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ORIGINAL ARTICLE

Tigecycline activity against metallo-β-lactamase-producing bacteria

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ABSTRACT

Backgound: Treatment of serious life-threatening multi-drug-resistant organisms poses a serious problem due to the limited therapeutic options. Tigecycline has been recently marketed as a broad-spectrum antibiotic with activity against both gram-positive and gram-negative bacteria. Even though many studies have demonstrated the activity of tigecycline against ESBL-producing Enterobacteriaceae, its activity is not well-defi ned against micro-organisms producing metallo-β-lactamases (MBLs), as there are only a few reports and the number of isolates tested is limited. **Aims:** The aim of the present study was to evaluate the activity of tigecycline against MBL-producing bacterial isolates. **Materials and Methods:** The isolates were tested for MBL production by (i) combined-disk test, (ii) double disc synergy test (DDST), (iii) susceptibility to aztreonam (30 μg) disk. Minimum inhibitory concentration to tigecycline was determined according to agar dilution method as per Clinical Laboratory Standards Institute (CLSI) guidelines. Disc diffusion susceptibility testing was also performed for all these isolates using tigecycline (15 μg) discs. **Results:** Among the total 308 isolates included in the study, 99 were found to be MBL producers. MBL production was observed mostly in isolates from pus samples (40.47%) followed by urine (27.4%) and blood (13.09%). MBL production was observed in *E. coli* (41.48%), *K. pneumoniae* (26.67%), *Proteus mirabilis* (27.78%), *Citrobacter spp*. (41.67%), *Enterobacter spp*. (25.08%), and *Acinetobacter spp*. (27.27%). The result showed that tigecycline activity was unaffected by MBL production and it was showed almost 100% activity against all MBL-producing isolates, with most of the isolates exhibiting an MIC ranging from 0.25-8 μg/ml, except 2 MBL-producing *E. coli* isolates who had an MIC of 8 μg/ml. **Conclusion:** To conclude, tigecycline was found to be highly effective against MBL-producing Enterobacteriaceae and acinetobacter isolates, but the presence of resistance among organisms, even before the mass usage of the drug, warrants the need of its usage as a reserve drug. The study also found that the interpretative criteria for the disc diffusion method, recommended by the FDA, correlates well with the MIC detection methods. So, the microbiology laboratories might use the relatively easier method of disc diffusion, as compared to the comparatively tedious method of MIC determination.

Key words: Gram-negative bacteria, metallo-β-lactamases, tigecycline

INTRODUCTION

In the present era of multidrug-resistant organisms, clinicians are facing an acute shortage of antibiotics with activity against these organisms. Resistance to carbapenems in *Enterobacteriaceae* can be caused by overproduction of Amp-C β-lactamases, associated with loss of outer membrane porins and/or overexpression of efflux pumps[1] or by production of β-lactamases with significant hydrolysis activity against carbapenem compounds. These carbapenemases can be divided into the metallo-β-lactamases (MβL; Ambler class B) and serine carbapenemases (class A or Bush class 2f) according to the functional requirements and the structure of their active site.[2,3] The genes encoding most of these carbapenemases reside on plasmids or transposons carrying additional genes encoding resistance to other classes of antimicrobial agents.[4] These transferable structures can readily be acquired by

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gram-negative pathogens, facilitating the dissemination of these potent resistance mechanisms and, in many cases, conferring on the isolate a multidrug resistance profile,[5] significantly reducing the treatment options for infections caused by carbapenemase-producing isolates.

Tigecycline is a semi-synthetic glycylcycline derived from minocycline that has documented activity against tetracycline-resistant gram-negative pathogens that are refractory as a result of both efflux and ribosomal protection mechanisms.[6] In addition, organisms that are resistant to other antimicrobial classes do not exhibit cross-resistance to tigecycline, supporting the potential therapeutic use of this antimicrobial agent for the treatment of infections caused by carbapenemase-producing *Enterobacteriaceae* isolates.[7]

In the present study, we tested the *in vitro* activity of tigecycline against MBL-producing Enterobacteriacae and Acinetobacter isolates.

MATERIALS AND METHODS

A total of 308 gram-negative bacterial isolates were tested between December 2010 and September 2011 in a tertiary care hospital in Kolkata. The isolates were recovered from the following sources: Wound, urine, sputum, body fluid, and blood cultures. Isolates were identified upto species level by standard laboratory procedures.[8] Antibiotic susceptibility was determined according to the interpretative criteria of the Clinical Laboratory Standards Institute 2010 (CLSI).[9] Antibiotic susceptibility was performed for tigecycline (15 μg), polymyxin B (300 units), imipenem (10 μg), piperacillin + tazobacatam (100/10 μg), amikacin (30 μg), ceftriaxone (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), amoxicillin + clavulanic acid (20/10 μg), aztreonam (30 μg), and cefoxitn (30 μg). All the isolates were tested for MBL production by phenotypic detection method using a single agar plate and comprised three components. (i) In the combined-disk test, two imipenem (IMP) disks (10 μg), one containing 10 μl of 0.1 M (292 μg) anhydrous EDTA (Himedia India), were placed 25 mm apart (center to center). An increase in zone diameter of >4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL. (ii) In the DDST, an imipenem (10 μg) disk was placed 20 mm (center to center) from a blank disk containing 10 μl of 0.1 M (292 μg) EDTA. Enhancement of the zone of inhibition in the area between the two disks was considered positive for an MBL. (iii) The final component was an aztreonam (30 μg) disk. Given the unique sensitivity of MBLs to this antibiotic, we studied the inhibition zone sizes of all isolates to determine the utility of this component in phenotypic MBL detection.[10]

