# In Vitro Activity of Tigecycline and Colistin against clinical isolates of Acinetobacter baumannii in Hospitals in Tehran and Bandar-Abbas, Iran

Shahin najar peerayeh [1], Afsaneh Karmostaji[2], Soraya sharifi sarasiabi[3], Sedigheh Javadpour[4], Parivash Davoodian[5], Nahid Moradi[6]

[1.] Ph.D. in Medical Bacteriology, Associate Professor, Department of Bacteriology, Faculty of Medical sciences, University of Tarbiat Modares, Tehran, Iran
[2.] Ph.D. in Medical Bacteriology, Assistant Professor, Infectious & Tropical Diseases Research Center , Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[3.] M.Sc. of Bacteriology, Sirjan University of Medical Sciences, Sirjan, Iran
[4.] Ph.D. in Medical Bacteriology, Associate Professor, Infectious & Tropical Diseases Research Center , Hormozgan university of Medical Sciences, Bandar Abbas, Iran
[5.] M.D. and Infectious Disease Specialist, Associate Professor, Infectious & Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[6.] M.Sc. of Bacteriology, Faculty of Medicine, Infectious & Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

**Corresponding author:**
Dr. Afsaneh Karmostaji. Infectious and Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran. Email: Afsanehkk@yahoo.com , Tel: +98.761-6666365-7 , Fax: +98761-6670724

**Abstract:**
**Background:** The *Acinetobacter* species, particularly *A. baumannii*, has emerged as one of the main causes of nosocomial infections in recent years. The high prevalence of drug resistance in *A. baumannii* limits the therapeutic options for treating infections caused by these bacteria. The objective of this study was to determine the *in vitro* activity of Tigecycline and Colistin against clinical isolates of *A. baumannii* in Tehran and Bandar Abbas, Iran.
**Methods:** This study was conducted from March 2009 to November 2010 at three hospitals in Tehran and Bandar Abbas, Iran, using 165 *Acinetobacter* species isolated from clinical specimens. All isolates were subjected to PCR to detect $bla_{\text{OXA-51}}$-like genes that are unique to *Acinetobacter baumannii*. Isolates that gave a band for the $bla_{\text{OXA-51}}$-like genes were identified as *A. baumannii*. Anti-microbial susceptibility tests were performed for Tigecycline, Colistin, and other antibiotics.
**Results:** Sensitivity rates to Colistin and Polymyxin-B were 100%. Resistance rates for Tigecycline were 4.2% in Tehran and 8.8% in Bandar-Abbas according to Jones criteria, whereas, according to U.S. FDA criteria, the resistance rates were 20.8% and 17.6%, respectively.
**Conclusions:** New alternative drugs are needed for the treatment of drug resistant *A. baumannii*. Although Colistin appears to be a good choice, adverse reactions have limited its usage. Tigecycline is effective against *A. baumannii* isolates, and it shows promise for solving the problem.

**Keywords:** Acinetobacter baumannii; Polymyxin-B, Colistin; Tigecycline

**Additional Information for citing this article:**
Title of Journal: Electronic physician; Abbreviated title of journal: Electron. Physician
doi: 10.14661/2014.919-924

**Editorial information:**
Type of article: Original
Received: March.12.2014
Revised: 1st Revision: May.01.2014; 2nd Revision: May.10.2014
Accepted: May.22.2014
Published: July.01.2014

## 1. Introduction

*Acinetobacter baumannii* is an important cause of nosocomial infections. Due to its significant ability to up-regulate or acquire resistant determinants, there are serious challenges associated with choosing and monitoring appropriate antibiotic treatments (1). Although Carbapenems are generally considered to be the most promising antibiotic to use against *Acinetobacter baumannii*, the increasing resistance to treatment with Carbapenems may lead to limitations od their use and effectiveness in some cases. The use of unconventional antibiotics, such as the Polymyxins, Rifampicin, and Tetracyclines has been described, but there are little data on *in vitro* antibiotic susceptibilities of these agents (2). Colistin appears to be very effective against multidrug-resistant (MDR) *A. baumannii* isolates *in vitro*; however, it is not used extensively in treatment because of side effects, such as neurotoxicity and nephrotoxicity (3). Colistin is now considered as the last line of treatment against MDR *Acinetobacter* infections, but there are still reports of these infections being resistant to Colistin. The mechanism of Colistin resistance is the modification of the lipid A of the outer lipopolysaccharide (LPS) membrane. More recently, it has been identified that mutations in PmrA and PmrB and complete loss of LPS production may mediate Colistin resistance by *A. baumannii* (4).

