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Original Research Article

In Vitro Activity of Tigecycline against Multidrug Resistant Gram Negative Bacilli as Evaluated by Disc Diffusion Method and E-Test

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ABSTRACT

Introduction: Tigecycline is an antibiotic belonging to the glycylcyclines class with *in vitro* activity against most gram negative bacteria, even multidrug resistant pathogens. It is considered to be a newer treatment option for emerging multidrug resistant pathogens.
Objectives: To evaluate the *in vitro* activity of tigecycline against Multidrug resistant gram negative bacteria isolated from various clinical specimens to compare with other antimicrobials.
Materials & methods: A total of 150 multidrug resistant isolates of Enterobacteriaceae (113) and Acinetobacter spp (37) were tested for tigecycline susceptibility by the E-test and disc diffusion method.
Results: Tigecycline showed good microbiological activity against all the isolates of multidrug resistant gram negative bacteria with 100% susceptibility in E. coli & Enterobacter species, 94% in Klebsiella species and 81.08% in Acinetobacter spp. isolates.
Conclusion: Tigecycline was found to be highly effective against multidrug resistant gram negative bacteria. 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 (MDR), *in vitro* susceptibility, ESBL, MBL

INTRODUCTION

The rates of antimicrobial drug resistance and particularly of multiple drug resistance are increasing among gram negative organisms, thus posing a difficult challenge to treat such infections. [(1)] Multi drug resistance in clinically important organisms particularly pathogens of family that produce β -lactamases with a broad profile of substrate activity such as extended-spectrum β-lactamases (ESBLs), AmpC β -lactamases, as well as carbapenemases, including metallo β-lactamases (MBLs) and non-fermentative gram negative bacilli (including Acinetobacter spp & Pseudomonas spp) have led to the limited therapeutic options,

resulting in increased morbidity and mortality. [2, 3]

Patient will respond to antibiotic if the pathogen is susceptible to the chosen antibiotic; however today situation is so worrisome that no agents available that are fully active against all the common pathogens. The key to antimicrobial development has been to design agents that elude the main bacterial resistance mechanisms. One such agent is Tigecycline, which is chemically the 9-t-butylglycylamido derivative of minocycline, is a member of a novel class of antibiotics, the glycylcyclines. Like the tetracyclines, tigecycline binds to the 30s subunit of bacterial ribosomes and inhibits protein synthesis by preventing the incorporation of aminoacid residues into elongating peptide chains. [4, 5]

Regarding Enterobacteriaceae, tigecycline has shown to evade common mechanisms of acquired tetracycline resistance, such as those conferred by efflux pumps encoded by the (A-D) resistance determinants and ribosomal protective mechanisms.[6] However it has been reported that tigecycline showed only bacteriostatic activity against bacterial isolates of Acinetobacter species.[7,8] Nevertheless, tigecycline clearly displays inhibitory activity against Acinetobacter spp.[9-12] and has been utilized for therapy against MDR strains despite the lack of US FDA approved clinical indication & interpretative criteria for in vitro susceptibility testing. [13]

The drug is not significantly active against Pseudomonas aeruginosa & Proteeae as it carry inherently encoded resistance-nodulation-division (RND) efflux pumps that confer decreased sensitivity. [14, 15]

The drug was approved for use by the US Food and Drug Administration (FDA) in June 2005 and by European medicine agency in April 2006 for empiric monotherapy of nosocomial and acquired intra-abdominal infection (IAI) and skin and soft tissue infections. Recently in 2009, the US Food and Drug Administration (FDA) approved tigecycline for community-acquired pneumonia. [16]

Therefore considering increasing rate of MDR gram negative pathogens, we evaluated the in-vitro activity of tigecycline against multiple-drug resistant E. coli, Klebsiella, Enterobacter and Acinetobacter spp. and compared its activity against other commonly used antibiotics.

MATERIALS & METHODS

A total of 150 Gram negative isolates included in this study were isolated from patients attended to a tertiary care teaching hospital in North Maharashtra. Among these 150 isolates, 29 were E. coli spp, 50 were Klebsiella spp, 34 were Enterobacter spp and 37 were Acinetobacter spp. All isolates were selected from an existing stock of organisms isolated retrospectively over 2 year period starting from January 2011. Only one isolate per patient was included for testing.

All the test strains were isolated and identified by conventional biochemical tests. [17]

Antimicrobial susceptibility testing was done by Kirby-baur disc diffusion method as per the Clinical and Laboratory Standard Institute (CLSI) guidelines [18] using the Mueller Hinton agar and antimicrobial discs. The following antimicrobial agents (µg) were used.

Amikacin (30 µg), amoxicillin/ clavulanic acid (20/10µg), aztreonam (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), gentamicin(10 µg), imipenem (10µg), meropenem (10µg), piperacillin/tazobactam(100/10µg), trimethoprim-sulphamethoxazole (25µg) and tigecycline (15 µg).

