

AN IN-VITRO STUDY OF TIGECYCLINE SUSCEPTIBILITY AMONG MULTIDRUG RESISTANT BACTERIA IN A TERTIARY CARE HOSPITAL

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This article is available online at www.ssjournals.com

ABSTRACT

Background & objectives: Emergence and spread of antibiotic resistance among bacteria pose a challenge to clinicians due to limited therapeutic options available. Multi drug resistant (MDR) bacteria are known to cause life threatening infections in intensive care settings. Tigecycline is a newer glycycline antibiotic, considered as therapeutic option for MDR gram positive and gram negative bacteria. Aim of this study was to evaluate in-vitro susceptibility of tigecycline in MDR bacteria.

Methods: We studied the in-vitro activity of tigecycline in 100 MDR bacteria isolated from blood, skin, soft tissue and surgical site infections, urine, sputum, bronchoalveolar lavage, CSF and other body fluids of patients admitted to a tertiary care hospital in South India. MDR bacteria tested for tigecycline susceptibility by disc diffusion and E-test included: Methicillin resistant *S.aureus* (MRSA) (35), extended spectrum β lactamase (ESBL) producing Enterobacteriaceae (36), *P.aeruginosa* (15) and *Acinetobacter spp.* (14).

Results: Tigecycline was found effective against all isolates of MRSA ($MIC_{90} \leq 0.25\mu\text{g}/\text{ml}$). All ESBL producing *E.coli*, *Klebsiella* and *Enterobacter* isolates were susceptible ($MIC_{90} \leq 1.0\mu\text{g}/\text{ml}$). However MDR isolates of *Acinetobacter spp.* showed reduced susceptibility to tigecycline (64.28% sensitive) with MIC_{90} of $2.0\mu\text{g}/\text{ml}$. 86.66% *P.aeruginosa* isolates were resistant to tigecycline.

Interpretation & conclusions: This study shows that tigecycline can be a useful reserve antibiotic against MDR MRSA and ESBL producing Enterobacteriaceae, but a higher prevalence of resistance is seen among members of *Acinetobacter spp.* and *P.aeruginosa*. Clinicians should however look out for adverse effects associated with tigecycline such as acute pancreatitis, severe nausea, vomiting and septic shock.

Keywords: Tigecycline, MRSA, *Acinetobacter spp.*, multi drug resistance, India.

1. Introduction:

Due to the emergence and spread of multidrug resistant (MDR) pathogens in health care settings clinicians are facing an acute shortage of antibiotics with broad spectrum activity that can be suitably used in initial empiric therapy¹. Resistance to currently available antibiotics is increasing at an alarming rate even among bacteria isolated from the community. MDR pathogens like methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *enterococci* (VRE), extended spectrum β lactamase (ESBL) and metallo β -lactamase (MBL) producing non-fermenting gram negative bacilli are known to harbour genetic elements that render them resistant to many antibiotics. Carbapenems were considered to be antibiotic of choice in the treatment of life threatening infections caused by MDR Enterobacteriaceae and non-fermenters such as *P.aeruginosa* and *Acinetobacter spp.*. However in recent years due to the emergence and spread of carbapenem resistant MBL

producing non-fermenters the only treatment option for these MDR pathogens is the potentially toxic Colistin/Polymyxin B group of antibiotics². Due to the alarming spread of these MDR bacteria both in the hospital and community settings there is a need for the development and evaluation of new broad spectrum antimicrobial agents.

With this scenario the development of tigecycline, a broad spectrum glycycline is a useful advancement in the treatment of MDR pathogens. Tigecycline (9-t-butylglyclamide minocycline) is a semi-synthetic glycycline with broad spectrum activity against clinically relevant MDR organisms like MRSA, *penicillin-resistant Streptococcus pneumoniae*, *beta hemolytic streptococci*, VRE, ESBL producing gram negative bacteria, MDR *P.aeruginosa*, *Acinetobacter spp.*, and anaerobes. It exerts its bacterostatic effect by binding to a single high affinity intracellular site of the bacterial 30S ribosome^{3, 4, 5}. Tigecycline is not affected by the common

mechanisms of bacterial resistance to tetracycline. However it has limited activity against *P.aeruginosa* and reduced activity against *Proteus mirabilis*. As it has an impressive microbiological, pharmacodynamic and pharmacokinetic profile tigecycline is being evaluated for empirical treatment of serious infections caused by MDR pathogens^{2,6}. Tigecycline was approved in 2005 by the U.S.Food and Drug Administration (FDA) and in 2006 by the European Medicines Agency for the treatment of complicated skin and skin structure infections and complicated intra-abdominal infections. In this study we evaluated tigecycline susceptibility among multidrug resistant bacteria isolated in a tertiary care hospital.

