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Original Article

Telavancin, a hope against Methicillin Resistant Staphylococcus aureus

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ABSTRACT

Objective: To determine the in vitro efficacy of Telavancin against Methicillin resistant *Staphylococcus aureus.*

Methods: This cross-sectional study was conducted at the Department of Microbiology, Army Medical College, Islamabad/Military Hospital, Rawalpindi, from 1st Nov 2012 to 30th June 2013. The authors aimed at assessing the in vitro efficacy of Telavancin against Methicillin resistant *Staphylococcus aureus* by E strip method as this study will be a help in determining guidelines for the interpretation of our local susceptibility breakpoint criteria for Telavancin against possibly emerging vancomycin intermediate *Staphylococcus aureus* and vancomycin resistant *Staphylococcus aureus*. Should vancomycin resistance become wide spread alternative therapies for methicillin resistant *Staphylococcus aureus* will be urgently needed.

Results: In this study 102 methicillin resistant *Staphylococcus aureus* were isolated, and all were found to be 100% susceptible to Telavancin.

Conclusion: Telavancin has excellent in vitro activity against methicillin resistant *Staphylococcus aureus*.

Keywords: MRSA, Telavancin, VISA and VRSA

Introduction

Infections caused by Staphylococcus aureus range from mild skin infections to life threatening conditions like sepsis, pneumonia, and toxic shock syndrome.[1] Although a part of the normal human flora, Staphylococcus aureus is capable of causing clinically important infections.[2] Mortality from Staphylococcus aureus diseases was high in the pre-antibiotic era, but introduction of penicillin had a dramatic impact, however, it was short lived due to the emergence of penicillinase producing S. aureus.[2] In order to counter the situation two new antibiotics were introduced. One was vancomycin, a glycopeptide introduced in 1956 and the other a beta-lactamase stable antibiotic, methicillin, which happened to be the first semisynthetic anti staphylococcal penicillin, in 1960.[3]

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However, this too did not last for long and soon this organism developed resistance to methicillin, with the first case of methicillin resistant Staphylococcus aureus (MRSA) reported in the United Kingdom in 1961.[4] By the mid 1990s, reduced susceptibility to vancomycin began to emerge and the first case of S. aureus showing reduced susceptibility to vancomycin, known as VISA (vancomycin intermediate Staphylococcus aureus), was reported from Japan in the year 1996. Till June 2002, eight VISA infections had also been documented in the USA. However, the first clinical isolate of vancomycin resistant S. aureus (VRSA) was reported from a patient in Michigan (USA) in year 2002.[5] Telavancin (Vibativ™; Theravance, San Francisco, CA, USA) is a new rapidly bactericidal, lipoglycopeptide that is structurally related to vancomycin. It shares the same glycopeptide core as possessed by vancomycin.[6] The difference lied in possessing an extra lipophilic side chain which is known to enhance its mode of action.[7]

Telavancin is effective against clinically important Gram positive pathogenic organisms like staphylococci (including MRSA, hVISA, and VISA strains) and streptococci, (including penicillin

resistant *Streptococcus pneumoniae* PRSP) as well as Gram positive anaerobic bacteria.[8]

This study will help in determining guidelines for the interpretation of our local susceptibility breakpoint criteria for Telavancin against possibly emerging vancomycin intermediate *Staphylococcus aureus* and vancomycin resistant *Staphylococcus aureus*. Should vancomycin resistance and reduced susceptibility to glycopeptides become widespread alternative therapies for methicillin resistant *Staphylococcus aureus* will be urgently needed.

Methods

This cross-sectional study was carried out in the Department of Microbiology, Army Medical College, National University of Sciences and Technology Islamabad, affiliated with an 1100 bedded tertiary care hospital from Nov1, 2012 to June 30 2013. Clinical specimens including blood, pus, wound swabs, sputum, pleural fluid, nasobronchial secretions, endotracheal secretions, cerebrospinal fluid, pericardial fluid, peritoneal fluid, high vaginal swabs, central venous lines and catheter tips were screened for the presence of MRSA. All clinical isolates of MRSA from routine samples of patients were included whereas the duplicate samples of the same patient during the same episode of illness and Methicillin sensitive *Staphylococcus aureus* were excluded.

