Int J Pharm Bio Sci 2015 April; 6(2): (B) 1087 - 1095

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFICACY OF LINEZOLID OVER VANCOMYCIN IN TREATING METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) INFECTIONS.

NAVODHYA JINDAL[1], SONIA JAIN[2] AND DHRITIMAN MAITRA[1*]

[1]Department of General Surgery, Medical College, Kolkata, West Bengal
[2]Department of Microbiology, Medical College, Kolkata, West Bengal

ABSTRACT

The purpose of this analysis was to compare the efficacy and safety of linezolid versus vancomycin in patients with nosocomial pneumonia, complicated skin & soft- tissue infections and sepsis by methicillin-resistant *Staphylococcus aureus* (MRSA).This study was conducted on hospitalized patients of a tertiary care hospital in Kolkata, West Bengal. Out of 191 patients, 130 received linezolid 600 mg (IV or oral) every 12 hours and 61 received vancomycin 1 g every 12 hours IV. Clinical success rates at the end of the treatment were 94.6% and 90.16% for the linezolid and vancomycin groups respectively (p=0.2564) and microbiological eradication rates were 93.07% and 88.52% respectively (p=0.2901) showing no significant difference in the efficacy of two drugs. Drug related adverse events were reported in 0.7% and 1.63% of the patients treated with linezolid and vancomycin respectively with thrombocytopenia (3.84%) reported only in patients who received linezolid. Linezolid is as effective as vancomycin however intravenous or oral linezolid was well tolerated.

KEYWORDS:**Linezolid,Vancomycin,Gram-positive infections, Meticillin-resistant Staphylococcus aureus MRSA.**

DHRITIMAN MAITRA
Department of General Surgery, Medical College, Kolkata, West Bengal

***Corresponding author**

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen worldwide.[1] In India cases of hospital acquired severe MRSA infection began to be reported from the mid-1960s. Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25 per cent in western part of India[2] to 50 per cent in South India[3]. It is one of the common causative agents of hospital and community acquired infections, particularly skin infections, pneumonia, surgical site infections, chronic bone infections and blood stream infections.[4] A network of microbiology laboratories (Indian Network for Surveillance of Antimicrobial Resistance - INSAR) at premier medical colleges and hospitals in India was formed with support from the World Health Organization. The network aims to monitor antimicrobial resistance and to review the magnitude of its problem in India. Initially, a few organisms of public health importance have been chosen for monitoring their prevalence and antimicrobial resistance patterns, with *S. aureus* being chosen among the Gram-positive organisms[5]. Drugs approved for the treatment of MRSA infections are vancomycin, linezolid, daptomycin, teicoplanin, quinopristine-dalfopristine and tigecycline.[6,7] However, all the drugs except linezolid have a narrow therapeutic range and require therapeutic drug monitoring, particularly in patients with renal dysfunction. Potential adverse effects of vancomycin include renal toxicity and 'red-man' syndrome.[8,9] Teicoplanin has advantages over vancomycin, such as a longer half-life allowing once-daily dosing and fewer adverse reactions. However, teicoplanin has a protein-binding rate of 90%, which may compromise therapeutic efficacy in some cases.[1] Indiscriminate use of vancomycin leads to the emergence and spread of vancomycin resistance in multidrug resistant strains is of growing concern in the recent years. The first case of heterogeneous VISA was reported in Japan in 1997 from a 62-year-old man with MRSA pneumonia who remained unresponsive after 12 days of vancomycin treatment. [11] The first report of VRSA emergence from a tertiary care hospital in North India was reported by Tiwari and Sen. [12] With the emergence of resistance to linezolid therapeutic options have become increasingly limited. Linezolid-resistant *Staphylococcus aureus* was first isolated in 2001 [13] Despite the availability of newer antimicrobial agents Linezolid, daptomycin, tigecycline for drug-resistant Gram-positive pathogens, clinicians and patients still need options for treatment of MRSA infection as daptomycin and tigecycline are very expensive. The study was conducted to evaluate the efficacy of two globally standard important therapeutic drugs linezolid and vancomycin for MRSA infections.

MATERIALS AND METHODS

The study was carried out at Medical College Hospital, Kolkata from Dec 2012 to Dec 2014. The goal of study was to compare the efficacy & safety of linezolid with vancomycin for the treatment of patients admitted in Medical College, Kolkata with nosocomial MRSA infections like Pneumonia, Sepsis , Surgical-site infections, complicated skin & soft- tissue infections (SSTIs).