The presence of ESBL in isolates of Enterobacteriaceae was screened by double

disc approximation test using ceftazidime and ceftazidime-clavulanic acid discs according to CLSI guidelines [18] and confirmed by E test. E.coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were used as negative and positive controls respectively for testing ESBL production.

Isolates of Acinetobacter species were screened for MBL production using imipenem and imipenem-EDTA combined disc diffusion method [19] and confirmed by E-test.

In this study, isolates were defined as multi-drug resistant when they demonstrated diminished susceptibility to ≥ 2 of drug classes tested in susceptibility testing panel. [20]

Tigecycline susceptibility screening was initially done by disc diffusion method using tigecycline disc (15µg). Tigecycline MIC was determined using the E-test according to manufacturer's instructions & CLSI guidelines. [18] All the antibiotic discs, media, E strips and ATCC strains were supplied by Himedia laboratories, Mumbai.

Interpretation of the Antimicrobial susceptibility testing was done as per CLSI criteria16. Since there were no CLSI recommended interpretative criteria for tigecycline, the US FDA breakpoints: Enterobacteriaceae (susceptible when MIC ≤ 2 µg/ml and resistant ≥ 8 µg/ml) were used.

RESULTS

A total of 150 MDR gram negative isolates were evaluated in this study. These included 113 of Enterobacteriaceae and 37 of Acinetobacter spp. The source of these isolates included pus (51), blood (9), Respiratory samples (29), urine (27), sterile body fluids (8), wound swabs (21) and other specimens (ear swabs, skin swabs - 5).

Since tigecycline has no or limited activity against Pseudomonas and Proteus species, [21] these were not included in this study.

Ninety six percent of multidrug resistant Enterobacteriaceae strains were positive for ESBL production and 67.56% of multidrug resistant Acinetobacter spp were positive for MBL production. The complete antibiotic susceptibility profile of the tested organism is given in Table-1.

Table 1: Antibiotic susceptibility for MDR GNB.

Name of Antibiotic	Enterobacteriaceae (113) {E. coli (29), Klebsiella (50), Enterobacter (34)}			Acinetobacter (37)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Amikacin	71	19	23	15	00	22
Amoxicillin-clav	5	7	101	12	07	18
Aztreonam	5	6	102	3	7	27
Ceftazidime	00	07	106	4	0	33
Cefepime	42	07	64	5	00	32
Ciprofloxacin	11	08	94	1	2	34
Gentamicin	34	02	97	9	0	28
Imipenem	108	01	04	22	01	14
Meropenem	108	03	02	25	00	12
Piperacillin-tazobactam	57	25	31	17	05	15
Tigecyclin	113	00	00	31	02	04
Trimethoprim-sulfamethoxazole	15	02	96	05	00	32

Table 2 shows the activity of tigecycline against MDR Gram negative isolates tested by E test method.

Organism (150)	Susceptible (%)	Intermediate (%)	Resistant (%)
E.coli (29)	29 (100)	-	-
Klebsiella spp (50)	47 (94)	2 (4)	1(2)
Enterobacter spp (34)	34 (100)	-	-
Acinetobacter spp (37)	30 (81.08)	2 (5.4)	5 (13.5)

Table 3- Distribution of tigecycline MICs against MDR gram negative isolates.

Organism (No.)	MIC50	MIC90	MIC range
E. coli (29)	0.25	0.50	0.047-6
Klebsiella (50)	0.50	2	0.25-16
Enterobacter (34)	0.25	0.50	0.047-8
Acinetobacter (37)	1	4	0.25-16

Thus when evaluated by disk-diffusion method, tigecycline was 100% active against isolates of E. coli, Klebsiella species and Enterobacter species. It was also active against 86.5% of Acinetobacter species.

Activity of tigecycline against MDR gram negative isolated tested by E-test is shown in Table-2 and MIC values are given in Table-3.

There are currently no interpretative MIC breakpoints available from the CLSI for tigecycline. Based on breakpoints recommended by US Food and Drug Administration for Enterobacteriaceae (susceptible ≤ 2mg/l, resistant ≥ 8mg/l), tigecycline was found to be 100% active against E. coli and Enterobacter spp. It was also active against 94% of Klebsiella spp.

At present, there are no interpretative breakpoints available for Acinetobacter species. If the same interpretative criteria for Enterobacteriaceae are arbitrarily applied, 81.08 % of the tested Acinetobacter species were susceptible.

The MIC 50 & MIC 90 for E. coli and Enterobacter species in this study was 0.25 and 0.5 µg/ml. Diameter of the zone of inhibition for tigecycline in these isolates ranged between 23-30 mm and MIC range was 0.047- 6 µg/ml for E. coli and 0.047-8 µg/ml for Enterobacter species.