Received specimens were inoculated on appropriate culture media. Blood for culture was enriched in the Brain Heart Infusion (BHI) broth and subcultured at 24, 48, 72 hours and 5^{th} and 7^{th} day on to Blood and MacConkey agar plates and incubated at 35°C aerobically for 18-24 hours before being reported as negative. Pus and wound swabs were inoculated on Blood agar and MacConkey agar, incubated at 35°C for 48 hours before being reported as negative. Sputum samples were inoculated on Blood and Chocolate agar. Nasobronchial secretions, endotracheal secretions and pleural fluid for C/S was inoculated on Blood agar, Chocolate agar and MacConkey agar. Nasal swabs were inoculated on Blood and Chocolate agar. Central venous lines and catheter tips were enriched in BHI broth and incubated at 35^{o} C for 24 hours. Then next day, the inoculated BHI was subcultured on Blood and MacConkey agar plates. High vaginal swab was inoculated on Blood agar and Sabouraud agar. All the inoculated plates were incubated for 48 hours aerobically at 35^{o} C before being reported as negative.

Detection of MRSA strains was carried out by checking the size of zone of inhibition around a 30µg cefoxitin disc. As per Clinical Laboratory and Standards Institute (CLSI) recommendations, isolates showing a zone diameter of ≤ 21mm were identified as MRSA strains.

After the detection of MRSA strains, MIC for Telavancin was determined by the Epsilometer strips (AB Biodisk, Solna, Sweden), that were stored in the freezer at a temperature of -20^{o} C. They were protected from moisture, heat and direct exposure to strong light when not in use. Upon removal from the freezer, package was allowed to equilibrate to room temperature for 30 minutes. A fresh suspension for each of the MRSA isolates was prepared keeping the turbidity equivalent to 0.5 McFarland turbidity standard (10^{6}cfu/ml). Within 15 minutes of mixing the MRSA colonies in normal saline, a sterile cotton swab was dipped into each suspension and pressed against the wall of the tube to remove excess liquid. A bacterial lawn was made by streaking on the surface of Muller Hinton Agar (MHA) plates, turning the plate approximately at 60° three times to attain an evenly distributed inoculum.

Using sterile forceps, an E strip of Telavancin was applied on the inoculated agar plate. It was made sure that the whole length of the strip was in complete contact with the agar surface. The air bubbles were removed by application of gentle pressure on the strip with forceps, moving from the minimum concentration upwards. Keeping in consideration the immediate release of the antibiotic from E strip into the agar medium, care was taken not to move the E strip once placed upon the agar surface. The inoculated plates for all the isolates were incubated at 35°C for 24 hours. An internal control of Methicillin resistant *Staphylococcus aureus* was used for quality control.

After incubation at 35°C for 24 hours bacterial growth became visible on the whole inoculated MHA plate and a symmetrical ellipse of inhibition along the strip was visible to the unaided eye. The plate was placed on a dark nonreflecting surface to determine the end points as shown in figure 1. The MIC value was read from the range as µg/ml. MIC was recorded where the epsilon intersected the E strip. The same procedure was repeated for all the MRSA isolates. The interpretive criteria of MIC values of Telavancin against MRSA according to FDA is ≤ 1 µg/ml. Before reading and recording the results obtained with clinical isolates, those obtained with

the quality control strain were checked to ensure that they were within acceptable ranges.

Statistical analysis of data was done by using Statistical Package for Social Sciences version 21 (SPSS 21). Frequencies (n) and percentages (%) were calculated for qualitative data for both ward wise and specimen wise distribution of isolates. The MIC_{50} and MIC_{90} values of Telavancin were calculated by cumulative percentage n(%) by calculating the percentage of isolates that exhibited a particular MIC value followed by their arrangement in ascending order. The MIC_{50} was defined as that value of MIC which corresponded to 50% of the isolates and MIC_{90} was defined as the value that corresponded to 90% of the isolates. The in vitro efficacy of Telavancin was based on the lowest value of MIC_{90} as a marker of better efficacy.