1. Study design

This is an institution based randomized (2:1) case control study.

2. Study population & their treatment

Patients with confirmed MRSA from various samples who were clinically suffering from the diseases were enrolled in the study and randomized in a 2:1 ratio to receive linezolid 600 mg (intravenous [IV] or oral) every 12 hours or vancomycin 1 g every 12 hours IV for 7 to 21 days. The investigator judged when it was appropriate to switch to oral linezolid after a minimum of 3 days of iv treatment. The duration of treatment was 7-28 days for sepsis and 7-21 days for other patients. Patients could receive aminoglycosides with no activity against the isolated MRSA for Gram-negative coverage. Patients were assessed after completing the course of therapy. Previous history of any antibiotic administered was also recorded. Exclusion criteria: Patients with depressed immunity like diabetes mellitus, HIV, Cancer, Transplant recipient, Those

on immunosupresive drugs etc are excluded from the study.

3. Efficacy variables

Patients were evaluated after completion of course of therapy. Primary efficacy variables were clinical outcome and microbiological outcome. Secondary efficacy variables included clinical findings and symptoms, body temperature and white blood cell count, lesion size for patients with cSSTI and summaries of chest radiographs or chest CT findings for patients with pneumonia. For the analysis in this study patient population that was chosen were having culture confirmed MRSA and the pathogen was not resistant to study medications .Clinical outcome was determined at the end of the therapy as cured, improved or failed. The success rate was defined as the number of cures and improvements divided by the number of cures, improvements and failures. Cured was defined as resolution of the clinical signs and symptoms when compared with baseline. Improved was defined as improvement in two or more, but not all, clinical signs and symptoms of infection when compared with baseline. Failed was defined as persistence or progression of baseline clinical signs and symptoms of infection.

3. Laboratory analysis

Samples from patients with suspected MRSA infection such as pus, wound swabs, sputum and other respiratory samples, blood and body fluid were collected and processed in microbiology department and analysed for the microbiological cure using various microbiological tests.

i. Phenotypic identification of staphylococcal isolates

Gram staining was performed and smears showing gram positive cocci in clusters were considered. Those samples were inoculated on blood agar, nutrient agar, Mac-conkey's agar and incubated overnight at 37^0C. *Staphylococcus aureus* was identified based on the colony morphology, slide coagulase test, tube coagulase test and anaerobic mannitol fermentation by using standard methods.

ii. Antibiotic susceptibility Testing

Disc Diffusion Method All the confirmed *Staphylococcus aureus* strains were subsequently tested for methicillin, resistance by Kirby-Bauer disc diffusion method using cefoxitin discs (30 µg) along with that vancomycin (30 µg/disc) and linezolid disc (30 µg) using other antibiotics (Hi- Media Laboratories Pvt Ltd, India) on Mueller-Hinton agar (Figure 1). The plates were incubated at 37^0C for 24 hrs and zone size was recorded. The isolates were considered methicillin resistant if the zone of inhibition was 24mm or less [15].

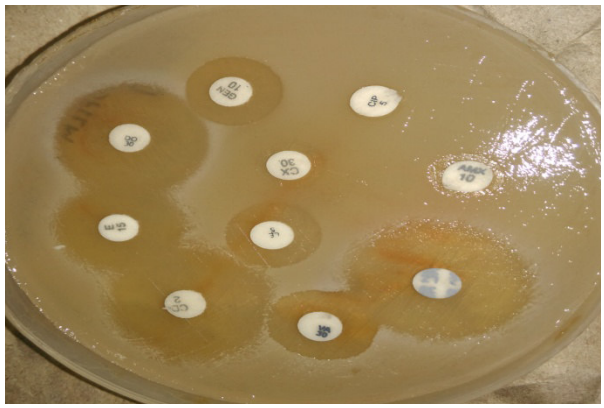


Figure 1
Disc diffusion method showing resistance to cefoxitin

iii. Molecular Analysis

Staphylococcus was confirmed by *16s rRNA* PCR (Figure 3). DNA was extracted as described by Phenol-Chloroform Method [16] with minor modification. DNA amplification was performed in total 20 µl of reaction mixture that contained 5 ng of template DNA,