MIC 50 & MIC 90 for MDR Klebsiella species was 0.50µg/ml & 2µg/ml respectively. Diameter of the zone of inhibition for tigecycline in these isolates ranged between 17-30 mm, and MIC range was 0.25-16 µg/ml for Klebsiella spp.

MIC 50 & MIC 90 for MDR Acinetobacter species was 0.25µg/ml & 16µg/ml respectively. Four out of 37 isolates had MIC values of ≥ 8 µg/ml i.e. in resistant range and all four showed ≤ 19 mm zone diameter by disc diffusion method i.e. resistant . Five isolates had MIC values in the intermediate range (3-8 µg/ml). Out of five isolates with MIC values of intermediate range, only two were resistant by disc diffusion method (≤ 19 mm).

DISCUSSION

Tigecycline has broad spectrum in-vitro activity against gram positive, gram negative and anaerobes. In addition, tigecycline demonstrates in-vitro activity against MRSA, VRE & ESBL producing pathogens. Also tigecycline has promising microbiological, pharmacodynamics & pharmacokinetic profile, therefore it is considered as a good alternative to treat infections due to multidrug resistant organisms. [(22)]

In present study, all the multidrug resistant E. coli and Enterobacter species isolates were found to be sensitive to tigecycline. MIC 50 & MIC 90 values of tigecycline were 0.25µg/ml and 0.50 µg/ml, which correlates with findings of previous Indian studies[(23-25)] and foreign studies.[(26-28)]

The susceptibility to tigecycline of Klebsiella species in present study was 94%, which correlates with findings in studies done by Souli M et al [(26)] who reported 92.6% and by Ralf et al [(29)] who reported 92.5% cumulative susceptibility rate of tigecycline in ESBL Klebsiella species. In the systematic review by Theodros Kelesidis, after evaluating 23 studies it was found that cumulative susceptibility rate to tigecycline of multidrug resistant Klebsiella species was 91.2% for 2627 isolates. [(21)]

In this study, MIC50 &MIC90 values of tigecycline against Acinetobacter species determined by E-test were 1 µg/ml & 4 µg/ml respectively, and among 37 isolates tested, susceptibility to tigecycline was 81.08%.

In another study from India, 70.6% of MDR Acinetobacter spp were susceptible to tigecycline, [(23)] whereas Manoharan et al

[24] reported a low rate of susceptibility (42%) to tigecycline among multidrug resistant Acinetobacter spp. Various authors have reported a resistance rate to tigecycline varying from being nonexistent to 66% in Acinetobacter spp. A recent study from PGI, Chandigarh, India [30] reported 85.8% and 36% susceptibility to tigecycline among MDR Acinetobacter spp and carbapenem resistant MDR Acinetobacter spp isolates respectively. They reported very high such as 6 µg/ml &32 µg/ml of MIC50 and MIC90 values of tigecycline against Acinetobacter spp. respectively. We too found higher MIC50 and MIC90 values of tigecycline like 1 µg/ml &4 µg/ml respectively against Acinetobacter spp. Other studies from Singapore [27] & Thiland [31] also reported found higher MIC50 and MIC90 values of tigecycline against Acinetobacter spp.

In India, infections caused by Acb complex pose a therapeutic challenge owing to their multidrug resistance. [32] Many studies reported most of Acinetobacter isolates showed complete or high resistance to multiple drugs including Carbapenems. Neelam Taneja et al [30] reported that 41.5% of Acinetobacter spp isolated from complicated UTI were MDR and showed high resistance to cefotaxime (74.1%), gentamicin (79.5%), amikacin (73.2%), ciprofloxacin (72.8%), piperacillin-tazobactam (31.7%) and imipenem (25.4%).

In another study, [33] Acinetobacter spp isolated from blood stream infections showed very low susceptibility to many drugs like ceftazidime (44.6%), piperacillin-tazobactam (32.1%), amikacin (46.4%) and ciprofloxacin (48.2%). Whereas Karthika et al [34] reported that most of active isolates showed complete or high resistance to imipenem (100%), meropenem(89%), amikacin(80%), cefotaxime(89%) and ciprofloxacin (72%). We too found high resistance by Acinetobacter spp to multiple drugs similar to these studies.

Few antimicrobial agents remain that are active against a wide range of organisms. For gram negative organisms, carbapenems & polymyxins are highly active. However in present study, high resistance has been reported for many antimicrobials including carbapenems. In another study by Mezzatesta et al [35] from Italy had reported 90% of the isolates from their hospital to be resistant to first line drugs, with imipenem resistance being 50%.

CONCLUSION

Thus tigecycline, with its ability to circumvent the common resistance mechanisms and its adequate micro-biological activity against gram negative organisms, may make a welcome alternative for the treatment of multidrug resistant gram negative organisms.

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