

## Susceptibility of Multi-resistant Gram-negative Bacilli in Singapore to Tigecycline as Tested by Agar Dilution

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### Abstract

**Introduction:** Tigecycline is an antibiotic belonging to the glycylycine class with *in vitro* activity against most Gram-negative bacteria, other than *Pseudomonas aeruginosa*. This study investigated the *in vitro* activity of tigecycline against multi-resistant isolates of Enterobacteriaceae and *Acinetobacter* spp. isolated from clinical specimens in Singapore. **Materials and Methods:** Minimum inhibitory concentrations (MICs) to tigecycline were determined for 173 isolates of multi-resistant *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp. and *Acinetobacter* spp. using agar dilution. **Results:** The MIC required to inhibit the growth of 90% of organisms varied from 0.5 to 4 mg/L for the study isolates. Based on a resistance breakpoint of  $\geq 8$  mg/L, resistance rates varied from 0% to 9%. **Conclusions:** Tigecycline demonstrates good *in vitro* activity against multi-resistant strains of Enterobacteriaceae, with more variable activity against multi-resistant strains of *Acinetobacter* spp.

Ann Acad Med Singapore 2007;36:807-10

**Key words:** *Acinetobacter baumannii*, Enterobacteriaceae, Microbial sensitivity tests

### Introduction

Tigecycline is a recently developed antibiotic that belongs to the glycylycine class, which is a novel analogue of the tetracyclines. Tigecycline is reported to have excellent *in vitro* activity against most Enterobacteriaceae,<sup>1</sup> although minimum inhibitory concentrations (MICs) are higher against *Proteus mirabilis*, *P. vulgaris*, *Morganella morganii*, and *Providencia* spp.<sup>2</sup> Based on *in vitro* data, tigecycline is also active against *Acinetobacter* spp., but not against *Pseudomonas aeruginosa*.

The prevalence of antibiotic resistance in Gram-negative bacilli is high in the Asia-Pacific region. Recent data from the SENTRY study showed that extended-spectrum beta-lactamases (ESBLs) were present in 30% to 35% of *Klebsiella pneumoniae* in China and Singapore.<sup>3</sup> Co-resistance to other antibiotics was also noted, particularly in countries with a high prevalence of ESBL-producing Enterobacteriaceae. Similarly, the SMART worldwide study reported that ESBL-producing strains of *Klebsiella* spp. and *Enterobacter* spp. were most prevalent in the Asia-Pacific region.<sup>4</sup> Antibiotic resistance in *Acinetobacter baumannii* also appears to be high: a Korean study reported imipenem resistance rates of 13% in *Acinetobacter baumannii*<sup>5</sup> while in Singapore, nearly half of all clinical

strains of *Acinetobacter* spp. are resistant to the carbapenems.<sup>6</sup>

This study investigated the *in vitro* activity of tigecycline against multi-drug resistant strains of *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Acinetobacter* spp. isolated from an 800-bed hospital located in Singapore, prior to the introduction of the antibiotic into the hospital formulary. The antibiotic resistance profile of Gram-negative bacilli in this hospital does not differ significantly from the national average.

### Materials and Methods

#### Collection of Isolates

Isolates of *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Acinetobacter* spp. were selected from an existing bank of organisms collected retrospectively over a 2-year period starting from 2004. Only 1 unique isolate per patient was included for testing. Test strains were previously identified using conventional biochemical tests.<sup>7</sup> Disc susceptibility testing was performed and interpreted according to Clinical Laboratory Standards Institute (CLSI)<sup>8</sup> guidelines for the following antibiotics: amikacin, amoxicillin-clavulanate (ampicillin-sulbactam for *Acinetobacter* spp.), aztreonam, cefepime, ciprofloxacin, ceftazidime, gentamicin, imipenem,

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minocycline, piperacillin-tazobactam and trimethoprim-sulphamethoxazole. The presence of ESBL in *E. coli* and *Klebsiella* spp. was inferred either by the double-disk approximation method,<sup>9</sup> or by the confirmatory tests as recommended by the CLSI.<sup>10</sup>

For the purposes of this study, isolates were defined as multi-drug resistant when they demonstrated diminished susceptibility to >1 of drug classes tested in the susceptibility testing panel.<sup>11</sup>

#### Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) to tigecycline were obtained by agar dilution, performed according to CLSI guidelines.<sup>10</sup> Tigecycline antibiotic powder (Wyeth, USA) in solution was added to molten Mueller-Hinton II agar (Becton-Dickinson, Maryland, USA), to provide 2-fold doubling concentrations (range, 0.016 to 16 mg/L). Plates for agar dilution were freshly prepared on each day of testing. Bacterial suspensions prepared from overnight cultures were adjusted to yield a final test inoculum of 10<sup>4</sup> colony forming units and applied to agar plates using a multi-point inoculator (Mast Diagnostics, England). Following incubation at 35°C for 16 to 20 hours for Enterobacteriaceae, and 20 to 24 hours for *Acinetobacter* spp., plates were inspected for growth. The presence of a single colony or a faint residual haze was disregarded. The lowest concentration of antibiotic that showed no bacterial growth was recorded as the MIC. Quality control was performed concurrently with each batch of testing using *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212. Quality control results obtained were within specified limits.<sup>8</sup>

#### Results

One hundred and seventy-three isolates were included in the study, comprising *Acinetobacter* spp. (n = 55), *E. coli* (n = 19), *Enterobacter* spp. (n = 35) and *Klebsiella* spp. (n = 64). One hundred and sixty-six (96%) isolates were defined as multi-resistant, and 16 isolates were resistant to all tested antibiotics. Ninety per cent to 95% of *Klebsiella* spp. and *E. coli* were positive for ESBL production. The full susceptibility profile of the tested organisms is listed in Table 1.

