In Vitro Antimicrobial Activity of Tigecycline.

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ABSTRACT

Introduction: Tigecycline is a novel glycylcycline derivative of the tetracycline with activity against a wide range of organisms including Methicillin resistant *Staphylococcus aureus* ,Vancomycin resistant *Enterococcus Spp* , Extended spectrum beta lactamase producing *(Escherichia coli , Klebsiella pneumonia)* and *Acinetobacter species*. The aim of the study was to assess effectiveness of the drug against methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), ESBL producers and carbapenem resistant *Acinetobacter baumannii* and to compare the efficacy of different methods of antimicrobial susceptibility testing for Tigecycline.

Methods: A total of 250 clinical isolates were processed and identified by conventional methods. In all the 250 isolates, antimicrobial susceptibility was carried out by disc diffusion method , Minimum inhibitory test by agar dilution method (MIC) and in 30 isolates of A baumannii MIC was also done by E test.

Results: Out of 250 isolates, 236 isolates were sensitive to tigecycline by agar dilution method while only 159 were sensitive by disk diffusion method.

Conclusions: Marked discordance was observed between the results of two different methods (DDT & Agar dilution method) for *E. coli*, *Klebsiella spp* and *A baumannii,* where significant number of isolates were resistant to tigecycline by DDT as compared to AD method. But results of MIC by agar dilution method & E test were in concordance for A. baumannii.

Keywords: gram positive; gram negative; in vitro susceptibility testing; tigecycline.

INTRODUCTION

In the present era of multidrug resistant organisms, the clinicians are facing an acute shortage of an effective broad spectrum agent for use. Moreover, treatment of life threatening infections due to multi drug resistant pathogens poses a difficult challenge to the clinician due to very limited efficient options.

Therefore research for newer tetracycline compounds with suitable modification of the chemical structure, lead to the development of tigecycline[1,2] a new glycylcycline compound with effective against important clinical pathogens, namely methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), penicillin resistant *Streptococcus pneumoniae*, as well as Gram negative bacilli producing extended spectrum beta lactamases (ESBLs) and also in the treatment of serious skin problems and intra abdominal bacterial infections in hospitals [3,4] thus offering renewed hope to the clinicians.

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Keeping this in mind, the present study was undertaken to determine the in vitro activity of tigecycline against commonly isolated bacterial pathogens in a clinical setting of a tertiary care centre.

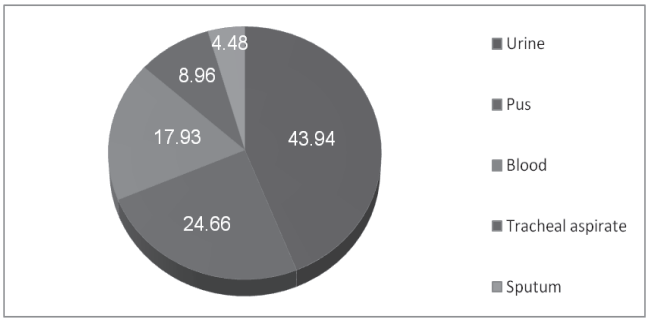
METHODS

The present study was undertaken in the department of Microbiology at a tertiary care hospital from May 2009 to May 2011. Total of 250 isolates from urine, blood, pus, sputum and tracheal aspirate were processed and identified by conventional methods. Antimicrobial susceptibility testing was carried by Kirby Bauer disk diffusion test (disc by BIO RAD), minimum inhibitory concentration (MIC) by agar dilution method (powder supplied by Weith) and by E- test (AB BIO disc) as per Clinical and Laboratory Standards Institute (CLSI) guidelines recommendation [5]. Interpretation was done as per criteria approved by US FDA. By DDT ≥19mm sensitive for *S. aureus, Enterococcus spp,Enterobacteriaceae* and *Acinetobacterspp*, by MIC ≤ 0.5 µg/ml sensitive for *S. aureus*, ≤ 0.25µg/ml for *Enterococcus spp,* and for *Enterobacteriaceae*≤ 2µg/ml (susceptible), 4µg/ml (intermediate), 8µg/ml (resistant).The same break point criteria of *Enterobacteriaceae* was also applied for *Acinetobacter spp*. *Escherichia coli* ATCC 25922, was used as quality control strain

RESULTS

Most commonly isolated sample was urine followed by pus, blood, tracheal aspirate and sputum respectively which is shown in Figure 1.