The isolates were also tested for production of AmpC, in order to rule out the resistance to carbapenems due to AmpC production. The detection of AmpC β-lactamases was done based on screening tests and confirmatory tests. For screening, disc diffusion zones of cefoxitin <18 mm were taken as cefoxitin-resistant. All cefoxitin-resistant isolates were tested further by AmpC disk test and modified three dimensional tests. Plates were examined for either an indentation or flattening of the zone of inhibition in the disc test. In the modified three-dimensional tests, three different kinds of results were recorded. Isolates that showed clear distortion of zone of inhibition of cefoxitin were taken as AmpC producers. Isolates with no distortion were taken as AmpC non-producers, and isolates with minimal distortion were taken as intermediate producers.[11] Disc diffusion susceptibility testing was performed for all these isolates using tigecycline discs (15 μg) purchased from Himedia (Mumbai India).[12,13] MIC of tigecycline was determined according to agar dilution method as per CLSI. Tigecycline was supplied as powder of known potency by the Wyeth Pharmaceuticals. Interpretation of zone diameter of all Gram-negative bacteria (including *Acinetobacter spp*) was using US FDA tigecycline susceptibility breakpoint listed for *Enterobacteriaceae* (MIC ≤ 2 μg/ml and ≥ 19 mm zone size).[6,12] Resistance was defined as MIC ≥ 8 μg/ml and zone size ≤14 mm).[12] Quality control was carried out by *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

RESULTS

Among the total 308 clinical isolates, 99 were found to be MBL producers. MBL production was observed mostly in isolates derived from pus samples (40.47%) followed by urine (27.4%) and blood (13.09%). MBL production was observed in *E. coli* (41.48%), *K. pneumoniae* (26.67%), *Proteus mirabilis* (27.78%), *Citrobacter spp.* (41.67%), *Enterobacter spp*. (25.08%), and *Acinetobacter spp*. (27.27%). Approximately, 50% (50/99) of the studied strains had Imipenem susceptibility results within the CLSI susceptible range despite their production of MBLs as tested by phenotypic tests.[10]

Tigecycline activity against these MBL producers is demonstrated in Table 1. All the isolates which did not show MBL production were susceptible to tigecycline activity (100%). Tigecycline was active against organisms producing MBL's, except for 2 (4.76%) *E. coli* isolates, showing MBL production.

The result showed that tigecycline activity was unaffected by MBL production, and it was the only compound,

Table 1: Tigecycline activity against *Enterobacteriace* and non-fermenters

***Enterobacteriace* (*n*=308)**	**Total number of isolates (*n*=308)**		**Susceptibility to tigecycline**	
	MBL producers (*n*=99) (%)	**Non-MBL producers (*n*=258)**	**MBL producers (%)**	**Non-MBL producers (%)**
E. coli (*n*=102)	42 (41.18)	60	95.24	100
K. pneumonae (*n*=120)	32 ((26.67)	88	100	100
P. mirabilis (*n*=36)	10 (27.78)	26	100	100
Citrobacter spp. (*n*=12)	5 (41.67)	7	100	100
Enterobacter spp. (*n*=16)	4 (25.00)	12	100	100
Non-fermenters				
Acinetobacter spp. (*n*=22)	6 (27.27)	16	100	100

MBL: Metallo-β-lactamases

which showed more than 90% activity against MBL-producing isolates, followed by polymyxin B (87.55%), piperacillin + tazobactum (79.2%), amikacin (79.2%), and cefepime (70.8%).

The MIC values for tigecycline ranged from 0.25-8 μg/ml, except for two *E. coli* isolates having an MIC of 8 μg/ml. MIC values of tigecycline against the MBL producers is demonstrated in Table 2. All the isolates having MIC ≤ 2 μg/ml also had a zone diameter ≥19 mm (cut-off for susceptibility), which corroborated well with the FDA guidelines of disc diffusion interpretation for tigecycline activity. The MIC values for 2 *E. coli* isolates were in the intermediate susceptibility range of 4 μg/ml as per the CLSI guidelines. These isolates showed a zone of inhibition of 15 and 16 mm respectively by the disc diffusion technique.

