

# In vitro activity of tigecycline against clinical isolates of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*, *Serratia marcescens* and *Enterobacter cloacae*

Yee-Huang Ku<sup>1</sup>, Yin-Ching Chuang<sup>2</sup>, Wen-Liang Yu<sup>3,4</sup>

<sup>1</sup>Division of Infectious Disease, Department of Internal Medicine, Chi Mei Medical Center, Liouying; Departments of <sup>2</sup>Medical Research and <sup>3</sup>Intensive Care Medicine, Chi-Mei Medical Center, Tainan; and <sup>4</sup>Department of Medicine, Taipei Medical University, Taipei, Taiwan

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**Background and Purpose:** Strains of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* have spread widely in Taiwan hospitals. In this study, we evaluated the in vitro antimicrobial activity of tigecycline against ESBL-producing *Enterobacteriaceae*, including *Klebsiella pneumoniae*, *Serratia marcescens* and *Enterobacter cloacae*.

**Methods:** 104 confirmed ESBL-producing bacteria were isolated from 4 hospitals in mid- and southern Taiwan between 2000 and 2006. The in vitro activity of tigecycline against these ESBL producers was tested by use of Etest strips.

**Results:** The minimal tigecycline concentration at which 50% of isolates were inhibited and minimal concentration at which 90% of isolates were inhibited for ESBL-producing isolates ranged from 0.38 to 0.75 µg/mL and 0.5 to 1.5 µg/mL, respectively.

**Conclusions:** Tigecycline, a new semisynthetic glycolcycline, may be considered an alternative drug of choice for patients infected with ESBL-producing bacteria.

**Key words:** beta-Lactamase resistance; beta-Lactamases; *Enterobacteriaceae*; Microbial sensitivity tests; Minocycline

## Introduction

In the current era of increasing use of broad-spectrum antimicrobial agents, the incidence of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* has increased worldwide at an alarming rate [1-3]. The multidrug-resistant properties of these bacterial isolates have limited the choice of antibiotics in clinical use, especially in nosocomial infections. Currently, carbapenems have been regarded as drugs of choice for therapy against serious infections caused by ESBL-producing organisms [3-7]. Increased use of carbapenems in areas with high prevalence of ESBL producers may be a contributory factor in the rising incidence of carbapenem-resistant *Pseudomonas*

*aeruginosa* and *Acinetobacter baumannii* [6,8]. As a result, newer agents such as tigecycline have been investigated and introduced as potential candidates for treating infections caused by ESBL producers.

The prevalence of ESBLs in Southern and Northern Taiwan is reportedly 1.5% and 5.6% in *Escherichia coli* isolates and 8.5% and 13.5%, respectively, in *Klebsiella pneumoniae* isolates [7,9,10]. Furthermore, several studies of clinical isolates of *Serratia marcescens* [11,12] and *Enterobacter cloacae* [13,14] have also identified various ESBLs in addition to the chromosomally mediated AmpC beta-lactamases. The additional ESBLs in the AmpC-producing organisms will potentially compromise the therapeutic efficacy of cefepime or ceftipime, which commonly exhibit excellent activity against the AmpC producers [15,16].

Tigecycline, a new glycolcycline antibiotic, has shown promising in vitro activity against many pathogens,

Corresponding author: Dr. Wen-Liang Yu, Department of Intensive Care Medicine, Chi-Mei Medical Center, Tainan, Taiwan.  
E-mail: Yuleon\_md@yahoo.com.tw

including multidrug-resistant strains [17-20]. In this study, we evaluated the *in vitro* activity of tigecycline against important ESBL-producing enterobacteria, including *K. pneumoniae*, *S. marcescens* and *E. cloacae*.

## Methods

### Bacterial isolates

A total of 104 clinical isolates of *Enterobacteriaceae* producing ESBL were collected from 4 hospitals and were identified individually on the basis of routine microbiologic methods, including 57 isolates of *K. pneumoniae* from Chi Mei Medical Center in southern Taiwan between March 2004 and January 2006 (Phoenix system, Becton Dickinson Company, Baltimore, MD, USA; and API 20E system, bioMérieux, Marcy l'Etoile, France), 36 isolates of *E. cloacae* from China Medical University Hospital and a district hospital in mid-Taiwan during 2000 and 2001 (VITEK<sup>®</sup> system, bioMérieux Vitek Inc, Hazelwood, MO, USA), and 11 isolates of *S. marcescens* from National Cheng-Kung University Hospital in southern Taiwan between August 1998 and August 2003 (API 20E system).

