JKIMSU, Vol. 1, No. 2, July-Dec. 2012
ISSN 2231-4261

ORIGINAL ARTICLE

Detection of *in-vitro* Activity of Linezolid in Methicillin Resistant *Staphylococcus aureus* Infections by E-test

Lakshmi S. Kakhandki[1], B V Peerapur[2], Smitha Bagali[1], Prashant Parandekar[1]*
[1]Department of Microbiology, BLDEU's Shri B. M. Patil Medical College, Solapur Road, Bijapur-586103(Karnataka), India. [2]Department of Microbiology, Raichur Institute of Medical Sciences, Raichur -584102, (Karnataka), India.

Abstract:

Aim: To determine the *in-vitro* activity of linezolid in methicillin resistant *Staphylococcus aureus*. *Material and Methods*: Samples such as pus, sputum, ear swab, pleural fluid that were received in microbiology laboratory were included in the study. Gram staining was performed on all the samples. Smears showing gram positive cocci in clusters were considered. 100 strains of *Staphylococci* were subsequently processed and identified using standard microbiological methods. *Staphylococcus aureus* so identified were subjected to screening for methicillin resistance using oxacillin disc (1µgm) and also *in-vitro* activity of linezolid by Kirby-Bauer disc diffusion method. Subsequently minimum inhibitory concentration for linezolid was determined by E test using Hi-Comb strips obtained from Hi-Media. *Results:* Out of 100 strains of *Staphylococcus aureus* isolated, 42 (42%) were methicillin resistant and 58 (58%) were methicillin sensitive. Majority of methicillin resistant *Staphylococcus aureus* isolated were from pus (35) followed by sputum (7). Among the 42 strains of methicillin resistant *Staphylococcus aureus* isolated, sensitivity to linezolid (30mcg) was 87.71% and minimum inhibitory concentration was in the range of 0.1 - 1µgm/ml. *Conclusion*: Linezolid is a novel alternative drug to vancomycin in the treatment of severe methicillin resistant *Staphylococcus aureus* infections.

Keywords: Methicillin Resistant *Staphylococcus aureus*, Linezolid, Minimum Inhibitory Concentration

Introduction:

Over the last two decades, the increasing incidence of methicillin resistant *Staphylococcus aureus* (MRSA) has caused significant clinical concern worldwide. Methicillin resistance in *Staphylococcus aureus* is also associated with resistance to several commonly used antimicrobial agents such as the macrolides, lincosamides, quinolones, trimethoprim-sulfamethoxazole and aminoglycosides [1]. Such resistance leads to increased mortality and also decrease in cost-effectiveness of treatment. Glycopeptide antibiotic vancomycin has been the drug of choice for the treatment of serious *Staphylococcal* infections for the decades with no resistance emerged until the late 90s. In Japan, the transmission within hospitals of methicillin resistant *Staphylococcus aureus* strains with heterogeneous resistance to vancomycin has been reported [2]. With the increasing incidence of multidrug resistant *Staphylococci* and emergence of resistance to gly-

copeptides in methicillin resistant *Staphylococcus aureus,* therapeutic options have become increasingly limited. Thus there is a clear need for novel agents as alternatives in the treatment of infections caused by methicillin resistant *Staphylococcus aureus* [3].

The only available antimicrobial with proved high activity against multi-resistant *Staphylococcus aureus*, including strains with reduced susceptibility to glycopeptides is linezolid. So this study was undertaken to determine the in-vitro activity of linezolid in methicillin resistant *Staphylococcus aureus* infections.

Material and Methods:

The study was carried out among the patients attending BLDEU'S Shri B. M. Patil Medical College Hospital, Bijapur, Karnataka. Samples such as pus, sputum, ear swabs, pleural fluid that were received in microbiology laboratory were included in the study. Gram staining was performed on all the samples. Smears showing gram positive cocci in clusters were considered. 100 strains of *Staphylococci* were subsequently processed. The samples were inoculated on blood agar, nutrient agar, Mac-conkey's agar, then incubated overnight at 37^0C. *Staphylococcus aureus* was identified based on the colony morphology, gram staining, tube coagulate test, slide coagulate test, phosphatase test, deoxyribonuclease test and mannitol fermentation test, by using standard methods.

All the confirmed *Staphylococcus aureus* strains were subsequently tested for methicillin resistance by Kirby-Bauer disc diffusion method using oxacillin discs (1µgm/disc) obtained from Hi- Media laboratories and the medium used was Mueller-Hinton agar with 4% sodium chloride. The plates were incubated at 35^0C for 24 hrs and zone size was recorded. The isolates were considered resistant if the zone of inhibition was 10mm or less [4].

Subsequently *in-vitro* activity for linezolid was tested by Kirby-Bauer disc diffusion method on Mueller-Hinton agar using linezolid disc (30mcg) obtained from Hi-Media laboratories. Minimum inhibitory concentration for linezolid was found out by E-test on Mueller-Hinton agar using Hi-Comb strips obtained from Hi-Media laboratories. The E test was performed according to the protocol supplied by the manufacturer and minimum inhibitory concentration was calculated as the value at which the zone of inhibition converged on the comb like projection of the strip [5]. Minimum inhibitory concentration within a range of ≤ 2µg/ml for the concentration gradients ranging between 8 –0.001µg was taken as sensitive.