200mM of dNPT mix, 1.2µl each of a pair of primers forward and reverse (10 pmol/µl, Sigma–Aldrich, USA), 1 U of Taq DNA polymerase, l0x buffer and 25 mM of $MgCl_2$. The amplification was carried out in Thermal cycler (Biometra, Germany).The amplification product (10µl) was analysed by electrophoresis on 1 % agarose gel and visualized with ultraviolet light after staining with ethidium bromide. Moreover, the genes encoding methicillin (*mecA*) were amplified using PCR. The reaction mixture composition was similar to that used for 16S-rRNA gene

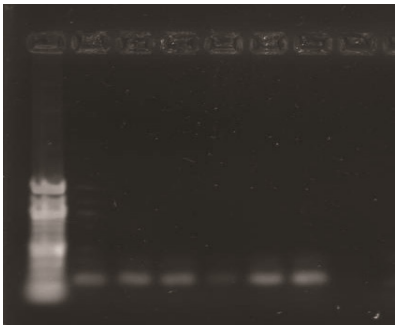


Figure 2
Agarose gel electrophoresis of PCR-amplified 16S rRNA gene of clinical isolates (a) Lane 1: 100 bp ladder; 7: S. aureus ATCC 25923, as positive control; (b) Lane 2-6:five clinical isolates, 8 : Negative Control

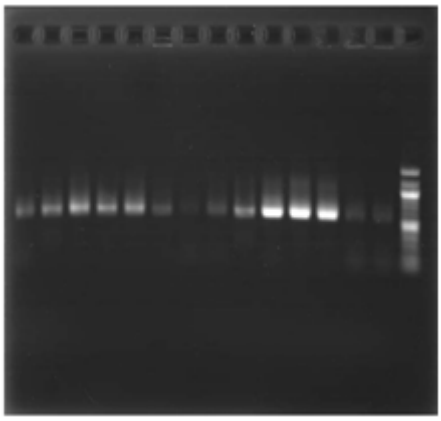


Figure 3
Agarose gel electrophoresis of PCR-amplified mecA genes of clinical isolates (a) Lane 15: 1000 bp ladder; Lane 1-14 : clinical isolates.

iv. Adverse event analysis

Vital signs, adverse events (AEs) and clinical laboratory values were evaluated. AEs were classified as mild, moderate or severe and considered related or unrelated to the study.

v. Statistical interpretation

Comparison was carried out after end of therapy using a Z test. Additionally 95% confidence interval (CIs) were calculated for the difference in success/cure rate and pathogen eradication rate. The incidence of AEs were compared between the two groups using a Z test.

RESULTS

Of the total 191 patients enrolled in the study, 130 were randomized to receive linezolid and 61 were randomized to receive vancomycin. The typical length of treatment for specific infections was 10-21 days for pneumonia and cSSTIs and 10-28 days for sepsis. The mean duration of treatment was 10.9 +- 5 days and 10.6 +- 5.1 days in the linezolid and vancomycin arms respectively.

Table I
Patients Demographics

	Linezolid n=130	Vancomycin n=61
Median Age Range (years)	35-45	40-48
Sex – Male	80 (61.53%)	40 (65.57%)
Female	50 (38.46%)	21(34.42%)

Table II
Distribution of MRSA isolates in relation to various specimens

Pus	No. of MRSA	No. of patients given Linezolid	No of patients givenVancomycin
Abscess	83	56	27
SSTIs	58	39	19
Total no. of pus samples positive for MRSA	**141** (73.82%)	**95** (73.07%)	**46** (75.41%)
Blood			
Septic Shock	20	14	6
Total no. of blood samples positive for MRSA	**20** (10.47%)	**14** (10.77%)	**6** (9.84%)
Sputum (Pneumonia)			
Total no. of sputum samples positive for MRSA	**30** (15.70%)	**21** (16.15%)	**9** (14.75%)

i. Clinical outcomes

After the end of therapy, overall success rates were 94.6% and 90.16% for the linezolid and vancomycin groups respectively (p= 0.2564). In patients with pneumonia, success rates were 90.47% (19/21) in the linezolid group and 77.78% (7/9) in the vancomycin group (p= 0.3488) (Fig 4). In patients with cSSTI, success rates were 98.94% (94/95) and 97.82% (45/46) in the linezolid and vancomycin arms (p=0.5989).(Fig 5) In patients with sepsis, the success rates were 71.42% (10/14) and 50% (3/6) in the linezolid and vancomycin groups (p= 0.3574).(Fig 6)

Table iii
Clinical outcome after end of therapy in patients treated with linezolid and vancomycin is as shown below.