A new Glycycline, Tigecycline also was found to have an excellent *in-vitro* activity against *A. baumannii* isolates. Tigecycline was licensed for use in the United States and Latin America in 2005, and it was licensed in Canada, Europe, the Asia/Pacific region, and the Middle East in 2006 and 2007 (5). Tigecycline is a substrate for the Ade ABC efflux pump system, and resistance to it can be mediated by efflux or ribosomal protection (6). Tigecycline resistance is rare. With the exception of the *Pseudomonas* species, Tetracycline-resistant bacteria are often sensitive to Tigecycline, and a broad spectrum of activity has been demonstrated against gram-negative bacteria, even extended-spectrum b-lactamase (ESBL)–producing *Escherichia coli* and *Klebsiella pneumonia* (7). Increasing Carbapenem resistance among *A. baumannii* isolates has led clinicians to look for new treatment alternatives. Many Carbapenemase-producing *A.baumannii* isolates are also resistant to all available antibiotics except Polymyxins. Tigecycline, which is active against most Carbapenemase-producing strains, may be a useful alternative to Polymyxins (3), but very little data have been published on the anti-microbial resistance among isolates of *Acinetobacter* species to Colistin and Tigecycline. The knowledge of changes in the resistance rates of bacteria in our local hospitals will improve empirical therapy. Since the mobility of the population is a risk factor for the distribution of antimicrobial, drug–resistant organisms, this study was conducted to evaluate the *in vitro* activity of Tigecycline and Colistin against clinical isolates of *A. baumannii* in hospitals of Tehran and Bandar-Abbas, two cities in Iran that have many immigrants.

## 2. Materials and methods

### 2.1. Hospital setting and collection of bacteria

The study was conducted from March 2009 to November 2010 at hospitals in Tehran and Bandar-Abbas, Iran. The capacities of the hospitals vary from 400 to 1000 beds. The clinical *Acinetobacter* isolates were identified by conventional methods (8), stored in sterile trypticase soy broth with 30% glycerol, and preserved at -70 °C. *Acinetobacter* isolates were collected from affected patients, (i.e., sputum, urine, cerebrospinal fluid, ascites, and pleural effusion).

### 2.2. Detection of the *bla*$_{\text{OXA-51}}$-like gene to identify the *Acinetobacter baumannii* species

All isolates were subjected to PCR to detect the *bla*$_{\text{OXA-51}}$-like gene that is unique to the *Acinetobacter baumannii* species (9). DNA was extracted from the strains by the boiling method. The PCR assay was conducted using the primer *bla* $_{\text{oxa-51}}$-like (353bp 5′ F- TAA TGC TTT GAT CGG CCT TG-3′ and 5′ R-TGG ATT GCA CTT CAT CTT GG-3′). PCR reactions were conducted in 25-µl reaction volumes with 3 µl of extracted DNA, 10 pmol of each primer, and 0.2 µl of 500 u Taq DNA polymerase in 10x PCR buffer containing 1 mM MgCl2 and 0.5 µl of a 25-mM solution of deoxynucleoside triphosphate. Conditions for the PCR were 95 °C for 4 min and then 34 cycles at 95 °C for 45 s, 34 cycles at 52 °C for 45 s, and 34 cycles at 72 °C for 30 s. This was followed by a final extension at 72 °C for 10 min (10).