Table 2: MIC levels of tigecycline against MBL-producing *Enterobacteriace* and non-fermenters

Organisms (number of isolates)	**Number of isolates with MIC (μg/ml) of**						
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**
E. coli (42)	0	0	17	12	9	2	2
K. pneumonae (32)	0	16	10	6	0	0	0
P. mirabilis (10)	0	8	2	0	0	0	0
Citrobacter spp. (5)	0	3	2	0	0	0	0
Enterobacter spp. (4)	0	0	2	2	0	0	0
Acinetobacter spp. (6)	0	0	1	4	1	0	0

MBL: Metallo-β-lactamases, MIC: Minimal inhibitory concentration

DISCUSSION

The widespread dissemination of carbapenemase-producing Enterobacteriaceae has profound implications for the clinical utility of the carbapenems.[14] Furthermore, carbapenemase-producing Enterobacteriaceae strains were generally resistant to the vast majority of antimicrobial agents available for clinical use, making the therapeutic options very limited.[15,16] MβL-producing Enterobacteriaceae have emerged in countries where MβL-producing *P. aeruginosa* strains have also become endemic, such as Greece, Turkey, Italy, Spain, and also in India.[16-18] This suggests that Enterobacteriaceae isolates are likely to have acquired these enzyme-encoding genes either from the MβL-producing *P. aeruginosa* strains or from other non-fermentative species that could be the primary reservoir for MβL genetic elements.

This study, in addition to other recent surveillance initiatives,[18] has determined that the antimicrobial activity of tigecycline is largely unaffected by metallo beta-lactamase in gram-negative organisms, confirming that this novel compound can be a valuable therapeutic option for the treatment of infections caused by these troublesome, resistant Enterobacteriaceae, as well as non-fermenters, which was similar to finding by Castanheira *et al.*[18] and Behera *et al.*[19]

Approximately 50% of the studied strains had Imipenem susceptibility results within the CLSI susceptible range despite their production of MBLs as per phenotypic tests, which corroborates with the findings of Castanheira *et al.*[18] Therefore, the study highlights the importance of routine testing of MBLs in order to prevent any disparity between *in vivo* and *in vitro* results.

The results of the present study suggests that tigecycline represents a significant step forward over other antibiotics currently in use for the treatment of MDR gram-negative organisms, showing excellent *in vitro* activity against strains, for which adequate therapy has been limited. It is a promising antimicrobial agent that will likely have a key role in treatment of nosocomial infections, provided that clinical efficacy in a variety of severe infection is documented. It is largely unaffected by MBL production in Enterobacteriacae and non-fermenters.[19]

Tigecycline was active against MBL-producing members of the family Enterobacteriaceae inhibiting 95.2% of them. Among Enterobacteriacae, resistance was observed only in *E. coli,* of which two isolates was resistant with MIC of 8 μg/ml, and one isolate was showing reduced susceptibility as demonstrated by zone diameter of <19 mm and >14 mm, and MIC of 4 μg/ml.

The susceptibility rates of *E. coli* was found to be 95.4%, which was similar to the study of Kelesidis *et al.*, who found 99.6% of *E. coli* isolates to be sensitive.[20] This study showed good activity of tigecycline against *Klebsiella, Enterobacter, Citobacter,* and *Proteus mirabilis*, which differs from the review done by Kelesidis *et al.*, in which he found the reduced activity against these organisms.[21]

However, it is important to note that tigecycline has not been approved for the treatment of bloodstream infections, and more clinical experience with this compound is necessary to better understand its role in the treatment of serious infections caused by carbapenemase-producing *K. pneumoniae* and other multidrug-resistant gram-negative bacilli. Recently, the US FDA has issued a warning describing an increased mortality risk associated with the use of tigecycline when compared with other drugs in the treatment of a variety of serious infections. The increased risk of mortality was determined using a pooled analysis of randomized clinical trials (RCTs) and was seen most clearly in patients treated for hospital-acquired pneumonia (HAP), especially ventilator-associated pneumonia (VAP), but was also seen in patients with complicated skin and skin structure infections (cSSSIs), complicated intra-abdominal infections (cIAIs), infections due to resistant pathogens, and diabetic foot infections.[1] Although for each indication, the mortality difference was not statistically significant, trends were present and, when pooled, a statistically significant difference was observed. Based on these data, the FDA recommends that alternatives to tigecycline should be considered in patients with severe infections. Notwithstanding the FDA approved indications for the drug, there are published data indicating that tigecycline's pharmacological and microbiological profiles encourage its use for off-label indications in severely ill patients in intensive care units [e.g. VAP due to multidrug-resistant (MDR) Acinetobacter spp.]. This practice is justified by the high regional resistance rates of MDR pathogens with limited therapeutic options [e.g. carbapenem-resistant Acinetobacter spp. and Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae]. In addition, there is good evidence that early effective therapy for such infections in critically ill patients improves outcomes.[20]

The study also signifies that the currently recommended zone interpretative criteria for disc diffusion method correlate well with agar dilution method for interpretation of MIC's. So, the microbiology laboratories might use the relatively easier method of disc diffusion, as compared to the comparatively tedious method of MIC determination.

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