The MIC values for tigecycline as obtained by agar dilution are shown in Table 2. There is currently no interpretative breakpoint available from the CLSI for tigecycline. Based on the breakpoints recommended by the US Food and Drug Administration for Enterobacteriaceae (susceptible ≤2 mg/L, resistant ≥8 mg/L), no tigecycline-resistant *E. coli* isolates were detected, while 4 (6%) isolates of *Klebsiella* spp. and 1 (3%) isolates of *Enterobacter* spp. were classified as resistant. At present, there are no interpretative breakpoints available for *Acinetobacter* spp. If the same interpretative criteria for Enterobacteriaceae are arbitrarily applied, 5 (9%) of the tested *Acinetobacter* spp. were resistant.

#### Discussion

The results of this study show that tigecycline displays *in vitro* activity against antibiotic-resistant strains of *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Acinetobacter* spp. However, interpretation of categorical susceptibility is hampered by the lack of formal breakpoint recommendations. The FDA has recommended susceptibility breakpoints for Enterobacteriaceae (MIC ≤2 mg/L), while European consensus statements suggest using a lower

Table 1. Antibiotic Susceptibility for Study Organisms

Antibiotic	Enterobacteriaceae			<i>Acinetobacter</i> spp.		
	%R	%I	%S	%R	%I	%S
Amikacin	31.4	15.7	52.9	47.2	3.8	49.1
Amoxicillin/Clavulanic acid	86.5	11.2	2.2	50	50	0
Ampicillin/Sulbactam	100	0	0	52.7	20	27.3
Aztreonam	87.7	6.2	6.2	85.7	0	14.3
Ceftazidime	92.9	7.1	0	85.5	7.3	7.3
Ciprofloxacin	82.2	5.9	11.9	96.4	0	3.6
Gentamicin	62.7	4.2	33.1	69.1	0	30.9
Imipenem	5.1	0	94.9	100	0	0
Minocycline	NT	NT	NT	31.5	24.1	44.4
Piperacillin/Tazobactam	50.8	23.7	25.4	100	0	0
Trimethoprim/Sulfamethoxazole	78	3.4	18.6	81.8	0	18.2

%I: % of isolates of intermediate susceptibility; %R: % of resistant isolates; %S = % of susceptible isolates; NT: not tested

Table 2. Distribution of Tigecycline MICs

Organism	Break-points	No.	R (%)	I (%)	S (%)	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC range	mg/L						
									0.25	0.5	1	2	4	8	16
<i>Klebsiella</i> spp.	S≤2 R≥8	64	4 (6%)	7 (11%)	53 (83%)	2	4	0.5-16		7	17	29	7	2	2
<i>Escherichia coli</i>	S≤2 R≥8	19	0 (0%)	0 (0%)	19 (100%)	0.5	0.5	0.25-0.5	9	10					
<i>Enterobacter</i> spp.	S≤2 R≥8	35	1 (3%)	1 (3%)	33 (94%)	1	2	0.25-16	3	11	11	8	1		1
<i>Acinetobacter</i> spp.	S≤2 R≥8	55	5 (9%)	11 (20%)	39 (71%)	2	4	0.25-16	2	5	6	26	11	3	2

FDA published breakpoints – I: intermediate; R: resistant; S: susceptible  
MIC: minimum inhibitory concentration

breakpoint for susceptibility (MIC ≤1 mg/L).<sup>12</sup> There are currently no interpretative breakpoints available for *Acinetobacter* spp. Based on the MIC distributions for our selected bacterial population, tigecycline has reduced *in vitro* activity against multi-resistant *Acinetobacter* spp.

The MIC required to inhibit the growth of 90% of organisms (MIC<sub>90</sub>) is higher for *Klebsiella* spp. and *Enterobacter* spp. in our study than those reported from other geographic regions.<sup>13</sup> A recent study reported a trend towards higher tigecycline MIC values when testing antibiotic-resistant Gram-negative bacilli.<sup>14</sup> Similarly, the MIC<sub>90</sub> for *Acinetobacter baumannii* ranges from 2 to 8 mg/L, depending on the strains tested.<sup>15</sup> However, other possible explanations for the higher MIC<sub>90</sub> values include the use of unique but banked isolates collected from a single institution over a 2-year period, or geographic variations in antibiotic susceptibility.

In contrast to the microbroth dilution testing methods commonly used for *in vitro* susceptibility testing, agar dilution was used in this study. There is limited data to show that the MIC obtained by agar dilution is lower than that obtained by broth dilution techniques.<sup>16</sup> Tigecycline is susceptible to the effects of oxidative reduction, and results of broth testing are affected by the age of the Mueller-Hinton broth used.<sup>17</sup> A similar effect was noted during the course of the agar dilution in this study. MIC values of control strains increased following overnight storage of pre-poured agar dilution plate (data not shown). Storing prepared agar plates in anaerobic conditions mitigated this effect. Reading the agar dilution MIC endpoints was complicated by the presence of trailing endpoints, which was particularly apparent in *Acinetobacter* spp. A few test isolates demonstrated the presence of a fine haze on agar plates at the MIC endpoint.

In conclusion, the results of this study confirm that tigecycline retains *in vitro* activity against multi-resistant strains of Enterobacteriaceae. The data from this study suggest that local *in vitro* susceptibility testing should always be performed, as local susceptibility rates may differ from those reported in other epidemiological studies.

#### Acknowledgements

This study was made possible by a research grant from Wyeth Pharmaceuticals.

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