Figure1. Distribution of various samples (%)



From table1, it has been observed that *E coli* was the most common pathogen isolated from clinical samples followed by *Klebsiella spp, A baumannii* and MRSA respectively.

Table1. Distribution of various species in the sample

Species	Number	Percentage %
Escherichia coli	69	27.6
Klebsiellaspp	58	23.2
Acinetobacter baumannii	48	19.2
MRSA	44	17.6
Enterococcus spp	8	3.2
Salmonella spp	7	2.8
Citrobacter freundii	2	0.8
Serratia marcescens	3	1.2
Pseudomonas spp	5	2
Proteus spp	6	2.4

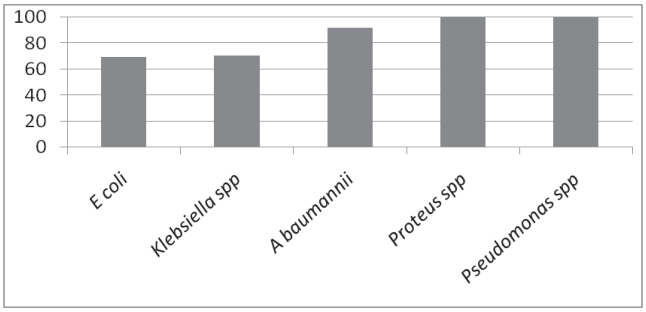
It has been observed that most resistant organism was *A baumannii followed by Klebsiella spp* and *E coli* respectively for tigecycline drug excluding *pseudomonas spp* and *proteus spp* which were known to have reduced susceptibility or non susceptible to tigecycline drug due to the multidrug efflux systems such as MexXY in *P. aeruginosa* and AcrAB in *Proteus mirabilis.*

Table 2.Susceptibility pattern of tigecycline for various isolates by DDT

Isolates	DDT		Total no of isolates
	S%	R%	
E coli	79.71	20.28	69
Klebsiella spp	39.65	60.34	58
Acinetobacter baumannii	39.58	60.41	48
MRSA	100	-	44
Salmonella spp	100	-	7
Enterococcus spp	100	-	8
Citrobacter freundii	50	50	2
Serratia marcescens	66.66	33.33	3
Peudomonas spp	-	100	5
Proteus spp	-	100	6

It has been observed that 100% *Pseudomonas spp*and *Proteus spp* were MDR followed by *91.66% A baumanni,* 70.68% *Klebsiella spp* and 69.56% *E. coli* respectively.

Figure 2.distribution of multidrug resistant isolates

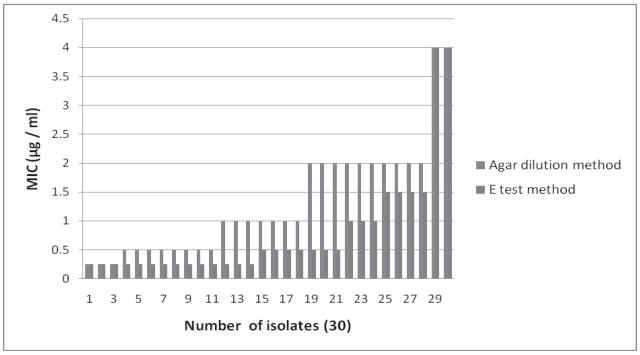


When the results of two different methods (DDT, AD) were compared for the different isolates, discrepancy was observed for *E coli, Klebsiellaspp* and *Abaumannii*which is shown in Table 3.