### 2.3. Susceptibility to Tigecycline and Colistin

The susceptibility tests for Tigecycline and Colistin were performed in the Microbiology Laboratory at Tarbiat Modares University using the disk agar diffusion (DAD) method, as recommended by the Clinical and Laboratory Standards Institute (11). *E. coli* ATCC 25922 was used as quality control strain. Tigecycline and Colistin disks were obtained from Mast Pharmaceutical Inc. U.K. Since no interpretive criteria have been approved for Tigecycline against *Acinetobacter* species, the results were interpreted by the criteria ( $S \geq 16$mm; I = 13-15 mm; $R \leq 12$ mm)

recommended by Jones et al. (12) and by criteria recommended by the U.S. FDA for Enterobacteriaceae, i.e., S ≥ 19 mm; I = 15-18 mm; R ≤ 14 mm) (Tygacil package insert [June, 2005], Wyeth Pharmaceuticals, Inc., Philadelphia, PA) (13).

**2.4. Susceptibility to antibiotics other than Tigecycline and Colistin**

We collected the data of the antibiogram pattern of the *A. baumannii* isolates to commonly-used antibiotics, e.g., Imipenem, Ciprofloxacin, Amikacin, Cefepime, Ceftazidime, and Polymyxin-B. The susceptibility testing of these antibiotics was performed by the disk diffusion method on Mueller-Hinton agar plates. The disks were obtained from Mast Pharmaceutical, Inc. U.K.

**3. Results**

This study included 165 *Acinetobacter* isolates that were collected from hospitals in Tehran and Bandar-Abbas. One hundred and fifty-seven isolates (95.15%) that gave a band for $bla_{\text{OXA-51}}$-like identified as *A.baumannii*, Figure (1). Thirty-four isolates were from Bandar-Abbas, and 123 were from Tehran. Nine of the isolates were from sputum (5.7%), 25 were from pus (15.1%), 12 were from urine (7.64%), four were from cerebrospinal fluid (2.54%), two were from ascites (1.27%), two were from ears (1.27%), three were from catheters (1.9%), 24 were from burn wounds (15.28%), and 84 were from pleural effusion (53.5%). The quantities of isolates related to wards were as follows: Among the isolates 114(72.6%) were from intensive care unit, 26 (16.56%) were from internal ward, 9 (5.73%) were from surgical ward, 3 (1.9%) from neaurosurgery ward, 2 (1.27%) from pediatric ward, 7 (4.45%) from emergency unit, 1 (1.9%) from coronary care unit, and 2 (2.27%) from ear, nose, and throat ward. For the two cities, Table 1 shows the results of susceptibility to Tigecycline and compares the susceptibility rates of the isolates to eight antibiotics.

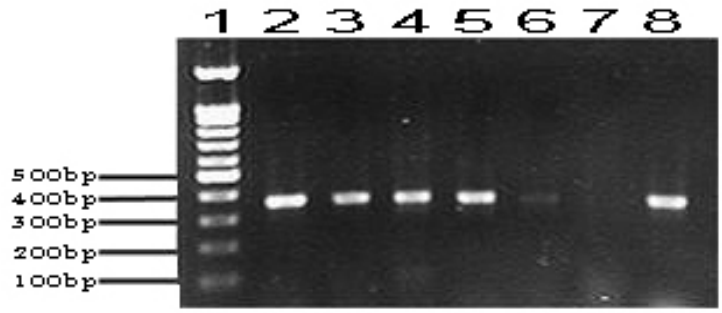


**Figure 1.** Polymerase chain reaction bla $-_{\text{oxa-51}}$ like gene (353 bp), line1:100-bp ladder, line 8: positive control, line 7: negative control and lines 2-6 subjects

