In Vitro Activity of Tigecycline against Methicillin Resistant Staphylococcus aureus (MRSA) and Vancomycin resistant Enterococci (VRE) As Evaluated by Disc diffusion method and E-test

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Abstract

Introduction: Tigecycline is an antibiotic belonging to the glycyclcyclines class with in vitro activity against most gram positive bacteria, even multidrug resistant pathogens. It is considered to be a newer treatment option for emerging multidrug resistant pathogens.

Aims & Objectives: To evaluate the in vitro activity of Tigecycline against Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) isolated from various clinical specimens to compare with other antimicrobials.

Materials & Methods: A total of 75 multidrug resistant isolates of MRSA (60) and VRE (15) were tested for Tigecycline susceptibility by the E-test and disc diffusion methods.

Results: Tigecycline showed good microbiological activity against all the isolates of MRSA and VRE with 100% susceptibility.

Conclusion: Tigecycline was found to be highly effective against multidrug resistant MRSA & VRE. Therefore it is an alternative option for treatment of complicated skin & soft tissue and intra-abdominal infections caused by such multidrug resistant pathogens.

Key words: Tigecycline, multidrug resistance, in vitro susceptibility, MRSA and VRE
Introduction

Significant changes in causative organisms of nosocomial bacterial infections have been observed globally over the past 100 years. In the first half of the 20th century, Gram-positive cocci, particularly Staphylococcus aureus and streptococci, were of primary concern. By the end of the 1970s, Enterobacteriaceae and Pseudomonas aeruginosa had gained prominence as nosocomial pathogens; however along with Acinetobacter spp, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) had also emerged with increasing resistance to most antimicrobial agents.\(^1\)

Methicillin-resistant Staphylococcus aureus (MRSA) is now endemic in India. The incidence of Hospital acquired MRSA varies from 25% in western part of India\(^2\) to 50% in south India.\(^3\) Isolates of Community acquired MRSA (CA-MRSA) has also been indentified and increasingly reported from India.\(^4\)

In 1988, Uttley et al. were the first to report the isolation of vancomycin-resistant \textit{E. faecalis} and \textit{E. faecium} in England. Shortly after the first isolates of vancomycin-resistant enterococci (VRE) were reported by investigators in the United Kingdom and France, similar strains were detected in hospitals located in the eastern half of the United States. Subsequently, VRE have spread with unanticipated rapidity and are now encountered by hospitals in most parts of the world.\(^5\) Another problem with Vancomycin-resistant enterococci (VRE) is the lack of available antimicrobial therapy for VRE infections as most VRE are also resistant to drugs previously used to treat such diseases (e.g. aminoglycosides and ampicillin).\(^6\) Serious infection with vancomycin-resistant enterococci (VRE) usually occurs in patients with significantly compromised host defenses and serious co-morbidities, and this magnifies the importance of effective antimicrobial treatment.\(^7\)

Methicillin-resistant Staphylococcus aureus (MRSA) has been a source of serious infections in Hospitals and are frequently resistant to other antimicrobial classes, complicating treatment and reducing therapeutic options.\(^8\) In one multicenter study from Coimbatore, 63.6 % of MRSA proved to be multi-drug resistant.\(^9\) Various other studies from different parts of India had reported a higher percentage of multidrug resistant MRSA.\(^10,11\)

Vancomycin is the main antimicrobial agent available to treat such infections, but unfortunately decrease in vancomycin susceptibility of Staphylococcus aureus and isolation of vancomycin intermediately sensitive and vancomycin resistant Staphylococcus aureus (VISA & VRSA) have recently been reported from many countries.\(^12\) This emphasizes the urgent need for a new compound.

Tigecycline (GAR-936) is the 9-t-butylglycylamino derivative of minocycline, a new generation of tetracyclines called glycyclycles. These glycyclycles overcome tetracycline resistance due to both ribosomal protection and efflux determinants.\(^13,14\)

The aim of this study was to assess the in-vitro activity of tigecycline against Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) in comparison with other antimicrobial agents.
Materials & Methods

A total of 75 Gram positive isolates included in this study were isolated from patients attended to Dr. VPMC, a tertiary care teaching hospital in north Maharashtra. Among these 75 isolates, sixty were Methicillin-resistant Staphylococcus aureus (MRSA) and 15 were vancomycin-resistant enterococci (VRE), selected from an existing stock of organisms isolated retrospectively over 1 year period starting from Jan 2012. Only one isolate per patient was included for testing.

