

Comparison of in vitro efficacy of linezolid and vancomycin by determining their minimum inhibitory concentrations against methicillin resistant *Staphylococcus aureus* (MRSA)

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Abstract

Objectives: To compare the in vitro activities of vancomycin and linezolid against methicillin resistant *Staphylococcus aureus* in our set up to help in formulating a better empirical treatment and reduce the emergence of vancomycin resistant *Staphylococcus aureus*.

Methods: The study was conducted over a period of 6 months (1st July 2009 - 31st Dec 2009). Fifty Methicillin resistant *Staphylococcus aureus* isolated from the clinical isolates of Military Hospital Rawalpindi were subjected to the determination of Minimum inhibitory concentrations of linezolid and vancomycin using E-strips.

Results: All the isolated organisms were uniformly susceptible to both the antibiotics. Vancomycin showed higher minimum inhibitory concentrations (MICs) as compared to linezolid MICs.

Conclusion: This study suggests that linezolid and vancomycin have similar in vitro efficacy for methicillin resistant *Staphylococcus aureus* infections.

Keywords: Linezolid, Minimum inhibitory concentrations, Methicillin resistant *Staphylococcus aureus*, Vancomycin (JPMA 61:356; 2011).

Introduction

Staphylococcus aureus is encountered very commonly in nosocomial and community acquired infections.^{1,2} Methicillin-resistant *Staphylococcus aureus* (MRSA) was reported as a major hospital acquired pathogen in the 1960s, and since then incidence of infections caused by this organism continues to rise.³⁻⁵

Methicillin resistant *Staphylococcus aureus* is a very versatile and dangerous organism causing infections ranging from relatively mild involvement of skin and soft tissue to life threatening serious infections.^{1,2} Nearly all the isolates are resistant to penicillin through production of β -lactamase enzymes⁶ and in recent years more than 50% of hospital acquired *Staphylococcus aureus* are resistant to all β -lactam antibiotics through production of an altered penicillin binding protein (PBP)2a. The infections caused by methicillin-resistant *Staphylococcus aureus* increase the length of hospital stay, and are also responsible for rising health care expenses, morbidity and mortality.^{2,4,7-9}

Resistance to all the available antibiotics against *Staphylococcus* has been reported with the exception of vancomycin. It was very much feared after the emergence of vancomycin resistant Enterococci that this resistance might also spread to *Staphylococci* and it became true very shortly, when low-level vancomycin resistance in *Staphylococcus aureus* was reported in 1996.¹⁰ Although this resistance is very rare, but it remains an imminent and important threat

because of its potential to disseminate. High level vancomycin resistance in *Staphylococcus aureus* in two reports is even a more alarming situation.^{11,12} The true impact of vancomycin resistance in *Staphylococcus aureus* is yet to be seen, but things are likely to get worse in the near future.

New molecular typing techniques have clearly documented the ability of epidemic, disease-producing clones of MRSA to populate hospitals and spread to diverse geographic regions very rapidly.^{13,14} The rapid spread and pathogenicity of these clones suggest that they may possess unique, as yet undefined, determinants of virulence. According to a recent multi-centre study, the frequency of MRSA in Pakistan is estimated to vary between 2-61%, with highest frequency seen in major cities of the country.¹⁵

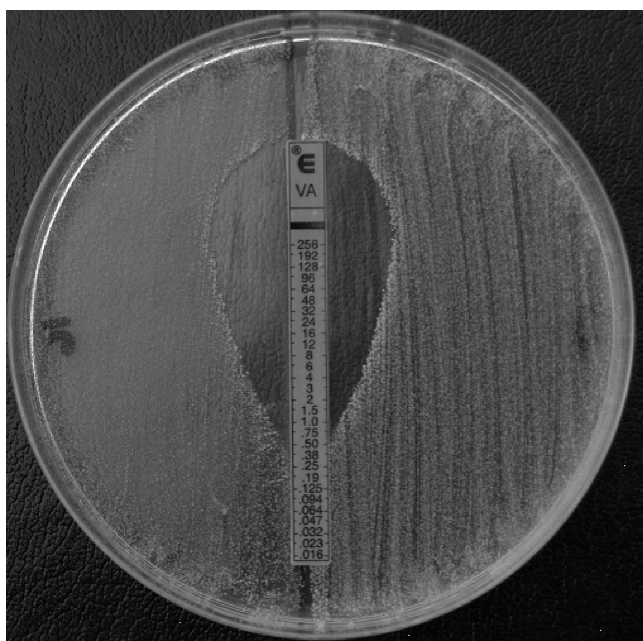
The glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of multidrug resistant Gram-positive bacteria. Use of this agent has increased dramatically in the past 20 years, because of the increasing prevalence of methicillin resistance in both *Staphylococcus aureus* and coagulase negative *Staphylococci*. Various alternate antimicrobials have been introduced in to clinical practice because of reports of high level resistance emerging against glycopeptides.¹⁶

Regarding the wide spread use of vancomycin and its emerging resistance, we planned a study to compare the in

in vitro activities of vancomycin with linezolid in our setup.

