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Microbiology Section

The in Vitro Activity of Tigecycline Against the Multidrug Resistant *Acinetobacter Spp*. at a Tertiary Care Hospital

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ABSTRACT

Background: The Acinetobacter spp., particularly *A.baumanii*, has emerged as one of the most troublesome pathogens in health care institutions globally, because they are often Multi Drug Resistant (MDR), which means that the therapy and the infection control are complicated. With the emergence of the carbapenemase-producing isolates which show resistance to all the available agents except the polymyxins, this genus deserves close attention. In this scenario, tigecycline, a glycylcycline which has a spectrum of activity which is unparalleled by any other broad spectrum agent, and is not affected by most of the known mechanisms of resistance to tetracycline which have been encountered in bacteria, is a useful alternative for the treatment of the infections which are caused by the Acinetobacter spp.

Aim: This study was conducted to investigate the in vitro activity of tigecycline against a collection of MDR isolates of Acinetobacter spp. from our hospital.

Material and Methods: A prospective, hospital based study was conducted from October 2010 to April 2012 in which all the Acinetobacter spp. isolates which were obtained from clinical samples, were subjected to the testing of their antimicrobial susceptibilities to different groups of drugs, which included tigecycline. Based on the susceptibility profile, the isolates which were labeled as MDR were further subjected to the Epsilometer test (E-test) to determine the minimum inhibitory concentrations (MIC) of tigecycline.

Results: A total of 85 Acinetobacter spp. isolates were obtained, out of which 38 (44.7%) were labeled as MDR. 91.8% of the total and 81.5% of the MDR isolates were sensitive to tigecycline and the MICs of tigecycline for these MDR isolates ranged from 0.25 to 32 µg/ml.

Conclusion: This study proved that tigecycline exhibited a good in vitro activity against the clinical isolates of the MDR Acinetobacter spp., and that it may be considered as a promising therapeutic option for the treatment of the nosocomial infections which were caused by these pathogens. But the tigecycline resistance among the isolates that had not previously been exposed to the drug is worrisome. So before starting the treatment, the in vitro susceptibility of the isolates to tigecycline and its MIC should be assessed.

Key Words: Acinetobacter spp., Tigecycline, MDR, MIC, E-test

INTRODUCTION

The Acinetobacter spp., particularly *A.baumanii*, has emerged as one of the most troublesome pathogens for the health care institutions globally. Hospital acquired pneumonia is still the most common infection which is caused by this organism. However, in the more recent times, the infections which involve the central nervous system, skin and soft tissue, and the bone have emerged as highly problematic for certain institutions [1,2].

The mortality which is due to the nosocomial infections which are caused by A. baumannii is high, reaching from 25 to 34% for bacteraemia and from 40 to 80% for nosocomial pneumonia [3,4]. This genus deserves close attention as it displays mechanisms of resistance to all the existing antibiotic classes, as well as a prodigious capacity to acquire new determinants of resistance [5].

Many carbapenemase-producing *A. baumannii* isolates are resistant to all the available therapeutic agents except the polymyxins and to the drugs with significant toxicity and poor penetration to respiratory secretions [6].

Acting in synergy with this emerging resistance profile is the uncanny ability of Acinetobacter spp. to survive for prolonged periods throughout the hospital environment, thus potentiating its ability for nosocomial spreads [2]. In this scenario, tigecycline, a 9-t-butylglyclamide derivative of minocycline, which has a spectrum of activity which is unparalleled by any other broad spectrum agent, and is not affected by most of the known mechanisms of resistance to tetracycline (ribosomal protection and active drug efflux) which have been encountered in bacteria, is a useful alternative to the polymyxins [6].

Tigecycline acts by the inhibition of the protein translation in bacteria, by binding to the 30S ribosomal subunit, and by blocking the entry of the amino-acyl tRNA molecules into the A site of the ribosome [7].

But in view of the increasing number of reports of the variable susceptibility of tigecycline against the Multiple Drug Resistant (MDR) Acinetobacter spp. isolates around the world and the few therapeutic options which are available for the treatment of the infections which are caused by this organism, this study was conducted to investigate the in vitro activity of tigecycline against a collection of MDR isolates of Acinetobacter spp. at our hospital.

