

## Tigecycline susceptibility report from an Indian tertiary care hospital

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**Background & objectives:** Treatment of serious life threatening infections due to multi-drug resistant pathogens presents a difficult challenge due to the limited therapeutic options. Therefore, we studied the *in vitro* susceptibility of tigecycline, a new glycylicycline with promising broad spectrum of activity against Gram positive and Gram negative bacteria at a tertiary care hospital in north India.

**Methods:** A total of 75 multi-drug resistant isolates of methicillin resistant *Staphylococcus aureus* (21), vancomycin resistant enterococci (14), vancomycin resistant *Streptococcus* spp. (3), extended spectrum  $\beta$  lactamase producing Gram negative bacteria (11) and multi-resistant *Acinetobacter* spp. (26) were tested for tigecycline susceptibility by the E- test and disc diffusion methods. An additional 83 multi-resistant Gram negative clinical isolates were screened by disc diffusion method alone.

**Results:** All the isolates of MRSA, VRE, vancomycin resistant *Streptococcus* spp. and ESBL producing enteric bacteria were sensitive to tigecycline by the E-test and disc diffusion methods. However, only 42 per cent of *Acinetobacter* spp. were found to be sensitive to tigecycline by the E-test method.

**Interpretation & conclusions:** In conclusion, tigecycline was found to be highly effective against Gram-positive bacteria and Gram-negative members of *Enterobacteriaceae*, but a high prevalence of resistance in members of *Acinetobacter* spp. is worrisome.

**Key words** *Acinetobacter* spp. - India - multi-drug resistance - tigecycline

In the present era of multi-drug resistant organisms (MDRO), clinicians are facing an acute shortage of antibiotics with activity against the MDROs. Pathogens like methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) and extended spectrum  $\beta$  lactamase (ESBL) producing Gram-negative bacilli harbour genetic determinants, which render them resistant to most of the available antimicrobials. With the emergence and spread of carbapenem resistant and metallo-  $\beta$  lactamase (MBL) producing *Pseudomonas aeruginosa*

and *Acinetobacter* spp., the only viable treatment option remains the potentially toxic colistin/polymyxin B group of antibiotics<sup>1</sup>. Infections by these MDRO lead to prolonged hospitalization, increased mortality, morbidity and cost of treatment.

In this scenario, the development of a new class of antimicrobial agents, glycylicyclines, represented by tigecycline, a 9-t-butylglyclamide derivative of minocycline is a significant advancement<sup>2-5</sup>. Tigecycline has a spectrum of activity unparalleled by any other broad spectrum agent and includes MRSA,

VRE, penicillin resistant *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria gonorrhoeae*, ESBL producing Gram-negative bacteria, MDR *Acinetobacter* spp., anaerobes and rapid growing Mycobacterial species<sup>2,4,6</sup>. Moreover, tigecycline is not affected by most of the known mechanisms of resistance to tetracycline encountered in bacteria. Because of the promising microbiological, pharmacodynamic and pharmacokinetic profile against clinically important bacteria, tigecycline is being evaluated for empirical therapy of seriously ill patients<sup>4,7</sup>.

At our institute, a 2000 bedded, tertiary care and teaching hospital, the prevalence of antimicrobial resistance is extremely high across both Gram-positive and Gram-negative bacterial genera due to the immense antibiotic pressure<sup>8-10</sup>. In critical care units, carbapenems are being used as last resort treatment of MDR *P. aeruginosa* and *Acinetobacter* spp.<sup>8</sup>. Currently, MDR *Acinetobacter* spp. remains the most problematic pathogen in our hospital, especially in the ICUs. In view of the increasing resistance in both Gram-positive and Gram-negative pathogens in India and across the world, this study was conducted to evaluate the *in vitro* activity of tigecycline against a contemporary collection of multiple drug resistant clinical isolates.

### Material & Methods

**Bacterial isolates:** The study was conducted in two parts:

For the first part of the study, stocks of confirmed bacterial strains kept in the Bacteriology laboratory of All India Institute of Medical sciences (AIIMS), New Delhi were used. In this part, tigecycline susceptibility testing was done on confirmed isolates of VRE, vancomycin resistant *Streptococcus* spp., MRSA, ESBL-positive Gram-negative bacteria (GNB) and MDR *Acinetobacter* spp. Identification of these isolates was done using routine microbiological methods and VITEK 2 (BioMérieux, Hazelwood, France) system. In general, antimicrobial susceptibility testing of all these isolates was done by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>11</sup> and the VITEK-2 system. Specifically, for *S. aureus*, screening for methicillin resistance was done by the oxacillin agar screen method using 6 µg/ml oxacillin (Sigma, USA) as recommended by the CLSI<sup>11</sup>. All the isolates were also screened for methicillin resistance by the cefoxitin (30 µg) disc method<sup>12</sup>. The *S. aureus* ATCC 25923 (Hi