Material and Methods

Clinical specimens including pus, blood, urine, sputum, high vaginal swabs, aspirates, central venous lines, chest tubes and catheter tips sent for culture and sensitivity to the Department of Microbiology Army Medical College, National University of Sciences and Technology (NUST) were inoculated on appropriate culture media and incubated at 37°C for 24 hours. *Staphylococcus aureus* was identified by recommended methods like Gram staining characteristics, morphology, catalase and coagulase tests. Methicillin resistance was tested by modified Kirby-Bauer disk diffusion technique according to Clinical and Laboratory Standards Institutes (CLSI) guidelines and minimum inhibitory concentration (MIC) was detected by the use of E-strips (AB-Biodisk). First an inoculum was prepared according to 0.5 McFarland turbidity standards (10⁶ cfu/ml). After inoculating the isolates onto the Muller Hinton agar, E-strips of vancomycin and linezolid were applied over it and incubated along with controls for 18-24 hours at 37°C aerobically. The MIC results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI).¹⁷ For vancomycin, isolates with MIC of ≤ 2 µg/ml were considered susceptible, isolates for which the MIC was 4-8 µg/ml were intermediate and isolates with MIC ≥ 16 µg/ml were considered resistant. Regarding linezolid isolates with the MIC of ≤ 2 µg/ml were considered susceptible.¹⁷ Methicillin resistant *Staphylococcus aureus* ATCC 33591 and methicillin sensitive *Staphylococcus aureus* (MSSA) ATCC 25923 were used as control strains.



Linezolid E-Strip

Vancomycin E-Strip

Results

Among a total of 50 MRSA isolates all were sensitive to both the drugs used but vancomycin showed comparatively higher MICs than linezolid. The MIC 50 and MIC 90 of both were calculated and compared. The range of MIC of Vancomycin and Linezolid against MRSA were 0.25-2ug/ml and 0.023- 0.75ug/ml respectively. MIC 50 of vancomycin and linezolid were 0.75ug/ml and

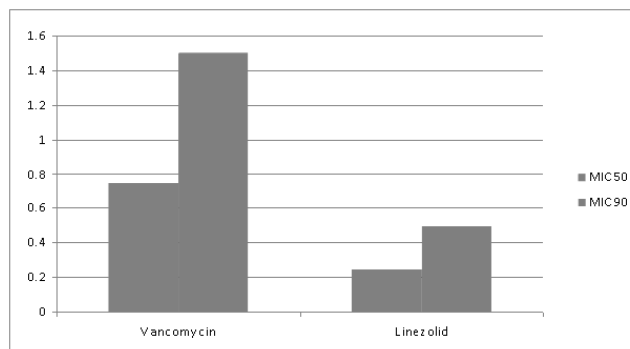


Figure: Graph showing comparison of MIC 50 and MIC 90 of vancomycin and linezolid. X axis shows drugs and Y axis shows MIC in ug/ml.

0.25ug/ml respectively. Results of MICs are represented in Table-1 and Figure.

Most of the MRSA isolates were retrieved from pus samples followed by nasobronchial lavage and urine (Table-2).

Table-1: Minimum inhibitory concentrations of linezolid and vancomycin against MRSA (n=50).

Antimicrobial Agent	% of isolates Susceptible at MIC (mg/L)												
	0.023	0.032	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2
Linezolid	2	2	4	6	4	14	28	22	14	4	-	-	-
Vancomycin	--	-	-	-	-	-	2	6	16	30	26	16	4

Table-2: Most of the MRSA isolates were isolated from pus samples followed by nasobronchial lavage and urine.

Samples which yielded growth of MRSA (n=50).	
Pus	64%
Nasobronchial Lavage	22%
Urine	8%
Vaginal Swab	6%

Discussion

MRSA is one of the major nosocomial pathogens causing significant morbidity and mortality. The important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient carriage on the hands of health care workers.^{4,5,10} In Pakistan, the significance of MRSA had been recognized relatively very late and it emerged as a problem in the 1980s and in the 1990s. Epidemic strains of these MRSA are usually also resistant to several other commonly used antibiotics. During the past 15 years, the appearance and world-wide spread of such clones have caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread.

Vancomycin has been the treatment of choice for methicillin-resistant *Staphylococcus aureus* (MRSA) infections. This agent, however, requires intravenous (IV) administration, continuous monitoring of levels and occasionally patients experience some unacceptable side effects. Linezolid, a member of the new oxazolidinone class of antibiotics, is highly active in vitro against MRSA and has excellent oral bioavailability and does not require monitoring of levels. Cumulative data indicates that linezolid and vancomycin have similar efficacy against MRSA infections.

A study carried out in USA in year 2005 indicated that intravenous or oral linezolid was well tolerated and is relatively superior to vancomycin in treating patients with MRSA-infected surgical-site infections and no dose adjustment for renal dysfunction patients is required.¹⁸ Our results are also comparable with a study carried out in Iran in 2009 which indicated that vancomycin and linezolid have same activity against MRSA.¹⁹ A study carried out in University of Health Sciences Lahore also concluded that Linezolid has a very good bacteriostatic activity against a broad range of multidrug resistant Gram-positive bacteria, including MRSA.²⁰ A study conducted in Japan indicated that

linezolid was very effective for the treatment of patients with pneumonia and sepsis caused by MRSA and may be better than vancomycin in achieving microbiological eradication in patients admitted in intensive care units.²¹ Occasional thrombocytopenia was associated with linezolid use, but it was mild and reversible on treatment completion and had no serious clinical implications and is only present in 2% of the patients getting treatment.²¹

Conclusion

This study suggests that linezolid and vancomycin have similar in vitro efficacy for MRSA infections. Linezolid's oral dosing option may allow earlier discharge of hospitalized patients thus reducing bed occupancy, over head charges and is a cost effective option from patient's point of view. The excellent in vitro activity of linezolid, its reported in vivo effectiveness and fewer side effects renders it an important therapeutic alternative to vancomycin in the treatment of methicillin resistant *Staphylococcus aureus* infection.

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