In vitro activity of tigecycline against Acinetobacter baumannii isolates from a teaching hospital in Malaysia

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The in vitro susceptibility of clinical and environmental isolates of Acinetobacter baumannii to tigecycline and other antibiotics was determined by disk diffusion method. The E-test was used to determine the minimum inhibitory concentration (MIC). The growth curves of tigecycline treated environmental and clinical strains were established. Fifty-seven percent and 75% of the clinical and environmental isolates were MDR strains, respectively. Ninety-five percent of the clinical isolates were susceptible to tigecycline and 5% showed intermediate resistance with MIC ranging between 0.032 and 3 mg/l. Tigecycline susceptible and intermediate resistance among the environmental isolates were 40% and 60%, respectively, with a significantly lower MIC range of 0.5–4 mg/l. The bacterial growth curves demonstrated the higher ability of the environmental strains to tolerate the antibiotic effects than the clinical strains. The relatively high resistance profile among the environmental isolate suggests an insidious emergence of tigecycline resistance amongst A. baumannii. Strict infection control procedures are imperative to prevent the dissemination of tigecycline-resistant A. baumannii strains in the hospital environment.

Keywords: Acinetobacter baumannii, Antimicrobial resistance, Tigecycline

Introduction

Acinetobacter baumannii, a Gram-negative non-fermentative cocobacillus, has emerged in the last two decades as a significant nosocomial pathogen responsible for several serious outbreaks especially in high dependency wards like the intensive care units (ICUs). The problems confronting clinicians and clinical microbiologists with this pathogen are made more acute with the increasing number of multidrug-resistant (MDR) A. baumannii being isolated. The years of misuse and abuse of broad-spectrum antimicrobial agents have greatly contributed to the spread of these MDR strains. The MDR strains have been implicated in serious life threatening nosocomial outbreaks of ventilator associated pneumonia, urinary tract infections, bacteremia, meningitis, and wound infections.¹² The outbreaks in ICUs have been extremely difficult to control. The hospital situation is further aggravated by the fact that A. baumannii is extremely resistant to disinfectants and drying procedures, thus, successfully adapting itself to the hospital environment ensuring long-term persistence and ease of transmission between patients within the same unit and other units of the hospital.³⁴

Routine infection control measures are often insufficient to halt the transmission of MDR A. baumannii particularly in the ICUs, but a strict strategy of enhanced nosocomial infection control measures that focus on controlling the environmental contamination of an outbreak strain should succeed in eradicating these organisms from the ICUs.⁵ Species of the genus Acinetobacter are among the most common isolates from ICUs of most Malaysian hospitals.⁶

Worldwide, A. baumannii clinical isolates have showed high resistance to most commonly used antibiotics including aminopenicillins, ureidopenicillins, broad-spectrum cephalosporins, most aminoglycosides, quinolones, chloramphenicol, and tetracycline. The emergence of MDR strains of A. baumannii resulted in carbapenems being more commonly used as the drug of choice for the treatment of Acinetobacter infections. However, the prevalence of hospital acquired infections caused by multidrug carbapenem resistant Acinetobacter strains is increasingly being reported.⁷–⁹ An earlier study conducted at
the University of Malaya Medical Centre (UMMC); a Malaysian teaching hospital, on antibiotic susceptibility of *Acinetobacter* spp., had recorded an increase in resistance at 10-year intervals for most antibiotics tested against these bacteria including but not limited to carbapenems.10,11 Another recent study from the same centre reported that *A. baumannii* isolates have high resistance rates to most commonly used antimicrobials in that medical center.6 The rapid emergence of imipenem-resistant *A. baumannii* in particular, necessitates the urgent search for alternative therapeutic options.

Tigecycline, a derivative of minocycline, with broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, has been reported to have potent activity against *A. baumannii* and other *Acinetobacter* spp.12–14 However, other reports have shown that the susceptibility of *A. baumannii* to tigecycline varies from one geographical area to another and also varies over time.15,16 The present study aims to determine the *in vitro* activity of tigecycline and other commonly used antibiotics against primary non-duplicate *A. baumannii* clinical isolates obtained from patients in the ICU and isolates collected from the ICU environment of the UMMC.