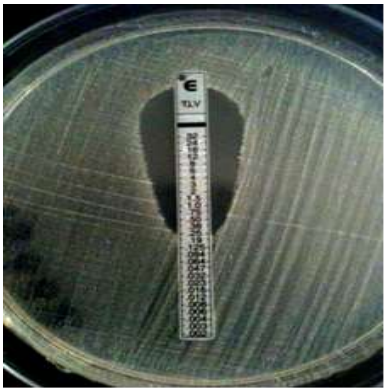

Figure 1. Telavancin E strip applied on MHA showing the zone of inhibition

Results

A total of 102 MRSA were isolated during the study period. The maximum number of isolates i.e. n=29 (28.43 %) were from the outpatient department and the minimum i.e. n=2(1.96 %) each from psychiatry and nephrology departments of the Military Hospital, Rawalpindi (Figure 2). Regarding the specimen wise distribution of the isolates, n=85(83.33%) of the MRSA isolates were from pus, n=9(8.82%) from blood, n=6(5.88%) from respiratory tract (n=3 from the sputum samples, n=2 from the pleural fluid and n=1 from a tracheal aspirate) and the minimum i.e. only n=2(1.96%) were from pericardial fluid aspirate as seen in Figure 3. All the isolates were found to be 100% susceptible to Telavancin as per the FDA guidelines i.e. all were found to be having MIC ≤ 1 µg/ml. The range, MIC_{50} and MIC_{90} values of Telavancin against MRSA are shown in the Table 1.

Table 1. Minimum Inhibitory Concentration (MIC) of Telavancin against Methicillin Resistant *Staphylococcus aureus* (n=102)

MIC (µg/ml)	0.19	0.25	0.38	MIC50 (µg/ml)	MIC90 (µg/ml)	Range (µg/ml)
% of isolates inhibited	n=6 (5.88%)	n=70 (68.62%)	n=26 (25.49%)	0.25	0.38	0.19-0.38

N, number of patients; %, percentage

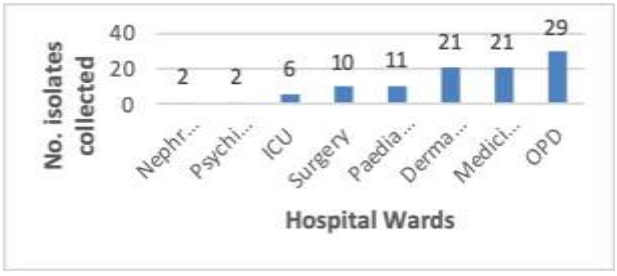

Figure 2. Ward wise distribution of the isolates (n=102).

ICU, intensivee care unit; No. number of patients; OPD out-patient department

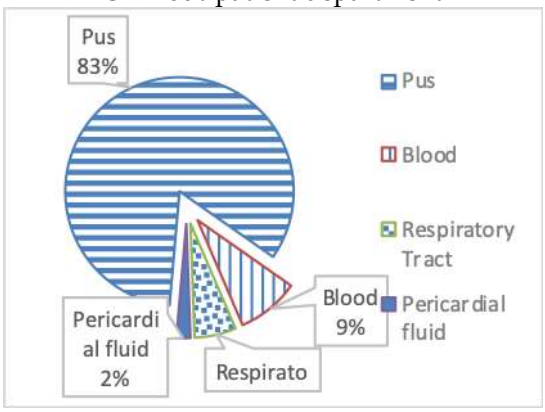

Figure 3. Specimen wise percentage distribution of the isolates (n=102)

Discussion

Telavancin, a novel semi synthetic lipoglycopeptide, derivative of vancomycin, is a bactericidal antibiotic approved for the use in complicated skin and skin structure infections. It is active against MRSA, VISA, and hVISA strains of *S. aureus*. It is currently in the late stages of clinical trials for the treatment of serious infections associated with the resistant Gram-positive bacteria. The increasing frequency of MRSA globally poses a serious threat in the form of VISA and VRSA.[9] Although VISA or VRSA have not yet been reported in Pakistan the recent spread of VISA strains in Turkey,(10) is alarming especially for the countries in its vicinity including Pakistan. This