EOT / response	Linezolid	Vancomycin	p-value
n (191)	130	61	-
Success n (%)	123 (94.6%)	55 (90.16%)	0.2564
Cured n (%)	60 (46.15%)	25 (40.98%)	0.5027
Improved n (%)	63 (48.46%)	30 (49.18%)	0.926
Failed n (%)	7 (5.38%)	6 (9.83%)	0.2547

Figure 4
Clinical outcomes for the pneumonia subgroups

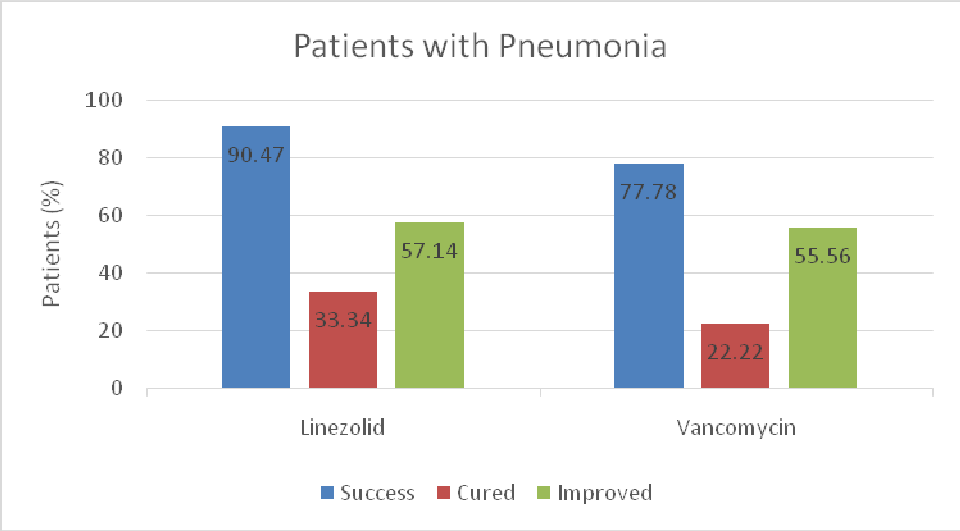


Figure 5
Clinical outcomes for the cSSTI subgroups

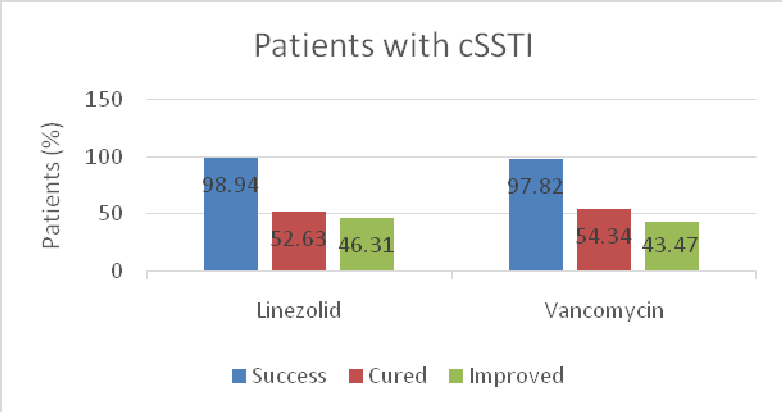
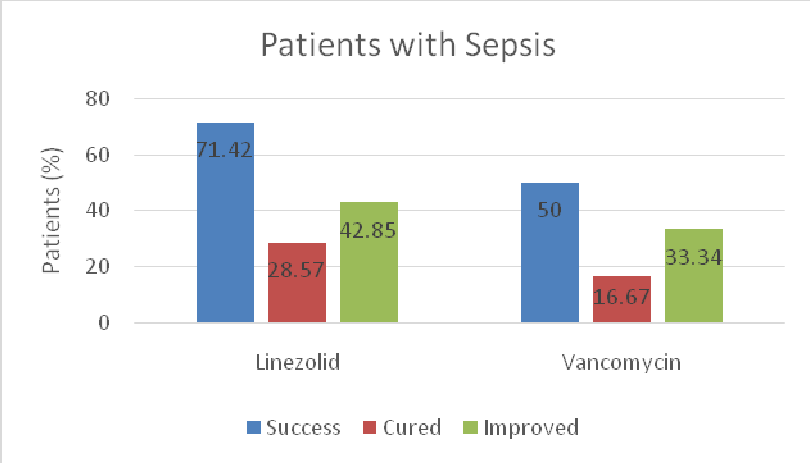