MATERIALS AND METHODS

A prospective study was conducted at the Acharya Shri Chander College of Medical Sciences and Hospital, Jammu, India, from October 2010 to April 2012. All the MDR Acinetobacter spp. isolates which were obtained from the clinical samples which were received in the microbiology laboratory of our hospital were included in this study. None of the patients had undergone any previous treatment with tigecycline and only one isolate per patient was included in the study. The isolates were identified by the standard laboratory methods [8]. The testing of the antimicrobial susceptibility of the isolated strains to the different groups of drugs was carried out on Mueller-Hinton agar by the Kirby Bauer disc diffusion method, and the results were interpreted as was recommended by the CLSI (Clinical Laboratory Standards Institute) guidelines [9].

The interpretation of the zone diameters of tigecycline was done by using the US FDA susceptible breakpoints [10]. Pseudomonas aeruginosa ATCC 27853 was used as a quality control. The following antimicrobial agents (µg) were used - cefotaxime (30), cefepime(30), ceftazidime (30) gentamicin (10), amikacin (30), ciprofloxacin (5), levofloxacin (10), co-trimoxazole (1.2/23.8), imipenem (10), piperacillin and tazobactam (75+10), cefoperazone and sulbactam (75+30), tigecycline (15), colistin (10) and nitrofurantoin (30), which was tested only for the urinary isolates.

The MDR phenotype was defined as the resistance to more than two of the following five drug classes: antipseudomonal cephalosporins, antipseudomonal carbapenems, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and aminoglycosides. The isolates which were resistant to all the drug classes which included the glycylcyclines and the polymixins were further labelled as pan drug resistant (PDR) [2].

Minimum inhibitory concentration testing (MIC):

The MIC of tigecycline was determined for all the MDR Acinetobacter spp. isolates by using the E-test strips according to the manufacturer's instructions. The MIC breakpoints which were used were ≤2, 4 and ≥8 mg/L for the susceptible, intermediate and the resistant strains, respectively [11].

RESULTS

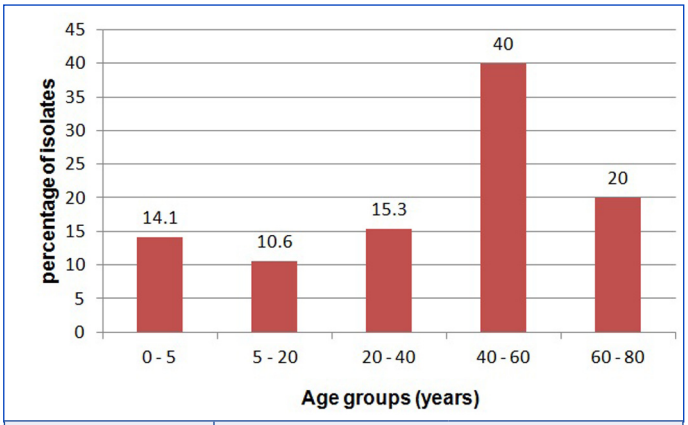
A total of 85 Acinetobacter spp. isolates were obtained during the study period, out of which 38 (44.7%) were labeled as MDR, based on the antibiotic susceptibility pattern of these isolates to various antibiotics, while 15(17.6%) showed resistance to imipenem [Table/Fig-1]. The origins of the Acinetobacter spp. isolates were (n/%): the respiratory tract (30/35.3), blood (25/29.4), skin and soft tissue (13/15.3), urine (9/10.6) and catheters (8/9.4). The ages of the patients ranged from 1-75 yrs, with the highest percentage of patients in the age group of 40-60 years [Table/Fig-2].The male to female ratio was 1.6:1 (52 males and 33 females).

The most significant finding was the reporting of two (2.4% of the total) PDR Acinetobacter spp. isolates which were resistant to both tigecycline and colistin. These PDR isolates were also resistant to imipenem. On being considered alone, 91.8% of the total isolates and 81.5% of the MDR isolates were found to be sensitive to tigecycline.