Media, Mumbai, India) and WHO-2<sup>13</sup> were used as negative and positive controls respectively for MRSA screening. Confirmation of methicillin resistance was done by demonstrating the presence of *mecA* gene by PCR using published primers<sup>14</sup>. For enterococci, screening for vancomycin resistance was done by the agar screen methods on Mueller-Hinton agar (MHA) (BBL™ BD, USA) using 6 µg/ml vancomycin (Hi-Media Laboratories, Mumbai, India)<sup>15</sup>. Vancomycin resistance was confirmed by determining the minimum inhibitory concentration (MIC) of vancomycin by E test (AB Biodisk, Sweden) and Vitek 2 system<sup>15</sup>. *Enterococcus faecalis* ATCC 29212 (Hi-Media, Mumbai, India) was used as control. All Gram negative isolates were screened for ESBL production by the disk potentiation test using ceftazidime (CAZ) and ceftazidime + clavulanic acid (CAZ+clav) disc (BBL™ BD, USA)<sup>11</sup>. ESBL E- test (CAZ/CAZ+clav) (AB Biodisk, Sweden) was also performed according to manufacturer's instructions to confirm the presence of clavulanic acid inhibitable ESBLs<sup>16</sup>. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls respectively for ESBL testing. Further, screening for MBL production was done in all isolates of *Acinetobacter* spp. by the imipenem- EDTA combined disk test. The test was performed according to published protocols<sup>17</sup>. Isolates with a positive EDTA-imipenem disc synergy test were subsequently evaluated by the MBL E-test strip containing a double sided seven-dilution range of imipenem (IP) (4 to 256 µg/ml) and imipenem (1 to 64 µg/ml) in combination with a fixed concentration of EDTA (320 µg/ml) (IPI)<sup>18</sup>. MIC ratio of IP/IPI of > 8 or > 3 log<sub>2</sub> dilutions was taken as MBL positive<sup>18</sup>.

**Tigecycline susceptibility testing:** In the first part of the study, tigecycline MIC was determined for all MRSA, VRE, ESBL producing GNB and MDR *Acinetobacter* spp. using the E-test (AB Biodisk, Sweden) method according to the manufacturer's instructions. For tigecycline, the MIC breakpoints used for susceptibility were taken as ≤ 0.5 µg/ml for *S. aureus*, ≤ 0.25 µg/ml for enterococci, and ≤ 2 µg/ml for Gram-negative bacteria, as approved by the FDA<sup>2,5,19</sup>. Disc diffusion susceptibility testing was also performed for all these isolates using tigecycline disks (15 µg; Oxoid Ltd, Basingstoke, Hants, UK). The interpretation of zone diameters for all Gram negative bacteria (including *Acinetobacter* spp.) was done using the US FDA tigecycline susceptible breakpoints listed

for *Enterobacteriaceae* (MIC  $\leq 2$   $\mu\text{g/ml}$ , and  $\geq 19$  mm zone size)<sup>5</sup>. Resistance was defined as MIC  $\geq 8$   $\mu\text{g/ml}$  and zone size  $\leq 14$  mm<sup>18</sup>. Interpretation of zone diameters of all Gram-positive bacteria was done using the US FDA tigecycline susceptible breakpoints listed for *S. aureus* (MIC  $\leq 0.5$   $\mu\text{g/ml}$  and  $\geq 19$  mm zone size) and *E. faecalis* (vancomycin susceptible only) (MIC  $\leq 0.25$   $\mu\text{g/ml}$  and  $\geq 19$  mm zone size)<sup>20</sup>.

For the second part of the study, consecutive clinical isolates of Gram-negative bacteria (*E. coli*, *Klebsiella* spp. and *Acinetobacter* spp.) obtained over a period of two months (July-August 2007) at the Microbiology laboratory of Trauma Centre, AIIMS, New Delhi, which were resistant to two or more of the most commonly used antimicrobial classes for the treatment of the indicated infection (MDR) were included. Antibiotic sensitivity patterns of these isolates were compared in order to exclude clonal origin of the clinical isolates. Only one isolate per patient was included for the study. Screening for tigecycline susceptibility in these isolates was done by the disc diffusion method alone, using tigecycline disks (15  $\mu\text{g}$ ; Oxoid Ltd., Basingstoke, Hants, UK).