Figure 6
Clinical outcomes for sepsis subgroups



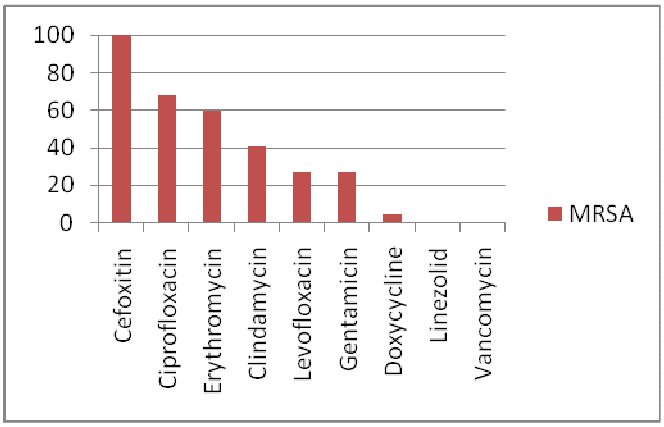
ii Microbiological outcomes

All the isolates were susceptible to both linezolid and vancomycin with no MICs>4mg/L.

At the end of therapy, microbiological eradication rates were 93.07% (121/130) and 88.52% (54/61) in the linezolid and vancomycin groups respectively (p=0.2901

).In the patients with pneumonia, pathogen eradication rates were 66.67% (17/21) and 66.67% (6/9) in the linezolid and vancomycin groups respectively (p=1).In patients with cSSTI, the eradication rates were 96.84% (92/95) and 95.65% (44/46) in the linezolid and vancomycin arms respectively (p=0.7203).Pathogen eradication rates were 50% (7/14) and 33.34% (2/6) in patients with sepsis treated with linezolid and vancomycin groups respectively (p=0.4925).

Figure 7
Antibiotic resistance profile of MRSA



The 191 strains of MRSA showed 100% resistance to penicillin & cefoxitin , 68.18% to ciprofloxacin, 60% to erythromycin, 40.90 % to clindamycin, 27.27% to levofloxacin, 27.27 % to gentamicin ,4.54% to doxycycline but all MRSA strains were sensitive to vancomycin & linezolid.

iii Adverse Events

Table iv
Drug related adverse events in patients treated with linezolid and vancomycin is as shown below.

Adverse events	Linezolid (n=130)	Vancomycin (n=61)	P value
Drug related events	21.53% (28)	19.67% (12)	0.7683
Serious adverse events	0.7% (1)	1.63% (1)	0.5464
Anemia	1.53% (2)	1.63% (1)	0.9586
Diarrhea	5.38% (7)	1.63% (1)	0.2274
Headache	2.30% (3)	1.63% (1)	0.7626
Rash	0.7% (1)	3.27% (2)	0.176
Thrombocytopenia	3.84% (5)	0	0.1209
Vomiting	1.53% (2)	1.63% (1)	0.9586

DISCUSSION

This study was conducted in West Bengal, India with the aim to compare linezolid with vancomycin for the treatment of MRSA infections. This study utilized an open label design as vancomycin could not be given orally and requires monitoring of levels and dosing adjustment.This study showed linezolid to be equally effective to vancomycin in treating patients with culture confirmed MRSA. Clinical outcomes were similar at EOT in both treatment groups. Both the groups were equally effective in achieving microbiological eradication also with no stastistical difference between them (p=0.2901) .The current study showed that in patients with cSSTIs the clinical success rate and microbiological eradication rates were 98.94% and 96.84% in the linezolid group and 97.82% and 95.65% in the vancomycin group respectively showing no significant difference between the two groups. In patients with pneumonia linezolid achieved 90.47% clinical success rate and

66.67% microbiological eradication while vancomycin achieved 77.78% clinical success rate and 66.67% microbiological eradication .Clinical success rate and microbiological success rate in patients with sepsis treated with linezolid was 71.42% and 50% respectively and in patients treated with vancomycin was 50% and 33.34% respectively .Overall rates of adverse events were similar in both linezolid and vancomycin treated patients. Thrombocytopenia was reported more frequently in linezolid treated patients but it caused no serious clinical consequences. The incidence of MRSA infection in India is high, with infection rates in hospitals as high as 60%, the problem is seen worldwide [1]. In the United States, nosocomial infection rates with MRSA are approaching 40%[14]. Furthermore, CA-MRSA is becoming an increasing problem with associated high morbidity and mortality. The emergence of resistant bacteria underlines the urgent need to develop new antibiotics for their treatment.