**Table 1**. Susceptibility rates of *A. baumannii* isolates (n = 157) for eight anti-microbials in two cities in Iran

| City | Tehran n = 120 | | | Bandar-Abbas n = 34 | | |
|---|---|---|---|---|---|---|
| antibiotics | S | I | R | S | I | R |
| Colistin | 120 (100%) | 0 | 0 | 34 (100%) | 0 | 0 |
| Polymyxin B | 120 (100%) | 0 | 0 | 34 (100%) | 0 | 0 |
| imipenem | 31 (25.8%) | 25 (20.8%) | 64 (53.3%) | 11 (32.3%) | 11 (32.3%) | 12 (35.3%) |
| Ciprofloxacin | 5 (4.2%) | 0 | 115 (95.8%) | 3 (8.8%) | 0 | 31 (91.2%) |
| Amikacin | 51 (42.5%) | 8 (6.7%) | 61 (50.8%) | 17 (50%) | 1 (2.9%) | 16 (47%) |
| cefepime | 7 (5.8%) | 16 (13.3%) | 97 (80.8%) | 3 (8.8%) | 4 (11.8%) | 27 (79.4%) |
| ceftazidime | 6 (5%) | 0 | 114 (95%) | 2 (5.9%) | 1 (2.9%) | 31 (91.2%) |
| Tygecycline Jones criteria | 80 (66.7%) | 35 (29.2%) | 5 (4.2%) | 26 (76.5%) | 5 (14.8%) | 3 (8.8%) |
| Tygecycline FDA criteria | 29 (24.2%) | 66 (55%) | 25 (20.8%) | 8 (23.5%) | 20 (58.8%) | 6 (17.6%) |

## 4. Discussion

A high prevalence of multi-drug resistance by *A. baumannii* limits the therapeutic options for the treatment of infections caused by these bacteria. It is clear that new drugs are needed for the treatment of MDR A. *baumannii.* In some studies, Tigecycline has been found to be active in about 87 to 93% in of *Acinetobacter* species (3). In our study, according to the Jones criteria, the susceptibility rate to Tigecycline in the hospital in Tehran was 66.7%, and it was 76.5% in the hospital Bandar-Abbas. However, based on the FDA criteria, the rates were 24.2% in Tehran and 23.5% in Bandar-Abbas. All isolates (100%) were susceptible to Colistin and polymyxin B. Several studies have reported the susceptibility to Colistin as 98 to 100% (3). There are two problems in evaluating the effectiveness of Tigecygline against *Acinetobacter baumannii*. First, the U.S. Food and Drug Administration (FDA) has not assigned Tigecycline breakpoints for *Acinetobacter* species, and no interpretive criteria are currently available (7). The U.S. FDA-approved breakpoints of Tigecycline were defined against *Enterobacteriaceae*, and the diameter of the inhibition zone is ≥ 19 mm and MIC ≤ 2 mg/L (14). The second problem, identified by Surapee in Thailand, is that the U.S. FDA-approved breakpoint of Tigecycline against *Enterobacteriaceae* was not applicable to Tigecycline against *A. baumannii*. The breakpoint for an inhibition zone diameter of ≥13 mm was more accurate in predicting the susceptibility of *A. baumannii* to Tigecycline with a sensitivity of 99% and a specificity of 100% (15). In Bouchillon's study, Tigecycline was the most active drug against all species of *Acinetobacter* (7).

The Tigecycline MIC90 of 2 µg/ml was 16-fold and 64-fold more potent than the b-lactams and Carbapenems, respectively, against all tested strains. Tigecycline was 8-fold and 16-fold more potent against *Acinetobacter* species than Levofloxacin and Doxycycline, respectively (7). A Taiwanese study showed that the overall susceptibility rate of *A. baumannii* to Tigecycline was 80.9%. The susceptibility rates determined in various hospitals to Tigecycline were 37-100%. The rates of susceptible, intermediate, and resistant isolates by the disk diffusion method, using the criteria of Jones et al., were 88.3, 9.9, and 1.8%, whereas they were 44.0, 51.7, and 4.3% by the U.S. FDA's criteria. By comparison with their previous report on Tigecycline activity against *A. baumannii* isolates in Taiwan, they observed a seven-fold increase (from 3% in 2003 to 20% in 2007) in the number of *A. baumannii* isolates that are not susceptible to Tigecycline (13). Since the most probable resistance mechanism for Tigecycline in *A. baumannii* is the presence of an efflux pump mechanism, exposure to sub-therapeutic levels of Tigecycline for even short periods of time may promote the rapid emergence of Tigecycline resistance and cause therapeutic failure. In our study, a possible explanation for increased Tigecycline resistance was the exposure to multiple antibiotics in our hospitals in which the efflux systems of these strains already are highly active. The disk diffusion method is the most commonly used (> 90%) susceptibility method in our hospitals, since automated susceptibility test systems are not available in most microbiology laboratories. This situation also exists in other countries (16). Therefore, selection of appropriate criteria for the disk diffusion method is crucial. There was no resistance to Colistin in our study. It appeared to be a good option in the treatment of MDR *A. baumannii*, but adverse reactions limit its extensive use. However, Colistin resistance is rare worldwide at the current time, which likely reflects its very limited use. Colistin-resistant isolates have recently been identified in several Gram-negative species, such as *A. baumannii*, *Klebsiella pneumoniae*, and *P. aeruginosa* (4). Only sporadic cases of Colistin resistance have been reported in the literature in clinical isolates of patients admitted to the ICUs of an Argentinian University Hospital. Colistin hetero-resistance was observed in 4% of these isolates (17).