2. Material

MDR bacteria included in this study were isolated from patients admitted to Yenepoya medical college hospital, a tertiary care teaching hospital in Karnataka, south India. These MDR strains were isolated from blood stream infections (BSI) (9), skin and soft tissue infections (SSTI) (37), urinary tract infection (29) sputum (17) and body fluids (8). Initial isolation and identification of these bacteria was carried out according to standard recommended procedures⁷. MDR bacteria tested are: MRSA (35), ESBL producing gram negative bacilli of family Enterobacteriaceae (*E.coli*, *Klebsiella* and *Enterobacter spp*) (36), *Acinetobacter spp*. (14) and *P.aeruginosa* (15). Only one isolate per patient was included in the study.

2.1. Method: Tigecycline susceptibility screening was initially done by disc diffusion method (15µg tigecycline disc HiMedia Mumbai). Tigecycline MIC was determined using the E-test (AB Biodisk, Sweden) according to the manufacturer's instructions and Clinical and Laboratory Standards Institute (CLSI) guidelines⁸. The MIC breakpoints for Tigecycline were taken as ≤ 0.5 µg/ml for *S. aureus* and ≤ 2 µg/ml for gram negative bacteria, as mentioned in CLSI 2008 guidelines.

All the MRSA isolates were screened for methicillin resistance using 30 µg cefoxitin disc and 1 µg oxacillin disc (HiMedia, Mumbai).⁹ *S. aureus* ATCC 25923 and MRSA *S.aureus* ATCC 43300 were used as negative and positive controls for MRSA detection respectively. Confirmation of methicillin resistance was done by demonstrating the

presence of *mecA* gene (amplified product of 604 bp) by PCR¹⁰.

All Gram negative bacilli were screened for ESBL production by disk potentiation test using ceftazidime (CAZ) and ceftazidime + clavulanic acid (CAZ+clav) disc according to CLSI guidelines⁸. *E.coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls respectively for testing ESBL production. Isolates of *P.aeruginosa* and *Acinetobacter spp*. were screened for MBL production using imipenem-EDTA combined disk test¹¹.

Interpretation of tigecycline sensitivity for gram negative bacteria was done using the tigecycline susceptibility breakpoints listed in CLSI guidelines for Enterobacteriaceae. Sensitive isolates were those with MIC ≤ 2 µg/ml, and ≥ 19 mm zone size and resistance was defined as MIC ≥ 8 µg/ml and zone size ≤ 14 mm. For *S. aureus* MIC ≤ 0.5 µg/ml and ≥ 19 mm zone size was considered sensitive⁸.

3. Results: (Table 1)

100 MDR clinical bacterial isolates were included in this study. These are MRSA (35), ESBL producing gram negative bacilli of family Enterobacteriaceae (*E.coli*, *Klebsiella* and *Enterobacter spp*) (36), *Acinetobacter spp*. (14) and *P.aeruginosa* (15). All MRSA isolates showed the presence of *mecA* gene by PCR and were sensitive to tigecycline by E-test method. The MIC₅₀ and MIC₉₀ for MRSA strains in this study was 0.12 and 0.25 µg/ml respectively. The zone of inhibition diameter of MRSA isolates for tigecycline disc ranged from 22-30 mm. All the ESBL producing members of family Enterobacteriaceae were sensitive to tigecycline by E-test and disc diffusion method. MIC₉₀ of *E.coli*, *Klebsiella spp*. and *Enterobacter spp*. was 0.19µg/ml, 1µg/ml and 0.5µg/ml respectively. Diameter of the zone of inhibition for tigecycline in these isolates ranged from 23-30 mm. Thus, tigecycline was sensitive against all isolates of MDR *Staphylococci* and Enterobacteriaceae by disk diffusion and E-test methods.

Out of the 14 MDR *Acinetobacter spp*, 11 were sensitive to tigecycline by disc diffusion test but only 9 (64.28%) were sensitive by E-test. 3/14 isolates had MIC values of ≥ 8 µg/ml i.e. in the resistant range. and 2/14 had MIC values in the intermediate range (between 3-6µg/ml). Of the 3 isolates with MIC of ≥ 8 µg/ml, only two were resistant by the disk diffusion method with a zone diameter ≤ 14

mm. Out of the 2 isolates with MIC values in the intermediate range, only one was resistant by disc diffusion (zone diameter 16 mm). All the 14 isolates of *Acinetobacter spp.* were resistant to carbapenems and 13/14 (92.8%) were MBL producers by IMP-EDTA double disc synergy test. 9/14 (64.28%) of *Acinetobacter* isolates had a tigecycline MIC of ≤ 2 $\mu\text{g/ml}$ by the E-test and zone size of ≥ 19 mm by disc diffusion test and were considered susceptible according to CLSI guidelines. Thus in case of *Acinetobacter spp.* discordance between the E-test and disc diffusion test results was evident. 2/15 (13.34%) isolates of *P.aeruginosa* were sensitive to tigecycline by disc diffusion and E test methods.