**Methodology**

*Isolation and identification of A. baumannii*

One hundred and twenty *A. baumannii* isolates obtained from the ICU of the UMMC in (2008–2009) were used in the current study. One hundred of these were clinical isolates collected from different sources which include respiratory secretions, catheter tips, pus/wounds swabs, urine, blood, and body fluids. The remaining 20 isolates were particularly isolated from randomly performed swabs of solid surfaces of the ICUs and its surrounding areas.

Duplicate isolates from the same patient or locations were strictly prohibited. All isolates were identified using standard microbiological protocol and then confirmed by PCR targeting 16S rDNA gene.

**Antimicrobial susceptibility tests**

The *in vitro* antimicrobial susceptibility testing for tigecycline, imipenem, ampicillin/sulbactam, ceftazidime, cefepime, piperacillin/tazobactam, amikacin, gentamicin, ciprofloxacin, and colistin was determined by disc diffusion method and the minimum inhibitory concentration (MIC) was evaluated using Etest (AB Biodisc, Solan, Sweden) on freshly prepared Mueller-Hinton agar plates (Oxoid Ltd, Hampshire, UK) with the bacterial suspension equivalent to 0.5 McFarland standard. The agar plates were then incubated at 37°C overnight. The MICs values obtained by the E-test were confirmed using broth dilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) with proper controls. The MIC values were cautiously interpreted according to the CLSI guidelines for all antibiotics except tigecycline, as the CLSI has yet to define the tigecycline MIC breakpoints for enterobacteriaceae.17 The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and US Food and Drug Administration (FDA) have defined the tigecycline MIC breakpoints for the Enterobacteriaceae as follows, EUCAST breakpoints (S/R; mg/l) at ([<1]/>[2]) and FDA breakpoints (S/R; mg/l) at ([<2]/[>8]). The latter breakpoints were used for the interpretation of tigecycline susceptibility in this study. The susceptibility rates >2 mg/l and <8 mg/l defined as an intermediate resistant.

**Growth kinetics**

The growth curves of both *A. baumannii* tigecycline intermediate resistant (TIGIR) and tigecycline susceptible (TIGS) of both environmental and clinical strains were determined in the presence of tigecycline at their respective sub-MIC levels under the same
growth conditions. In brief, stationary phase cultures were used to inoculate 75 ml of Mueller-Hinton broth to an initial OD$_{600}$ of 0.05. These cultures were then divided into 5.0 ml aliquots and then left to grow at 37°C. At the log phase of the bacterial growth (OD$_{600}$=0.3), 2 and 4 mg/l of tigecycline were added to the TIG$^S$ and TIG$^{IR}$ cultures of both environmental and clinical strains. The bacterial cultures were then incubated for a further 3–4 hours, whereas bacterial densities were evaluated turbidimetrically at 30-minute intervals until they reached the stationary phase. Bacterial cultures were grown at 37°C and liquid cultures were continuously aerated by proper shaking. Growth kinetic assays for each strain were recorded using biological triplicate. Growth kinetic curves were plotted using the OD values and time. Untreated bacterial cultures of tested strains were used as a control.

**Results**

In the current study, respiratory secretion and wound swabs were the most common sources of *A. baumannii* clinical isolates, 40% and 30%, respectively, followed by body fluids (14%), tissue biopsies and bones (8%), urine (5%), and blood (3%). The susceptibility profile of *A. baumannii* isolates against tigecycline and nine other selected antibiotics was determined as a pool, not segregated by the respective isolation sources due to limitation in the sample numbers.