The MICs of tigecycline for the MDR Acinetobacter spp. isolates ranged from 0.25 to 32 µg/ml [Table/Fig-3]. All the isolates which had an MIC of ≤ 2 µg/ml also had a zone diameter of ≥ 19mm (the cut-off for the susceptibility). Similarly, the three isolates which had

Antimicrobial agent	No. of Sensitive isolates (%)	No. of resistant isolates (%)	No. of intermediate sensitive isolates
Cefotaxime	19(22.4)	62(72.9)	4(4.7)
Cefepime	15(17.6)	65(76.5)	5(5.9)
Ceftazidime	21(24.7)	63(74.1)	1(1.2)
Gentamicin	20(23.5)	63(74.1)	2(2.4)
Amikacin	21(24.7)	61(71.8)	3(3.5)
Ciprofloxacin	24(28.2)	60(70.6)	1(1.2)
Levofloxacin	25(29.4)	58(68.2)	2(2.4)
Cotrimoxazole	12(14.1)	72(84.7)	1(1.2)
Imipenem	67(78.8)	15(17.6)	3(3.5)
Piperacillin/tazobactum	61(71.8)	18(21.2)	6(7)
Cefoperzone/sulbactum	63(74.1)	17(20)	5(5.9)
Tigecycline	78(91.8)	4(4.7)	3(3.5)
Colistin	83(97.6)	2(2.4)	Nil
Nitrofurantoin (Only for 9 urinary isolates)	1(11.1)	7(77.8)	1(11.1)

[Table/Fig-1]: Antimicrobial susceptibility pattern of Acinetobacter spp. isolates (n=85)



Age in years	Acinetobacter spp.	
	No. of isolates	Percentage
0-5	12	14.1
5-20	9	10.6
20-40	13	15.3
40-60	34	40
60-80	17	20

[Table/Fig-2]: Age distribution of the Acinetobacter spp. isolates
Distribution of Acinetobacter spp. isolates(n=85) according to age of patients

MIC of tigecycline (µg/ml)	No. of isolates
0.25	11
0.5	12
1	6
2	2
4	3
8	2
16	1
32	1

[Table/Fig-3]: MIC of tigecycline for MDR Acinetobacter spp. isolates (n=38)

an MIC of 4 µg/ml were found to be intermediate (15-18mm) and the isolates with an MIC of ≥8 µg/ml were found to be resistant (≤ 14mm) by the disc diffusion method.

DISCUSSION

In the present study, the predominant source of the Acinetobacter spp. isolates was the respiratory tract, which is consistent with the findings of various other studies which were done in different parts of the world [12-14]. The prevalence rate of the multi drug resistance in the Acinetobacter spp. isolates was 44.7%, which is comparable to that which was reported by Taneja et al., [15], but it was quiet high as compared to that in a study which was done by Kuo et al., [16] , who reported MDR rates of 21.4 and 8.9 per cent in catheterized patients and in respiratory samples respectively.

Further, various authors have reported the resistance rate to tigecycline to vary from being nonexistent to 66 % [13-15], [17-19]. But in the present study, tigecycline was shown to have a good sensitivity (81.5%) against the MDR Acinetobacter spp., which was almost comparable to that which was reported by Insa et al., [12].

In our study, the E test correlated 100 percent with the inhibition zone diameters, which was in contrast to the findings of a study which was done by Behera et al., [19] but it was similar to the findings of a study which was done by Venezia et al., [18].

Inspite of the high sensitivity rate, the finding of the increased tigecycline MIC values (8-32µg/ml) for four Acinetobacter spp. isolates in our study was a cause of concern, since this organism was not only totally unexposed to tigecycline but also to the tetracycline group of antibiotics in our hospital. It has been described that mutations of tet(A) selected in vitro could enable the efflux of glycylcyclines and that the up-regulation of the chromosomally-mediated efflux pumps could lead to the resistance of the Acinetobacter spp. to tigecycline [6,20].

In the present study, we reported 2.3% of the Acinetobacter spp. to be pan drug resistant, which although was lower as compared to the 3.5% which was reported by Taneja et al., [15] , was significant, as it signified the beginning of the era where only a few therapeutic options would be available for their treatment.

CONCLUSION

The treatment options for the infections which are caused by multidrug resistant organisms are very limited, and tigecycline is rapidly finding a role in the treatment of severe infections, as this antimicrobial has a favourable in vitro activity against a wide variety of organisms, which include the MDR Acinetobacter spp. But the tigecycline resistance among the MDR isolates that had not previously been exposed to this drug and also the emergence of PDR isolates is worrisome.

So before starting the treatment, the in vitro susceptibility to tigecycline should be assessed, to prevent the development and the dissemination of resistance against this one of the last available promising and safe therapeutic options which is available to the clinicians for combating these bacteria.

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