Results:

Out of 100 strains of *Staphylococcus aureus* isolated 42(42%) showed resistance to methicillin (methicillin resistant *Staphylococcus aureus*) and 58(58%) were sensitive (methicillin sensitive *Staphylococcus aureus*). Majority of methicillin resistant *Staphylococcus aureus* isolated were from pus (35), followed by Sputum (7). Among the 42 methicillin resistant *Staphylococcus aureus* isolates, sensitivity to linezolid (30mcg) was 87.71% by Kirby-Bauer disc diffusion method and minimum inhibitory concentration was in the range of 0.1-1µg/ml.

Discussion:

Staphylococcus aureus is a major cause of nosocomial infections including pneumonia,

post-operative wound infections, bacteremia and other infections. Over past 50 years *Staphylococcus aureus* has acquired resistance to all the previously effective antimicrobials, and has today emerged as one of the most important nosocomial pathogen [6]. Vancomycin remains the drug of choice in the treatment of these infections, but vancomycin has potential for toxic side effects like renal impairment besides its prohibitive cost. Concerns about its reduced effectiveness and development of resistance are mounting [7]. So this study was carried out to detect the efficiency of a novel drug linezolid in the treatment of methicillin resistant *Staphylococcus aureus* infections which could be effective if vancomycin fails.

Linezolid belongs to a new synthetic class of antimicrobials, the oxazolidinones. They are active against a wide variety of gram positive organisms including methicillin resistant *Staphylococcus aureus* . Linezolid binds the 50S ribosomal subunit and inhibits bacterial protein synthesis by interfering with the formation of initiation complex in bacterial translation systems. Because this agent possesses a novel structure and unique mechanism of action, it does not display cross resistance with other classes of antimicrobial agents [3]. Linezolid has an acceptable safety profile for both intravenous and oral administration and has proven to be effective in the treatment of infections due to methicillin resistant *Staphylococcus aureus* in critically ill patients [8]. Side effects to linezolid have been mostly pain in abdomen and hematological adverse events. Linezolid also helps in diabetics when vancomycin fails [9].

In the present study the sensitivity of methicillin resistant *Staphylococcus aureus* to linezolid has been 87.71% by Kirby- Bauer disc diffusion method and minimum inhibitory concentration has been in the range of 0.1-1µg/ml. All the patients receiving linezolid have had full recovery as shown by negative follow up cultures. These findings suggest that linezolid could be as effective as vancomycin in the treatment of methicillin resistant *Staphylococcus aureus* infections and may be more effective than vancomycin in achieving the microbiological eradication. Our results are consistent with the studies of Carmen Betriu *et al* [3]. and Srinivasan S *et al* [7]. Thus linezolid is a promising therapeutic option in the era of rapidly growing antibiotic resistance due to its cost effectiveness and comparatively lower side effects.

References:

1. Biavasco F, Vignaroli C, Varaldo P E. Glycopeptide resistance in coagulate – negative staphylococci. European Journal of Clinical Microbiology and Infectious Diseases 2000; 19: 403-17.
2. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. Lancet 1997; 350: 1670-3
3. Carmen Betriu, Montserrat Redondo, Ana Boloix, Maria Gomez, Esther Culebras and Juan J. Picazo. Comparative activity of linezolid and other new agents against MRSA and teicoplanin-intermediate coagulase negative staphylococci. Journal of Antimicrobial Chemotherapy 2001; 48: 911-913.
4. Clinical and laboratory standards institute.

Performance standards for antimicrobial disc diffusion tests. Approved standards. 9th ed. CLSI document M2-M9. Wayne PA: CLSI; 2006.

5. Menezes G A, Harish B N, Sujatha S, Vinothini Parija S C. Emergence of Vancomycin intermediate *Staphylococcus* species in southern India. *J Med Microbiol* 2008 Jul; 57(pt 7): 911-912.
6. Mehta A P, Rodrigues C, Sheth K, Jani S, Hakimlyan A, Fzalbhoy N, Control of Methicillin-resistant *Staphylococcus aureus* in a tertiary care Center-A-five-year study. *J med Microbial* 1998; 16: 31-4.
7. Srinivasan S, Sheela D, Shashikala, Mathew R, Bazroy J, Kanungo Risk factors and associated problems in the managements of infections with MRSA. *IJMM* 2006; 24:182-185.
8. Chien J W, Kucia M L, Salata R A. Use of linezolid , an oxazolidinone, in the treatment of multidrug-resistant gram-positive bacterial infections. Clinical Infectious Diseases 2000; 30: 146-51.
9. MRSA Watch: Treatment-Linezolid. file:///G: MRSA%20 Watch%20 Treatment%20 -%linezolid.htm

***Author for Correspondence:** Dr. Lakshmi. S. Kakhandki, Assistant Professor, Department of Microbiology, BLDEU's Shri B. M. Patil Medical College, Solapur Road, Bijapur-586103, Karnataka, (India). Cell : 09986133560 Email: drlaxmikanbur@yahoo.com*