## 5. Conclusions

The findings of this study showed that Colistin and Polymyxin-B still show the highest potency against *A.baumannii* in our hospitals. However, the resistance of *A.baumannii* to Tigecycline may limit their use in the future. The broad-spectrum *in vitro* activity of Tigecycline and Colistin may make them a suitable candidate for use in the empirical treatment of serious infections. However, it should be noted that *in vitro* data do not equate to clinical efficacy, and resistance rates should be monitored continuously. Finding cases of resistance to Tigecycline suggests an efflux-based mechanism, and further work is ongoing to elucidate this mechanism.

**Acknowledgments:**
This research was funded by the medical faculty of Tarbiat Modares University.

**Conflict of Interest:**
There is no conflict of interest to be declared.

**Authors' contributions:**
All of authors contributed to this project and article equally. All authors read and approved the final manuscript.

**References:**


1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clinical microbiology reviews. 2008;21(3):538-82. Epub 2008/07/16. PMCID: 2493088. PMID:18625687. DOI: 10.1128/CMR.00058-07, URL: http://dx.doi.org/10.1128/CMR.00058-07.
2. Tan TY, Ng LS, Poh K. Susceptibility testing of unconventional antibiotics against multiresistant Acinetobacter spp. by agar dilution and Vitek 2. Diagnostic microbiology and infectious disease. 2007;58(3):357-61. Epub 2007/05/01. PMID:17467946. DOI: 10.1016/j.diagmicrobio.2007.02.008, URL: http://dx.doi.org/10.1016/j.diagmicrobio.2007.02.008.
3. Dizbay M, Altuncekic A, Sezer BE, Ozdemir K, Arman D. Colistin and tigecycline susceptibility among multidrug-resistant Acinetobacter baumannii isolated from ventilator-associated pneumonia. International Journal of Antimicrobial Agents. 2008;32(1):29-32. PMID:18539006. DOI: v, URL: http://dx.doi.org/http://dx.doi.org/10.1016/j.ijantimicag.2008.02.016.
4. Park YK, Choi JY, Shin D, Ko KS. Correlation between overexpression and amino acid substitution of the PmrAB locus and colistin resistance in Acinetobacter baumannii. International Journal of Antimicrobial Agents. 2011;37(6):525-30. PMID: 21497062. DOI: 10.1016/j.ijantimicag.2011.02.008, URL: http://dx.doi.org/http://dx.doi.org/10.1016/j.ijantimicag.2011.02.008.
5. Wang YF, Dowzicky MJ. In vitro activity of tigecycline and comparators on Acinetobacter spp. isolates collected from patients with bacteremia and MIC change during the Tigecycline Evaluation and Surveillance Trial, 2004 to 2008. Diagnostic microbiology and infectious disease. 2010;68(1):73-9. Epub 2010/08/24. PMID: 20727474. DOI: v, URL: http://dx.doi.org/10.1016/j.diagmicrobio.2010.04.002.
6. Jamal W, Salama M, Dehrab N, Al Hashem G, Shahin M, Rotimi VO. Role of tigecycline in the control of a carbapenem-resistant Acinetobacter baumannii outbreak in an intensive care unit. Journal of Hospital Infection. 2009;72(3):234-42. http://dx.doi.org/http://dx.doi.org/10.1016/j.jhin.2009.03.023.