Table3.Comparison of results by DDT and AD

Isolates	DDT		AD		Total no of isolates
	S%	R%	S%	R%	
E coli	79.71	20.28	100	-	69
Klebsiellaspp	39.65	60.34	98.27	1.72	58
A baumannii	39.58	60.41	95.83	4.16	48
MRSA	100	-	100	-	44
Salmonella spp	100	-	100	-	7
Enterococcus spp	100	-	100	-	8
C freundii	50	50	100	-	2
S marcescens	66.66	33.33	100	-	3
Pseudomonas spp	-	100	-	100	5
Proteus spp	-	100)	-	6 (100)	6

Figure 3. Comparison between the results of agar dilution and E test for *Abaumanni*



DISCUSSION

Tigecycline, a semisynthetic derivative is an ideal antimicrobial agent which is unaffected by a number of defence mechanism such as protein binding, β lactamase production (ESBL production, Amp Chyperproducers) DNA gyrase alterations, the Van resistance genes and others that are used by many organisms which are isolated in community-acquired and nosocomial infections.

From this study it was found that 79.71% of *E coli* were sensitive by disc diffusion test. Of total, 69.56% were MDR of which 66.66 % were sensitive to tigecycline which was much lower as compared to the findings of Behera et al [100% sensitivity] [6]. 33.33% were ESBL producers of which 78.26% were sensitive which was lower as compared to the Taneja et al and Ahmed et al [7,8] [100% sensitivity]. By AD method, 100% of *E coli* were sensitive which was similar with observations of Tan et al and Pillar et al [100% sensitivity] [9,10].

Of total *Klebsiella spp,* only 39.65% were sensitive which was much less than finding of Behera et al and Taneja et al [100% sensitivity] [6,7] , Ahmed et al [94.29% sensitivity] [8], Tan et al [83% sensitivity] [9]. Out of total, 70.68% were MDR, 13.79% were ESBL producer and 1.72% was MBL producer. Of ESBL producer, only 12.5% were sensitive by DDT as compared to Taneja et al [100% sensitivity][7]. By AD, 1.72% was intermediate resistant (4 µg/ml) while all others sensitive.In the present study, a discordance was observed in the results of DDT and AD for *Klebsiella spp* [byDDT 39.65% sensitivity, by AD 98.27%]. While Behera et al [6] have reported 100% concordance in the results of DDT and E test.

Of total *A. baumannii*, only 39.58% were sensitive by DDT which was less as compared toTan et al [9] [71% sensitivity]. Out of total, 91.66% were MDR. MIC of *A baumannii* showed 95.83% sensitive and 4.16% intermediate resistant (4µg/ml) while Tan et al [9] reported significantly higher intermediate resistance 20%, Crucio et al only 2.4% resistant in TEST global programme[11]. In our study, marked discordance was observed in the results of two different methods. By DDT, 39.58% sensitivity, by AD 95.83 % sensitivity. For 30 isolates results of AD and E-test were in 100% concordance. MIC by E test was found to be 1 to 2 log dilution less as compared to MIC by AD in most of the isolates while Bolmstrom et al [12] reported E test MIC results of > 1log 2 dilution higher than the broth

dilution method.

Behera et al [6] also reported a significant discordance between the results of DDT and E-test (DDT- 65% sensitive, E-test- 42% sensitive). On the other hand Venezia et al [13] reported 100% concordance in the results of DDT and E test which differed from this study and from Behera et al [6].

Out of 44 MRSA, 100% were sensitive to tigecycline by DDT and by AD which was similar to Behera et al [6] [100% by DDT and E test], Boucher et al and Kitzis et al [100% by AD] [14 15].

CONCLUSION

Tigecycline is found to be effective against various Gram positive and Gram negative bacteria which are multiple drug resistant (MRSA, ESBL producing *E coli* and *Klebsiella spp* and MDR *A baumannii,*) by MIC method which is considered the standard method for testing drug susceptibility testing.

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