The source of these isolates included pus (22), blood (14), Respiratory samples (10), urine (11), Tissue (2), sterile body fluids (5) and other specimens (ear swabs, nasal swabs and skin swabs - 11). All the test strains were isolated and identified by conventional biochemical tests.15

Specifically for Staphylococcus aureus, screening for methicillin resistance was done by cefoxitin (30μg) disc method16 and confirmed by oxacillin screening agar method. The Staphylococcus aureus ATCC 25923 and ATCC 29213 were used as reference strains.

For enterococci, screening for Vancomycin resistance was done by the agar screen method on MHA, using 6μg/ml vancomycin. Vancomycin resistance was further confirmed by determining the minimum inhibitory concentration (MIC) of vancomycin by E test. Vancomycin sensitive strain E. faecalis ATCC 29212 was used as negative control and vancomycin resistant strain E. faecalis ATCC 51299 was used as positive control.16

Antimicrobial susceptibility testing was determined by the disc diffusion (DD) technique using different antimicrobial agents; penicillin G (10 U), ceftazidime (30 μg), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), co-trimoxazole (25 μg), tetracycline (30 μg), ciprofloxacin (5 μg), vancomycin (30 μg), linezolid (30 μg) and tigecycline (15 μg) (Hi-media, Mumbai) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI).16 The standard S. aureus strains ATCC 25923 and Enterococcus faecalis ATCC 51299 were used as reference strains for MRSA and VRE respectively.

Along with disc diffusion testing, tigecycline MIC was determined by E-test method according to manufacturer’s instructions. All antibiotic discs, media, reference strains and E-strips were procured from Hi-media Laboratories, Mumbai.

Interpretation of the Antimicrobial susceptibility testing was done as per CLSI criteria.16 Since there were no CLSI recommended interpretative criteria for tigecycline, the US FDA breakpoints: Staphylococcus aureus (susceptible when MIC ≤0.5 μg/ml and ≥19mm zone size) and Enterococci (susceptible when MIC ≤0.25 μg/ml and ≥19mm zone size) were used.

Results

Using the cut-off established by the US FDA in 2005 for Staphylococcus aureus and Enterococcus spp., it was observed that all MRSA and VRE isolates were susceptible to tigecycline by Disc Diffusion method and E-test method. All MRSA isolates were inhibited by a
concentration of $\leq 0.5 \, \mu g/ml$ of tigecycline and presented a zone of inhibition of $\geq 20$ mm around the disc of tigecycline.

The MIC$_{50}$ and MIC$_{90}$ values of tigecycline for the isolates of MRSA in our study were found to be $0.125 \, \mu g/ml$ and $0.38 \, \mu g/ml$ respectively, much below the US FDA cut offs for susceptibility. The isolates had a zone diameter varying from 20-27 mm around tigecycline-disc by disc diffusion method.

Of the 15 isolates of VRE, 12 were E. faceum and 3 were E. gallinarum. The MIC$_{50}$ and MIC$_{90}$ of these isolates were found to be respectively $0.094 \, \mu g/ml$ and $0.125 \, \mu g/ml$. All these isolates had tigecycline zone diameter ranging from 24-36 mm by disc diffusion method (Table 1).

The percentage of MRSA and VRE isolates minimum inhibitory concentration (MIC) of tigecycline obtained by the E-test is shown in Table 2.

The 63.3% of MRSA isolates were found to be multidrug resistant, showing total resistance to penicillin G and high resistance to ceftazidime (78.3%), ciprofloxacin (71.6%), co-trimoxazole (70%), gentamicin (75%), tetracycline (80%), and erythromycin (65.3%). The three most active agents in vitro against MRSA identified in this study were tigecycline, vancomycin and linezolid, with 100% susceptibility reported for each (Table 3).