shows that VISA is present almost everywhere, but due to the lack of standardized methods for their identification they are not being detected in routine.[11]
Vancomycin was so far considered the gold standard for the treatment of invasive methicillin resistant *Staphylococcus aureus* (MRSA) infections. However, the development of resistance against vancomycin has led to clinical failure mainly due to the injudicious use of all the antimicrobials including vancomycin. The several hypothesized reasons for the failure of vancomycin include poor penetration of the antibiotic into the tissues, production of biofilms by several strains of *S. aureus* thus decreasing the antimicrobial activity of vancomycin and emergence of resistance to vancomycin in enterococci and staphylococci further leading to the rising MICs of vancomycin for MRSA.[12,13]
Many alternatives for the treatment of MRSA infections, including linezolid, daptomycin, tigecycline, and the latest of all Telavancin are currently approved by the US Food and Drug Administration (FDA).
In our study, 102 clinical isolates of MRSA diagnosed as per Clinical Laboratory and Standards Institute (CLSI) recommendations isolates showing a zone diameter of ≤ 21mm were evaluated for the in vitro efficacy of Telavancin.[14,15] It was found to be effective against Methicillin resistant *Staphylococcus aureus* with MIC ranging from 0.19 to 0.38µg/ml. The MIC_{50} was 0.25µg/ml and MIC_{90} was 0.38µg/ml. both these values fall into the range of susceptibility as approved by FDA.[10,16]
In a study conducted by Kevin *et al* in the year 2008, the range of MIC of Telavancin against MRSA was from 0.06 - 1µg/ml.[17] It was a study based on Telavancin and its comparator agents in which Telavancin was reported to be the most active agent tested against MRSA. Both in the reported study and in ours all the isolates were inhibited by ≤1 µg/ml of Telavancin and the value of MIC_{50} was also the same i.e. 0.25µg/ml. However, MIC_{90} was found to be different i.e.0.5 µg/ml as reported by Kevin *et al* which in our study was found to be 0.38µg/ml.
Jansen *et al* in his comparative study in the year 2007 reported both MIC_{50} and MIC_{90}, to be 0.25µg/ml for MRSA strains, which was partly the same as the results in our study i.e. MIC_{50} was the same. MIC_{90} was however, different being 0.38µg/ml in our study which in their study was reported to be 0.25µg/ml.[18] Based on the MIC_{90}, Jansen reported Telavancin as the most active agent against MRSA. It was reported to be 8 and 16 times more active than linezolid and teicoplanin respectively, 4 times more active than vancomycin and twice as active as daptomycin. Similar results have been obtained in other studies.[19]
In another comparative study conducted by Rodrigo *et al* in the year 2011-2012, when tested against MRSA, Telavancin had MIC_{50} of 0.25µg/ml similar to our results whereas MIC_{90} was different being 0.5 µg/ml and all the isolates, similar to our study were found to be 100% susceptible, whereas the comparator agents like daptomycin (MIC50/90, 0.25/0.5 mg/L; 98.4% susceptible), and 2 to 4 fold more potent than vancomycin (MIC50/90, 1/1 mg/L; 100% susceptible) and linezolid (MIC50/90, 1/1 mg/L; 100% susceptible).[20]
Regarding the isolation of MRSA from admitted and outdoor patients, the maximum number of the isolates i.e. twenty nine (28.43 %) were received from the outpatient department of Military Hospital, Rawalpindi in our study, in contrast to which a study conducted in India by Sanjay Kumar showed that the maximum number of the MRSA isolates were from the indoor patients (84.8%).[20]
Still in another study conducted by Kaur *et al* in 2013, the same results were observed which were contrary to the findings in our study that maximum number of the isolates of MRSA were from the IPD patients (76), while in our study it was the OPD patients constituting the maximum number of the MRSA isolates.
The specimen wise distribution of the MRSA isolates showed that the maximum number was isolated from pus (i.e. 83.33%), followed by blood cultures (8.82%) and respiratory tract in our study, and similar results were seen in the study by Sanjay Kumar showing the maximum number of the isolates of MRSA being isolated from pus i.e. (61.4%) followed by blood cultures (15.9%) and then were the respiratory tract pathogens.[20]
In contrast to our study, Joshi *et al* in the year 2013 revealed that the majority of methicillin resistant *S. aureus* isolates were obtained from blood cultures and respiratory tract samples both being 44% followed by urine 41% and finally the pus samples constituting 36% of the total MRSA isolates, while in our study pus samples constituted the bulk of MRSA isolates.[21]
Without extensive and effective control measures, emergence and spread of VISA and VRSA may be the next stage in the global crisis of antimicrobial resistance. Keeping in mind the challenge our study has proven to be very useful in which although Telavancin has shown excellent in vitro activity against MRSA, but its clinical use will establish its efficacy.

Conclusion

Telavancin has excellent in vitro activity against MRSA.