7. Bouchillon SK, Hoban DJ, Johnson BM, Stevens TM, Dowzicky MJ, Wu DH, et al. In vitro evaluation of tigecycline and comparative agents in 3049 clinical isolates: 2001 to 2002. Diagnostic microbiology and infectious disease. 2005;51(4):291-5. PMID: 15808321. DOI: 10.1016/j.diagmicrobio.2004.11.006, URL: http://dx.doi.org/http://dx.doi.org/10.1016/j.diagmicrobio.2004.11.006.
8. Schreckenberger PC vGA. Manual of Clinical Microbiology,7th ed. 1997: 539-60.
9. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by Detection of the blaOXA-51-like Carbapenemase Gene Intrinsic to This Species. Journal of Clinical Microbiology. 2006;44(8):2974-6. PMID:16891520. PMCID: 1594603. DOI: 10.1128/jcm.01021-06, URL: http://dx.doi.org/10.1128/jcm.01021-06.
10. Feizabadi MM, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghifard N, Aligholi M, et al. Antimicrobial susceptibility patterns and distribution of blaOXA genes among Acinetobacter spp. Isolated from patients at Tehran hospitals. Japanese journal of infectious diseases. 2008;61(4):274-8. Epub 2008/07/26. PMID: 18653968
11. PA W. Clinical and Laboratory Standard Institute: Performance standards for antimicrobial susceptibility testing, Seventeenth informational supplement. CLSI document M100 S17. USA 2007
12. Jones RN, Ferraro MJ, Reller LB, Schreckenberger PC, Swenson JM, Sader HS. Multicenter studies of tigecycline disk diffusion susceptibility results for Acinetobacter spp. J Clin Microbiol. 2007;45(1):227-30. Epub 2006/11/10. PMCID: 1828968. PMID:17093026. DOI: 10.1128/jcm.01588-06, URL: http://dx.doi.org/10.1128/jcm.01588-06.
13. Liao CH, Kung HC, Hsu GJ, Lu PL, Liu YC, Chen CM, et al. In-vitro activity of tigecycline against clinical isolates of Acinetobacter baumannii in Taiwan determined by the broth microdilution and disk diffusion methods. Int J Antimicrob Agents. 2008;32 Suppl 3:S192-6. Epub 2008/12/17. DOI: 10.1016/s0924-8579(08)70027-x , URL: http://dx.doi.org/10.1016/s0924-8579(08)70027-x.
14. Halstead DC, Abid J, Dowzicky MJ. Antimicrobial susceptibility among Acinetobacter calcoaceticus-baumannii complex and Enterobacteriaceae collected as part of the Tigecycline Evaluation and Surveillance Trial. The Journal of infection. 2007;55(1):49-57. PMID: 17250897. Epub 2007/01/26. DOI: 10.1016/j.jinf.2006.11.018, URL: http://dx.doi.org/10.1016/j.jinf.2006.11.018.
15. Tiengrim S, Tribuddharat C, Thamlikitkul V. In vitro activity of tigecycline against clinical isolates of multidrug-resistant Acinetobacter baumannii in Siriraj Hospital, Thailand. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2006;89 Suppl 5:S102-5. Epub 2007/08/28.
16. Curcio D, Fernandez F, Jones RN, Ferraro MJ, Reller LB, Schreckenberger PC, et al. Tigecycline disk diffusion breakpoints of Acinetobacter spp.: a clinical point of view. J Clin Microbiol. 2007;45(6):2095;

author reply -6. Epub 2007/06/06. PMCID: 1933103. DOI: 10.1128/jcm.00107-07, URL: http://dx.doi.org/10.1128/jcm.00107-07.

17. Rodriguez CH, Bombicino K, Granados G, Nastro M, Vay C, Famiglietti A. Selection of colistin-resistant Acinetobacter baumannii isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. Diagnostic microbiology and infectious disease. 2009;65(2):188-91. Epub 2009/09/15. PMID: 19748431. DOI: 10.1016/j.diagmicrobio.2009.05.019, URL: http://dx.doi.org/10.1016/j.diagmicrobio.2009.05.019.