The *in vitro* assessment of tigecycline activity and the other antibiotics against *A. baumannii* are shown in (Table 1). The majority (95%) of the clinical isolates were susceptible to tigecycline and 5% of the isolates showed intermediate resistance. High resistant profile was noted for *A. baumannii* clinical isolates for the following antimicrobial agents: piperacillin/tazobactam (60%); ceftazidime (59%); imipenem (55%); cefepime (51%); ciprofloxacin (51%), and gentamicin (48%). Ampicillin/sulbactam demonstrated moderate activity with 50% of the clinical isolates being susceptible and 42% were intermediate resistant. Among the remaining antibiotics, cefepime, ciprofloxacin, and gentamicin possess strong activity against at least 40% of the clinical isolates, whereas amikacin possesses strong activity against 63% of the isolates. A high percentage (60%) of the hospital environment isolates showed intermediate resistance to tigecycline. Among the hospital environment isolates, 75% were resistant to all antibiotics tested except for gentamicin (55%). All isolates from both clinical and environmental sources were colistin sensitive.

The antimicrobial susceptibility profiles obtained by disc diffusion were in agreement with those obtained by E-test. The MICs for tigecycline obtained by broth dilution method were comparable to those obtained by E-test. The MIC range for tigecycline against *A. baumannii* clinical isolates was 0.032–3.0 mg/l with MIC$_{50}$ and MIC$_{90}$ of 0.5 and 2 mg/l, respectively (Table 2), whereas for the hospital environment isolates, the MIC range was 0.5–4 mg/l with MIC$_{50}$ and MIC$_{90}$ of 2 and 4 mg/l, respectively. The MIC$_{50}$ for amikacin, ceftazidime, and gentamicin against the hospital environment isolates were >256 mg/l, and the corresponding numbers for the clinical isolates were 1.5, 96, and 3 mg/l, respectively. The MIC$_{50}$ and MIC$_{90}$ of ciprofloxacin and imipenem were 32 mg/l for both the clinical and environmental isolates. The MIC$_{90}$ of colistin was 0.5 mg/l. Fifty-seven of the clinical strains (57%) were MDR *A. baumannii* (MDR *A. baumannii* is defined when the bacterium is resistant to at least three classes of antibiotic) and five (8.8%) of them showed intermediate resistance to tigecycline, while among the hospital environment isolates, 15 isolates (75%) were MDR *A. baumannii*, and 16 (80%) showed intermediate resistance to tigecycline.

**Growth kinetics**

The growth curves were established following treatment of TIG$^{IR}$ and TIG$^S$ *A. baumannii* isolated from both environmental and clinical sources with or

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**Table 1**: Antibiotic susceptibility profile of *Acinetobacter baumannii* clinical ($n=100$) and hospital environment ($n=20$) isolates from the UMMC

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Susceptibility (clinical isolates) (%)</th>
<th>Susceptibility (environmental isolates) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Amikacin</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Colistin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

*Note*: S: susceptible; R: resistant; I: intermediate.
without tigecycline at their respective sub-MIC levels. Both treated and untreated TIGIR environmental strains demonstrated higher growth rates compared to the clinical ones. The treated strains showed reduced growth rates only when it reached an earlier stage of the stationary phase compared with the untreated strains. The TIGIR clinical strains, both treated and untreated, exhibited a lower growth rates profile than the TIGIR environmental resistant ones. On the other hand, the TIGIR treated clinical strains showed an earlier reduced growth rates compared with the TIGIR treated environmental strain (Figure 1). Interestingly, the growth rates of the TIGS environmental strain were severely reduced by 30 minutes following exposure to the antibiotic compared to the TIGS clinical treated strain which exhibited longer tolerance to the antibiotic and growth was only reduced after 60 minutes (Figure 2). A statistical significant difference (P<0.005) in the growth kinetic rates was observed among treated TIGIR and treated TIGS environmental and clinical isolates.