**Discussion**

Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) are the important causes of nosocomial infections. Many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptides antibiotics such as vancomycin & teicoplanin.$^{17}$

The percentage of MDR strains among MRSA isolates is increasing and burden of such stains has ranged from 23.2% to 73% as reported by various studies.$^{9,11,18,19}$ In our study, we found that 38/60 (63.3%) isolates were multidrug resistant MRSA isolates.

Glycopeptides are generally the antibiotics of choice for the treatment of MRSA infections. However recent reports of vancomycin intermediate sensitive (VISA) and vancomycin resistant Staphylococcus aureus (VRSA)$^{12,20,21}$ emphasizes the need of development and testing of new agents such as tigecycline, for the appropriate treatment of serious infections caused by highly resistant pathogens.

Tigecycline has previously been shown to be highly active in vitro against MRSA and VRE. Behera et al$^{22}$ reported MIC$_{90}$ values of tigecycline against MRSA and VRE as $0.38 \, \mu g/ml$ and $0.094 \, \mu g/ml$ respectively with 100% susceptibility to tigecycline. Many other Indian and foreign studies reported MIC 90 of tigecycline ranging from $0.125 \, \mu g/ml$ to $0.5 \, \mu g/ml$ against MRSA with 98.9 to 100% susceptibility rate.$^{23-26}$ Our study showed similar results.
Recent comparative studies have also demonstrated the clinical efficacy of tigecycline in complicated skin and skin-structure infections (SSSIs) and complicated intra-abdominal infections (cIAIs). Tigecycline was the most active antimicrobial agent in one study by Bradford et al, with an MIC90 of 0.25 μg/ml reported for MRSA isolates collected from patients with either SSSIs or cIAIs.27

Several studies show that tigecycline is as active as the combination of vancomycin and aztreonam in skin and soft tissue infections (where it is necessary to cover the presence of MRSA and Gram-negative pathogens).28

The activity of tigecycline against staphylococci is completely unaltered by the presence of Methicillin or glycopeptides resistance genes and remains fully effective against enterococci, expressing one or more vancomycin resistance determinants. It is the most potent antimicrobial when tested against glycopeptides-intermediately resistant S. aureus.29

A recent in vitro study shows that tigecycline has in vitro activity against VRE and suggests that this drug may have an important role in the treatment of severely ill patients infected with VRE.30

Conclusion

Thus results of this study indicate that tigecycline has excellent in vitro activity against MRSA and VRE. This new antibiotic with the characteristics of broad spectrum, relatively low toxicity, and post-antibiotic effect is a promising antimicrobial agent. It may have an important role in the therapy of infections caused by multidrug resistant MRSA and VRE. Continued surveillance is necessary to generate local and epidemiological data on resistance profile of clinically important pathogens including MRSA and VRE.

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Conflict of Interest: None declared.

References


**Table 1:** MIC range and MIC for 50% and 90% of the organisms (MIC50 and MIC90, respectively) obtained by the E-test, and range, mean and S.D. of the diameter of the zone of inhibition obtained by the disk diffusion method for MRSA and VRE isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/mL)</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>MRSA</td>
<td>0.125</td>
<td>0.38</td>
</tr>
<tr>
<td>VRE</td>
<td>0.094</td>
<td>0.125</td>
</tr>
</tbody>
</table>

MIC, minimum inhibitory concentration; S.D., standard deviation; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

**Table 2:** The percentage of MRSA and VRE isolates minimum inhibitory concentration (MIC) of tigecycline obtained by the E-test

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC of tigecycline (µg/mL)</th>
<th>0.047</th>
<th>0.064</th>
<th>0.094</th>
<th>0.125</th>
<th>0.19</th>
<th>0.25</th>
<th>0.38</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (60)</td>
<td></td>
<td>2</td>
<td>7</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>VRE (15)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3:** Antibiotic susceptibility pattern of MRSA and VRE isolates to tigecycline along with other antimicrobials by Disc Diffusion (DD) method

<table>
<thead>
<tr>
<th>Name of Antibiotic</th>
<th>MRSA (60)</th>
<th>VRE (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>13</td>
<td>47</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>17</td>
<td>43</td>
</tr>
<tr>
<td>Erythromycin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>17/49</td>
<td>32/49</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>e</sup>-Erythromycin was not tested for urine samples.