4. DISCUSSION:

Tigecycline has emerged as a useful alternative antibiotic in the treatment of intra-abdominal and skin and soft tissue infections caused by MDR gram negative bacilli and MRSA. This is further supported by the low MIC₉₀ values of tigecycline in these isolates. Tigecycline is active against members of family Enterobacteriaceae irrespective of their ESBL production status.¹² In this study all the isolates of *E.coli*, *Klebsiella* and *Enterobacter spp.* tested had tigecycline MIC₉₀ values $< 1\mu\text{g/ml}$. Conventionally tigecycline is thought to be effective against MDR non-fermentative GNB such as *Acinetobacter spp.*¹³, however studies from India do not completely reflect this finding. In a study by Behra B *et al* (2009)¹², 58% of *Acinetobacter spp.* tested were resistant to tigecycline by E-test. A study by Manoharan A *et al* (2010) reported that 88% of *Acinetobacter spp.* tested were sensitive to tigecycline¹⁴. In our study 9/14 (64.28%) MDR isolates of *Acinetobacter spp.* tested were sensitive to this antibiotic. However discordance between results of disc diffusion and E test were evident in this study. Similar discordance has been observed and reported by Behra *et al*¹² and Pillar *et al.*¹⁶. More in-vitro tigecycline studies on a larger number of *Acinetobacter* isolates are required to confirm the agreement between disc diffusion and MIC testing, so that MIC breakpoints and disc diffusion guidelines can be formulated for *Acinetobacter spp.*. Resistance to tigecycline among *P.aeruginosa* isolates in this study was 86.6%. This high incidence of resistance among *Pseudomonas* could be attributed to the efflux pump mechanism¹⁶. Thus even though

tigecycline monotherapy is not suitable for the treatment of infections caused by *Pseudomonas* its role as part of a combination regimen including a fluoroquinolone or aminoglycoside to which the isolate is sensitive needs to be evaluated. In case of MRSA tigecycline activity against *S.aureus* is unaffected by the presence of methicillin resistance genes. Studies across India have reported MIC₉₀ for MRSA isolates between 0.19 -0.38 $\mu\text{g/ml}$ ^{12, 14}. MIC₉₀ for MRSA in our study was 0.25 $\mu\text{g/ml}$. A study by Pillar C M *et al* reported 99.6% tigecycline sensitivity among MRSA with MIC₉₀ value of 0.25 $\mu\text{g/ml}$, which is similar to findings of this study.

Tigecycline being a recently introduced antibiotic for use in nosocomial infections where MDR pathogens are suspected, constant monitoring of its sensitivity patterns is of great importance. Since tigecycline has a long half-life it can be conveniently administered every 12h. As it also has a large volume of distribution, it does not require dose adjustments in patients with impaired renal function. Due to these properties it can be used to treat severe poly-microbial infections caused by gram positive and gram negative bacteria in intensive care settings⁶. However there are a few reports of adverse effects associated with tigecycline such as severe nausea, vomiting¹⁷ and tigecycline induced acute pancreatitis.¹⁸ Use of tigecycline needs to be monitored routinely to track the development and dissemination of resistance. It should be used as a reserve antibiotic to treat life threatening infections and when MDR bacteria are suspected. The present study shows that tigecycline is a useful antimicrobial agent that can be used against MDR ESBL and MBL producing gram negative bacilli and MRSA causing intra-abdominal, blood stream and skin and soft tissue infections. However continuous local monitoring to look for the development of resistance against this antibiotic is essential. Potential of this antibiotic to be used as part of combination therapy needs to be evaluated in-vitro and clinically.

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Table 1: In vitro activity of Tigecycline against MDR clinical bacterial isolates

Organism	No. of isolates	MIC(μ g/ml)		Disc diffusion (%)		
		MIC ₅₀	MIC ₉₀	S	I	R
<i>S. aureus (MRSA)</i>	35	0.12	0.25	100	-	-
<i>E. coli</i>	19	0.12	0.19	100	-	-
<i>K. pneumoniae</i>	11	0.25	1	100	-	-
<i>Enterobacter spp.</i>	6	0.25	0.50	100	-	-
<i>Acinetobacter spp.</i>	14	1	2	64.28	-	35.72
<i>P.aeruginosa</i>	15	16	48	13.34		86.66