Discussion

In the last two decades, hospital acquired infections and outbreaks caused by MDR A. baumannii have escalated worldwide, causing major concerns amongst clinical practitioners. In Malaysia, a previous study from the UMMC has evaluated the antimicrobial susceptibility profile of several commonly used antibiotics against A. baumannii isolates obtained at two different time intervals, first in 1987 and second over the period of 1996–1998.10 The study showed that the isolates from the first group were highly susceptible to ciprofloxacin, imipenem, and amikacin, whereas isolates from the second isolation period showed drastically higher resistance to the same antimicrobials. The rapid increased in resistance of the A. baumannii isolates over a period of 10 years is significant and alarming.

In the current study, the A. baumannii isolates showed slightly higher resistance percentages to imipenem (55% versus 36.4%) compared to the isolates collected between 1996 and 1998 in the same hospital in which the percentage of resistance to imipenem, however, increased by 18.6%.10 The MICs of the isolates, however, remained at >32 mg/l. This slight increase in the number of resistant isolates could be due to the increasing use of imipenem as empirical choices for treatment of serious infections especially for patients in geriatric, surgical, and ICUs. This is likely as the UMMC pharmacy records showed a steady increase in the use of imipenem for treatment of A. baumannii infections in the last 10 years (data not shown). Interestingly, the decreasing percentage of A. baumannii resistance to amikacin (~8.5%), ceftazidime (~38.7%); ciprofloxacin, (~39.9%), and gentamicin (~47.5%) were noted among the current clinical isolates against those isolated from the same hospital during the period of 1996–1998. This decreased resistant profile could perhaps be due to the improved antibiotic policy of their use as empirical choices for the treatment of Gram-negative bacterial infections in the UMMC.

The increasing emergence and spread of carbapenems, in particular imipenem-resistant Acinetobacter strains, worldwide, suggests that the continuing use of carbapenems might eventually render these antibiotics ineffective in treating A. baumannii infections, hence, newer antibiotics and novel treatments maybe needed.18 Tigecycline, a broad-spectrum glycyclcline antimicrobial agent is among the alternative antibiotics currently suggested against MDR Acinetobacter.15 Although tigecycline has not been included in the official formulary of antimicrobial

### Table 2 Minimum inhibitory concentration (MIC) values (mg/l) of Acinetobacter baumannii clinical and hospital environment isolates from the UMMC

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC (mg/l) clinical isolates (n=100)</th>
<th>MIC (mg/l) environment isolates (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0.032–&gt;256</td>
<td>1.5</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>0.25–128</td>
<td>8</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.38–&gt;256</td>
<td>24</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.75–&gt;256</td>
<td>96</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.035–&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.125–2</td>
<td>0.38</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.023–256</td>
<td>3</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.094–32</td>
<td>32</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>0.016–&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.032–3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Note: MIC breakpoints of selected antibiotics based on CLSI (S: susceptible; I: intermediate; R: resistant).

Amikacin (S<16 mg/l; R>64 mg/l), Ampicillin/sulbactam, (S<8 mg/l; R>32 mg/l).
Cefepime (S<8 mg/l; R=32 mg/l), Ceftazidime (S<8 mg/l; R=32 mg/l).
Ciprofloxacin (S<1 mg/l; R=4 mg/l), Colistin (S<2 mg/l; R=4 mg/l).
Gentamicin (S<4 mg/l; R>16 mg/l), Imipenem (S<4 mg/l; R>16 mg/l).
Piperacillin/tazobactam (S<16 mg/l; R=128 mg/l), Tigecycline (S<2 mg/l; R>8 mg/l by US-FDA).
agents available for use in the UMMC, it is made available when needed especially for the treatment of those with suspected or confirmed infections due to MDR Acinetobacter since 2007. Tigecycline has a higher affinity than tetracycline for binding to the ribosome and evades the tetracycline efflux pump resistance mechanisms.\(^\text{18}\) Since its introduction in the UMMC, there is a concern that resistance against tigecycline will eventually emerge.