### Antimicrobial susceptibility testing

All antimicrobial compounds were provided by their respective manufacturers. Minimal inhibitory concentration (MIC) results for selected antimicrobials, including cefotaxime, cefotaxime-clavulanate, ceftazidime, ceftazidime-clavulanate, were determined by use of agar dilution methods as defined by the Clinical Laboratory Standards Institute (CLSI) [21]. The ESBL phenotype was confirmed by a reduction of at least 3 log<sub>2</sub> dilutions in an MIC for either cefotaxime (Sigma Chemical Co., St. Louis, MO, USA) or ceftazidime (Glaxo Group Research Ltd., Greenford, UK) in the presence of clavulanic acid 4 µg/mL (Sigma Chemical Co., St. Louis, MO., USA) [22]. Antimicrobial susceptibility testing of tigecycline for ESBL-producing isolates was performed by use of Etest<sup>®</sup> strips (AB BIODISK, Solna, Sweden). The antimicrobial susceptibility tests, including Etest of tigecycline, were performed at Chi Mei Medical Center. Quality control was performed by testing *Escherichia coli* American Type Culture Collection (ATCC) 25922.

## Results

A total of 104 strains of ESBL-producing *Enterobacteriaceae* isolated from 4 hospitals were tested for their

susceptibility to tigecycline using Etest strips. Table 1 indicates the ranges of MIC, MIC at which 50% of isolates were inhibited and MIC at which 90% of isolates were inhibited (MIC<sub>90</sub>) for compounds tested against ESBL-producing isolates. ESBL production among isolates was confirmed by a significant reduction of MIC level either for cefotaxime (*S. marcescens*) or ceftazidime (*E. cloacae*), or both (*K. pneumoniae*) in the presence of clavulanic acid. Around 97% of all ESBL-producing isolates were susceptible to tigecycline according to the FDA susceptibility breakpoint of 2 µg/mL [23]. However, there were 3 isolates showing higher MIC values for tigecycline, including 2 strains of *K. pneumoniae* (MICs, 3 and >256 µg/mL) and one strain of *E. cloacae* (MIC, 3 µg/mL).

## Discussion

Since the introduction of broad-spectrum cephalosporins into clinical practice in the early 1980s, the selective pressure of the use and overuse of antibiotics has resulted in the emergence of beta-lactamases [3,24], resulting in the rapid development of resistance to the expanded-spectrum beta-lactam antibiotics. Nowadays, ESBL-producing *Enterobacteriaceae* are increasing steadily and spreading worldwide [2,3]. ESBL producers have also been reported in Taiwan, and include *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli*, *E. cloacae*, *S. marcescens*, *Proteus mirabilis*, non-typhoid *Salmonella* and *Citrobacter koseri* [25]. Among hospitalized patients, the multidrug-resistant properties of the ESBLs limit therapeutic options, and there is an urgent need for new agents that are able to combat these pathogens.

Tigecycline is a glycylcycline derivative of minocycline possessing the central 4-ring carbocyclic skeleton that is essential for antibacterial activity [26]. The drug acts by binding to the bacterial 30S ribosomal subunit and by blocking entry of amino-acyl tRNA molecules into the A site of ribosomes. Amino acid residues are prevented from becoming incorporated into elongating peptide chains, thus leading to inhibition of protein synthesis [27].

Glycylcycline is bacteriostatic *in vitro* and exhibits potent activity against a broad spectrum of Gram-positive and Gram-negative bacteria, atypical and anaerobic bacteria, including strains for which the mechanisms of resistance to other antibiotics include drug efflux pumps and protection of ribosome [17-20,28]. It has promising activity against multidrug-resistant

**Table 1.** In vitro activity of tigecycline and third-generation cephalosporins with and without clavulanate against 104 extended-spectrum beta-lactamase-producing *Enterobacteriaceae* isolates

| Bacteria (no. of strains)         | MIC ( $\mu\text{g}/\text{mL}$ ) |                   |                   |
|-----------------------------------|---------------------------------|-------------------|-------------------|
|                                   | Range                           | MIC <sub>50</sub> | MIC <sub>90</sub> |
| <i>Klebsiella pneumoniae</i> (57) |                                 |                   |                   |
| Tigecycline                       | 0.19-256 <sup>a</sup>           | 0.38              | 1.5               |
| Cefotaxime                        | $\leq 0.03$ ->512               | 64                | 256               |
| Cefotaxime + clavulanate          | $\leq 0.03$ -56                 | 0.12              | 16                |
| Ceftazidime                       | 0.12->512                       | 256               | 512               |
| Ceftazidime + clavulanate         | 0.12->512                       | 4                 | 16                |
| <i>Serratia marcescens</i> (11)   |                                 |                   |                   |
| Tigecycline                       | 0.25-1.5                        | 0.75              | 1.0               |
| Cefotaxime                        | 8-256                           | 128               | 256               |
| Cefotaxime + clavulanate          | 0.5-32                          | 4                 | 32                |
| Ceftazidime                       | 1-128                           | 2                 | 16                |
| Ceftazidime + clavulanate         | 0.12-2                          | 1                 | 2                 |
| <i>Enterobacter cloacae</i> (36)  |                                 |                   |                   |
| Tigecycline                       | 0.125-3                         | 0.38              | 0.5               |
| Cefotaxime                        | 8-512                           | 16                | 128               |
| Cefotaxime + clavulanate          | 0.12-128                        | 0.5               | 64                |
| Ceftazidime                       | 8->512                          | 128               | 512               |
| Ceftazidime + clavulanate         | 2-128                           | 4                 | 32                |