### Results & Discussion

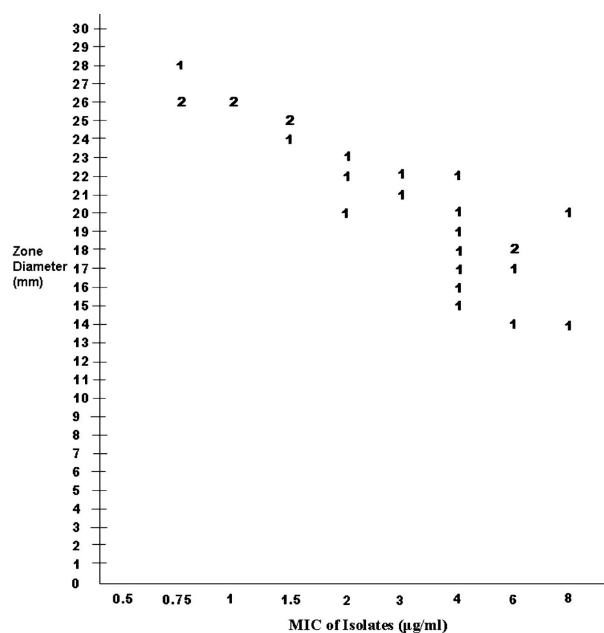
A total of 75 MDR clinical isolates were evaluated in the first part of the study. These included 21 confirmed isolates of MRSA, 14 of VRE (vancomycin MIC  $>256$   $\mu\text{g/ml}$ ), three of vancomycin resistant *Streptococcus uberis* (vancomycin MIC  $> 256$   $\mu\text{g/ml}$ ), 11 isolates of ESBL producing members of enterobacteriaceae (6 *K. pneumoniae* and 5 *E. coli*) and 26 of MDR *Acinetobacter* spp. Since tigecycline has no or limited activity against *Pseudomonas* and *Proteus* spp., these were not included in our study.

All the isolates of MRSA were *mecA* positive by PCR, showing a 310 base pair size band, using published primers<sup>14</sup>. All of them were found to be sensitive to tigecycline by the E-test method. The MIC<sub>50</sub> and MIC<sub>90</sub> of the isolates of MRSA in our study was found to be 0.25 and 0.38  $\mu\text{g/ml}$  respectively, much below the US FDA cut-offs for susceptibility. The isolates had a zone diameter of tigecycline by disc diffusion method varying from 23-30 mm. Of the 14 isolates of VRE, 13 were *E. faecium* and one was *E. gallinarum*. The MIC<sub>50</sub> and MIC<sub>90</sub> of these isolates was found to be respectively 0.064 and 0.094  $\mu\text{g/ml}$ . All these isolates had tigecycline zone diameters ranging from 24-38 mm by the disc diffusion method. The relatively low MICs of tigecycline for Gram-

positive cocci reflect their unexposed status to this new antimicrobial<sup>21</sup>.

All the 11 MDR *K. pneumoniae* and *E. coli* were found to be sensitive by the E-test and disc diffusion method. The MIC<sub>50</sub> and MIC<sub>90</sub> of these isolates were found to be 0.38 and 0.75  $\mu\text{g/ml}$  respectively. The zone diameter of tigecycline in these isolates varied from 22-29 mm. Thus, tigecycline was sensitive against all isolates of MDR Gram positive and Gram negative enteric bacteria by the E-test and disk diffusion method in the first part of this study. Isolates of ESBL producing members of *Enterobacteriaceae* having a low MIC for tigecycline signifies its potential utility in clinical infections due to these notorious MDR organisms.

