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

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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 blaOXA-51-like genes that are unique to Acinetobacter baumannii. Isolates that gave a band for the blaOXA-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

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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 of 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 Glycyccline, 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 trypctase 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 blaOXA-51-like gene to identify the Acinetobacter baumannii species

All isolates were subjected to PCR to detect the blaOXA-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_oxa-51_like (353bp 5 end TAA TGC TTT GAT CGG CCT TG-3 and 5-R TG 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 25mM 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 ≥ 16mm; I = 13-15 mm; R ≤ 12 mm)

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recommended by Jones et al. (12) and by criteria recommended by the U.S. FDA for Enterobacteriaceae, i.e., \( S \geq 19 \) mm; \( I = 15-18 \) mm; \( R \leq 14 \) 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 \textit{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 \textit{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 \textit{bla}\textsubscript{OXA-51}-like identified as \textit{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 neurosurgery 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\textsubscript{OXA-51} like gene (353 bp), line1:100-bp ladder, line 8: positive control, line 7: negative control and lines 2-6 subjects](image)

| Antibiotics   | City          | Tehran n = 120 | Bandar-Abbas n = 34 |
|---------------|---------------|----------------|---------------------|
|               | 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 Tigecycline 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.

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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.
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