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References

1. Zhang, K., J. Sparling, B. L. Chow, S. Elsayed, Z. Hussain, D. L. Church, D. B. Gregson, T. Louie, and J. M. Conly. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. J. Clin. Microbiol. 2004; 42:4947-4955.
2. Lowy, F. D. Staphylococcus aureus infections. N. Engl. J. Med. 1998; 339:520- 532.
3. Cosgrove, S. E., Qi, Y., Kaye, K. S., Harbath, S., Karchmer, A. W. and Carmeli, Y. The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect. Control Hosp. Epidemiol. 2005; 26:166–174.
4. Francis JS, et al. Severe Community-Onset Pneumonia in Healthy Adults Caused by Methicillin-Resistant Staphylococcus aureus Carrying the Panton-Valentine Leukocidin Genes. Clin Infec Dis. 2005; 40:1378-9.
5. Chang S, Dawn M, Hageman J C, Matthew L, Tenover C. Infection with vancomycin-resistant Staphylococcus aureus containing the van A resistance gene. N. Engl. J. Med. 2003; 348:1342-47.
6. Theravance Vibativ package insert. Theravance, South San Francisco, CA:http://www.vibativ.com. 2009.
7. Beauregard DA, Williams DH, et al. Dimerization and membrane anchors in extracellular targeting of vancomycin group antibiotics. Antimicrob Agents Chemother. 1995; 39: 781-5.
8. Higgins DL, Chang R, et al. Telavancin, a multifunctional lipoglycopeptide, disrupts both cell wall synthesis and cell membrane integrity in methicillin- resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2005; 49: 1127–34.
9. Clinical and Laboratory Standards Institute. M07-A8 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 8th ed Clinical and Laboratory Standards Institute, Wayne, PA. 2009.
10. Food and Drug Administration. FDA labelling information. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/ label/2009/022110s000lbl.pdf.
11. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant Staphylococcus aureus. Trends Microbiol. 2001; 9:486–493.
12. Sancak, B., S. Ercis, S. Menemenlioglu, D. Colakoglu, S and Hascelik, G. Methicillin-resistant Staphylococcus aureus heterogeneously resistant to vancomycin in a Turkish university hospital. J. Antimicrob. Chemother. 2005; 56:519–523.
13. Appelbaum, P.C. Reduced glycopeptide susceptibility in methicillin resistant Staphylococcus aureus (MRSA). Int. J. Antimicrobials. Agents 2007; 30:398-408.
14. Drew RH. Emerging options for treatment of invasive, multidrug resistant Staphylococcus aureus infections. Pharmacotherapy, 2007; 27:227–49.
15. Moellering RC Jr. Vancomycin: a 50-year reassessment. Clin Infect Dis, 42 (Suppl 1): 2006; S3–4.
16. Krause KM, Renelli M, Difuntorum S, Antimicrob. Agents Chemother. 2008; 52(7):2647.
17. Jansen WT, Verel A, et al. In vitro activity of telavancin against gram-positive clinical isolates recently obtained in Europe. Antimicrob Agents Chemother. 2007; 51: 3420–4.
18. King, A., I. Phillips, and K. Kaniga. Comparative in vitro activity of telavancin (TD-6424), a rapidly bactericidal, concentration-dependent anti-infective with multiple mechanisms of action against Gram-positive bacteria. J. Antimicrob. Chemother. 2004; 53:797-803.
19. Mendes R, Sader HS, Flamm RK, Farrell DJ, Jones RN Telavancin In Vitro Activity Against a Contemporary Collection of Gram-positive Pathogens Antimicrob Agents Chemother 2015;59(3)1811-1814
20. Mallick, S. K. and S. Basak. S. "MRSA—Too Many Hurdles to Overcome: A Study from Central India," Tropical Doc- tor, Vol. 40, No. 2, 2010, pp. 108-110. doi:10.1258/td.090440.
21. Joshi, S., Ray, P., Manchanda, V., Bajaj, J., Chitnis, D.S., Gautam, V., et al. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res. 2013; 137:363–69

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- **Nazish Babar:** Conception, Study Designing, Planning, B) Study Conduction, C) Interpretation, Analysis, Discussion,
- **Maria Mushtaq Gill:** Study Conduction, Interpretation, Analysis, Discussion,
- **Mehreen Gilani:** Conception, Study Designing, Planning, Interpretation, Analysis, Discussion
- **Javaid Usman:** Conception, Study Designing, Planning, Study Conduction,
- **Rubina Riaz:** Conception, Study Designing, Planning, Interpretation, Analysis, Discussion,
- **Hijab Shah:** Conception, Study Designing, Planning, Interpretation, Analysis, Discussion