A total of 26 *A. baumannii* isolates were included in the first part, all of which were MDR. Additionally, carbapenem resistance was seen in 20 (77%) isolates, of which 17 (85%) were found to be MBL producers. MBL production was confirmed by the imipenem-EDTA combined disc test and E-test methods, both of which showed 100 per cent concordance. Of the 26 isolates of *A. baumannii*, 11 (42%) had a tigecycline MIC of  $\leq 2$   $\mu\text{g/ml}$  by the E-test and were deemed susceptible according to US FDA breakpoints; two (7.6 %) had an MIC of 8  $\mu\text{g/ml}$  (resistant) and 13 (50%) had MICs ranging between 3-6  $\mu\text{g/ml}$  (intermediate). All the isolates having MIC  $\leq 2$   $\mu\text{g/ml}$  also had a zone diameter  $\geq 19$  mm (cut-off for susceptibility). However, of the two isolates showing MIC of 8  $\mu\text{g/ml}$ , only one was found to be resistant by the disk diffusion method (zone diameter 14 mm). Similarly, of the 13 isolates found to have MICs ranging from 3-6  $\mu\text{g/ml}$ , 7 (54%) were intermediate (15-18 mm), 5 (38%) were sensitive ( $\geq 19$  mm) and one was resistant ( $\leq 14$  mm) by the disc diffusion method. When a cut-off of  $\geq 13$  mm was considered as breakpoint for susceptibility as recommended by some authors<sup>22</sup>, all the isolates were found to be sensitive by disk diffusion method. The tigecycline zone diameter for *A. baumannii* by disc diffusion method ranged from 14-26 mm. Thus, we found discordance in the results of E-test and disc diffusion susceptibility testing methods in *A. baumannii*. With the interpretative zone diameters of  $> 19$  mm for sensitive, 17 (65 %) isolates were sensitive, 7 (27%) were intermediate and 2 (7.6 %) were resistant; with the interpretative zone diameter of  $\geq 13$  mm<sup>22</sup>, all the isolates of *A. baumannii* were sensitive by the disc diffusion method. We also found



**Fig.** Comparison of zone diameters and MICs of tigecycline for *Acinetobacter* spp. (N= 26). The numbers in the plot indicate the number of isolate.

a gradual reduction of tigecycline zone diameter as the MICs for the *Acinetobacter* isolates increased (Fig.).

In the second part of the study, 83 consecutive MDR Gram-negative clinical isolates were screened for tigecycline susceptibility by the disc diffusion method over a period of two months. Only one isolate per patient was included. The antibiogram of the isolates differed, signifying their non-clonal origin. These included a total of 25 isolates of ESBL producing *Enterobacteriaceae* (16 *E. coli* and 9 *Klebsiella* spp.) and 58 isolates of MDR *Acinetobacter* spp. All the isolates of *E. coli* and *Klebsiella* spp. were found to be sensitive to tigecycline by the disk diffusion method. However, of the 58 isolates of *Acinetobacter* spp., only 22 (38%) had a zone diameter  $\geq 19$  mm; 21 (36%) had a diameter between 15-18 mm and 15 (26%) had a zone diameter of  $\leq 14$  mm. When a zone diameter of  $\geq 13$  mm was considered as breakpoint for susceptibility<sup>22</sup>, 13 (22%) isolates were found to be resistant by the disc diffusion screening. We could not perform E-test on these isolates, therefore no association could be drawn about the MICs.

A high prevalence of tigecycline resistance amongst *Acinetobacter* spp. in our study is worrisome since the organism is not only totally unexposed to tigecycline but also to the tetracycline group of antibiotics in our hospital. Although tigecycline is

generally effective against MDR *Acinetobacter* spp.<sup>2,4</sup>, a recent report has found an unusually high rate of tigecycline resistance in these organisms<sup>23</sup>. These authors had also used the E-test method, which correlated 100 per cent with inhibition zone diameters. In our study, E test reported a higher rate of resistant and intermediate results as compared to disc diffusion method. This is again in contrast to another study, which found an unacceptably high minor error rate (false intermediate results) by the disc diffusion for *Acinetobacter* isolates with tigecycline MIC of 1 or 2  $\mu\text{g/ml}$ <sup>19</sup>. The tigecycline evaluation test (TEST), a global programme, is reported to have only 2 per cent of the 4247 *Acinetobacter* isolates showing tigecycline MIC  $\geq 2$   $\mu\text{g/ml}$ <sup>24</sup>. However, very limited clinical data are available to draw conclusion on the utility of tigecycline in treatment of MDR *Acinetobacter* infections.

More studies are needed to investigate the inter-method agreement of tigecycline *in vitro* susceptibility testing so that breakpoints and disc diffusion guidelines can be formulated for *Acinetobacter* spp. This will minimize interpretative difference amongst various studies and provide the true magnitude of resistance in this genus. Reporting of tigecycline resistance in *Acinetobacter* from two geographically distinct locations may have important therapeutic implications where it has become a last resort antimicrobial. Since tigecycline has a long terminal half-life and a large volume of distribution, it can be used as a life saving antimicrobial in polymicrobial infections due to Gram-positive and enteric Gram-negative bacteria<sup>7</sup>. However, its use needs to be strictly monitored to prevent development and dissemination of resistance against this one of the last available antimicrobial molecules.

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Conflicts of interest - None

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