Abbreviations: MIC = minimal inhibitory concentration; MIC<sub>50</sub> = MIC at which 50% of isolates were inhibited; MIC<sub>90</sub> = MIC at which 90% of isolates were inhibited

<sup>a</sup>One strain of *Klebsiella pneumoniae* showed MIC >256  $\mu\text{g}/\text{mL}$ .

isolates, including oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Enterobacteriaceae* that produce ESBLs and carbapenem-resistant *Acinetobacter* spp. [20,29-31]. Tigecycline is somewhat less active against *P. aeruginosa* and *Proteae*, as a result of bacterial resistance related to chromosomally-mediated efflux [20]. In addition, reduced susceptibility has been reported in isolates of *K. pneumoniae* and *Enterobacter* spp., mostly due to an elevated expression of ArcAB efflux pumps [32].

In Taiwan, the most prevalent types of ESBLs are SHV-5, SHV-12, CTX-M-3 and CTX-M-14 [25]. Among documented reports, SHV-5, SHV-12 or CTX-M-3 was reported as the most common ESBL in *K. pneumoniae* [9,33], while CTX-M-3 and SHV-12 were the most common ESBLs in *S. marcescens* [11,12] and *E. cloacae* [13,14], respectively. Owing to different hydrolyzing abilities of ESBLs against various beta-lactam antibiotics, certain cephalosporin resistance profiles may be helpful as a guide to the type of ESBL present. CTX-M-3-producing *S. marcescens* isolates generally showed higher levels of cefotaxime resistance than ceftazidime [11,12], while SHV-12-producing *E. cloacae* showed more resistance to ceftazidime [13, 14]. Table 1 shows the various resistance profiles to cephalosporins among strains of *K. pneumoniae*,

*S. marcescens* and *E. cloacae*, which were generally in accordance with the ESBL types previously reported.

In global surveillance studies of the in vitro activity of tigecycline against ESBL-producing *Enterobacteriaceae*, *E. coli* and *K. pneumoniae* are the only strains that have been investigated. There is no previous known study of the activity of tigecycline against ESBL-producing *Serratia* spp. and *Enterobacter* spp. [29,31]. This study is the first to document the excellent in vitro activity of tigecycline against ESBL-producing *S. marcescens* and *E. cloacae*.

The documented MIC<sub>90</sub> values of tigecycline ranged from 1 to 4  $\mu\text{g}/\text{mL}$  for ESBL isolates [29]. The clinical data we provide here show similar values for MIC<sub>90</sub>, which ranged from 0.5 to 1.5  $\mu\text{g}/\text{mL}$ . When a tigecycline susceptibility breakpoint of 2  $\mu\text{g}/\text{mL}$  was defined [23], 97% of the experimental isolates were fully susceptible to this compound, except for the 3 resistant strains, two strains of *K. pneumoniae* (MICs, 3 and >256  $\mu\text{g}/\text{mL}$ ) and one strain of *E. cloacae* (MIC, 3  $\mu\text{g}/\text{mL}$ ). This may imply that increased use of tigecycline could potentially further select resistant strains. Overall, this study demonstrates the broad applicability of tigecycline in treating infections caused by various enterobacteria producing diverse types of ESBLs.

The FDA has approved the use of tigecycline in the treatment of complicated skin/skin structure and intra-abdominal infections, and the drug can be used as monotherapy for empirical coverage of multidrug-resistant pathogens [17,31]. The major argument against the expanded availability of this compound is the potential for the development of drug-resistant strains. Furthermore, there is concern that the unrestricted availability of this agent might result in other commonly used antimicrobials, such as fluoroquinolones, cephalosporins, and carbapenems, becoming more prone to causing collateral damage in hospitalized patients [34]. Although the phenomenon of coresistance has not been observed at present, the potential for the emergence of drug resistance with tigecycline is unknown [35].

In conclusion, tigecycline has promising in vitro activity against ESBL-producing *Enterobacteriaceae* and has been a therapeutic option in the treatment of cases where multidrug-resistant pathogens are suspected. However, as with all antimicrobials, clinicians need to prescribe tigecycline appropriately, in order to avoid the emergence of resistant strains.

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