CONCLUSION

In conclusion, linezolid and vancomycin are equally effective in treating patients with pneumonia, cSSTIs and sepsis caused by MRSA with equal microbiological eradication rates. Both the drugs are equally safe to administer except for thrombocytopenia which is more in patients treated with linezolid however the efficacy of the two drugs is non comparable with almost similar adverse event. But since linezolid can be given orally with same bioavailability as intravenous linezolid, patient compliance with linezolid is much more than intravenous vancomycin.

CONFLICT OF INTEREST

Conflict of Interest: None.

REFERENCES

1. Diekema DJ, Pfaller MA, Schmitz FJ et al. SENTRY Participants Group. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis; (32) Suppl 2: S114–32 , (2001)
2. Patel AK, Patel KK, Patel KR, Shah S, Dileep P.Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. J Assoc Physicians India ;58(Suppl):37–40, (2010)
3. Gopalakrishnan R, Sureshkumar D., Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India, 58 (Suppl):25–31, (2010)
4. Kreienbuehl, L., E. Charbonney and P. Eggimann. Community-acquired necrotizing pneumonia due to methicillin-sensitive Staphylococcus aureus secreting panton-valentine leukocidin: A review of case reports. Ann. Intens. Care,(1): 52-52. PMID: 3259061,(2011)
5. Perichon, B. and P. Courvalin. VanA-Type vancomycin-resistant Staphylococcus aureus. Antimicrob. Agents Chemotherapy, 11: 4580-4587. PMID: 19506057 , (2009)
6. Catherine Liu, Arnold Bayer, Sara E. Cosgrove, Robert S. Daum. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant *Staphylococcus Aureus* Infections in Adults and Children. Clinical Infectious Diseases Advance;CID 2011:52, (2011)
7. Liang Beibei, Cai Yun, Chen Mengli, Bai Nan, Yu Xuhong, Wang Rui. *Linezolid versus vancomycin for the* treatment of Gram-positive bacterial infections: meta-analysis of randomised controlled trials Department of Clinical Pharmacology, General Hospital of Chinese People's Liberation Army. International Journal of Antimicrobial Agents, (35):3–12, (2010)
8. Kohno S, Yamaguchi K, Aikawa N, et al. Linezolid versus vancomycin for the treatment of infections caused by methicillin-resistant Staphylococcus aureus in Japan. J Antimicrob Chemother, (60): 1361-9 , (2007)

9. Hoelen DW, Tjan DH, van Vugt R, Geert van der Meer Y, van Zanten AR. Severe local vancomycin induced skin necrosis.Br J Clin Pharmacol,64(4): 553–554 , (2007)
10 Crossley K. Vancomycin-resistant enterococci in long-term-care facilities. Infect Control Hosp Epidemiol; (19) : 521–525,(1998).
11 Hiramatsu, K., H. Hanaki, T. Ino, K. Yabuta, T. Oguri, and F. C. Tenover. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother , (40) **:**135-136, (1997)
12 Tiwari HK, Sen MR. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infect Dis; 6:156, (2006)
13 Tsiodras, S., H.S. Gold, G.Sakoulas, G.M. Eliopoulos, C.Wennersten and L.l. Venkataraman. Linezolid resistance in a clinical isolate of Staphylococcus aureus. The Lancet. 358 (9277): 207–8,(2001)
14 Centers for Disease Control and Prevention. Vancomycin-resistant Staphylococcus aureus—New York. MMWR 2004; (53): 322–3,(2004)
15 Mathew.A.Wikler,Frankrill R.Cockerill,et al. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically- Approved standards, M7-A7. 7th edition, USA, (2007)
16 Sambrook J, Fritsch EF, and Maniatis T. Molecular Cloning: A Laboratory Manual. 2nd edition, Cold Spring Harbor Laboratory Press publisher: NewYork,1989,pp.1.21-1.41.