Recent worldwide studies on the in-vitro activity of tigecycline against *Acinetobacter* spp. have revealed a variety of susceptibility patterns. The MICs ranged from 0.125 to 128 mg/l with MIC\(_{50}\) and MIC\(_{90}\) results ranging from 0.5 to 16 and 2 to 32 mg/l, respectively.\(^\text{15}\) Our study results are consistent with previous findings where the MIC of tigecycline ranged from 0.032 to 4 mg/l with MIC\(_{50}\) of 0.5 mg/l and MIC\(_{90}\) of 2 mg/l for the clinical isolates and MIC\(_{50}\) of 2 mg/l and MIC\(_{90}\) of 4 mg/l for the environmental isolates. In the present study, the MICs obtained by the E-test and the broth dilution methods were consistent. However, Liu et al., reported that only 76.6% of the cases gave consistent MICs by the two methods.\(^\text{19}\) Although our findings showed a high tigecycline susceptibility of 95% with the remaining 5% showing intermediate resistance, particularly among the clinical isolates, the emergence of highly resistant profile to tigecycline in MDR *A. baumannii* reported by Navon-Venezia et al., is a matter of high concern.\(^\text{20}\) The findings that isolates of the hospital environment also showed increased resistance to tigecycline further add to the concern. Intermediate resistance to tigecycline among the environmental isolates could be due to the continuous antibiotic selective pressure along with the harsh condition that the bacteria is being subjected to in the hospital settings.\(^\text{18,21,22}\)

The environmental and clinical TIG\(^\text{S}\) strains showed higher sensitivity to tigecycline as the bacterial growth rates showed a sharp and rapid decline soon after exposure to the antibiotic. In fact, the growth rates of the environmental TIG\(^\text{S}\) strains were more rapidly reduced compared to the TIG\(^\text{S}\) clinical strains. This reflects the acute inhibition effect of tigecycline on strains which were highly susceptible towards this antibiotic. In comparison, the TIG\(^\text{IR}\) environmental strains exhibited greater ability to attain a steady growth rate in the presence of the antibiotic than the TIG\(^\text{IR}\) clinical strains. This could indicate that these bacteria might have developed or possessed a better adaptive mechanism, which makes the bacteria have a better manoeuvre to the selective pressure of the antibiotic and the dry environmental conditions. Furthermore, these bacteria might be evolved to develop a resistant mechanism as part of a more general response to the environmental stress it experiences in the hospital settings.\(^\text{23–25}\) This finding is consistent with other studies; Nucleo et al. found that the exposure of *A. baumannii* to sub-MIC concentration of the antibiotic stimulates a bacterial regulatory elements to produce or express certain environmental survival receptors to protect itself from being killed either by antimicrobials or by desiccation and/or disinfection procedures.\(^\text{24}\) Another study has reported that the ability of *A. baumannii* to survive an external environmental stress is indicative of the fact that these bacteria must possess a regulatory mechanism to sense and respond to that kind of stress and, thus, contributing to the resistance build-up.\(^\text{25}\)

**Conclusions**

The antibiotic choices for treating infections caused by MDR *A. baumannii* is becoming increasingly limited due to the increasing antimicrobial resistance particularly to the carbapenems. Up to date, there is very limited Malaysian data to support the clinical use of tigecycline in *A. baumannii* infections. The result of the current study suggests that tigecycline in general is effective for the treatment of MDR *A. baumannii* especially against the clinical infections. Hence, tigecycline is a promising alternative to carbapenem in the treatment of nosocomial infections caused by MDR *A. baumannii*. The observed tolerance amongst the hospital environment isolates is of a real concern to the medical community as these isolates might develop resistance with ease. Judicious use of tigecycline is therefore recommended when necessary with strict control measures. The dissemination of TIG\(^\text{IR}\) *A. baumannii* strains within the hospital environments has to be aggressively controlled with strict infection control measures to prevent the risk of its transmission to hospitalized patients.

However, despite the promising *in vitro* susceptibility pattern of *A. baumannii* towards tigecycline, its effectiveness clinically needs to be evaluated.

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