been isolated. [18].In a multicentre study done in2008-2009 also, no isolate was found to be resistant to linezolid by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) [18]. Previous studies have reported that strains with upper levels of minimum inhibitory concentrations (MICs) of vancomycin were in the sensitive range (1-4 µg/ml); which would result in more morbidity and mortality among patients, as compared to those which had lower ranges of vancomycin MICs (less than 1 µg/ml). [19]. Further this agent, however, requires intravenous (i.v.) administration, continuous monitoring of levels and occasionally, patients experience some unacceptable side effects. Linezolid, on the other hand, is also available in oral form [6]. This drug is rapidly and completely absorbed after oral administration, with a mean bioavailability of approximately 100% and it does not require continuous monitoring. Further, the oxazolidiones have a unique mechanism of action and they do not exhibit cross resistance with existing agents. [20] In a study done by Benjamin P Howden, he described improved outcomes obtained with linezolid for MRSA

infection as compared to those which were obtained with use of vancomycin [21]. So, we suggest that linezolid is a good alternative for treatment of multidrug resistant *Staphylococcus aureus* strains. Our study was comparable with other studies which have also concluded that linezolid was superior to vancomycin in treating patients.

CONCLUSION

This study suggests that linezolid and vancomycin have similar in vitro efficacies for MRSA infections. Oral dosing option of linezolid allows earlier discharges of hospitalized patients and so, it is cost effective from point of view of patients.The excellent in vitro activity of linezolid, its reported in vivo effectiveness and fewer side effects, makes it an important therapeutic alternative to vancomycin in treatment of MRSA infections.

We would also stress here again that the indiscriminate use of antibiotics without doing antibiotic susceptibility testing and prolonged and inappropriate use of antibiotics lead to emergence of resistance. A good antibiotic policy should be laid down between clinicians and microbiologists in all tertiary care hospitals and a strict antibiotic regimen should be applied by clinicians. As only limited drugs are available for the treatment of VISA, irrational use of antibiotics should be avoided and a rational antibiotic policy must be adopted.

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