



Emergence of Tigecycline Resistant *Acinetobacter baumannii* From an Intensive Care Unit (ICU) in Tehran

Afsaneh Karmostaj¹, Shahin Najar Peerayeh^{1,*}, Ali Hatf Salmanian²

¹ Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, IR Iran

² National Institute of Genetic Engineering and Biotechnology, Tehran, IR Iran

*Corresponding author: Shahin Najar Peerayeh, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, IR Iran. Tel.: +98-2182883870, 82883560, Fax: +98-2182884555, E-mail: najarp_s@modares.ac.ir.

ABSTRACT

Background: *Acinetobacter* species, specially, *Acinetobacter baumannii*, is known, as an important opportunistic pathogen, with a variety of infections such as pneumonia, bacteraemia, meningitis, urinary tract, skin and soft tissue infections, associated with high mortality. High prevalence of multidrug resistance in *A. baumannii*, limits our therapeutic choices in the treatment of infections caused by this bacterium.

Objectives: The current study aimed to determine in vitro activity of tigecycline and colistin against clinical isolates of MDR *A. baumannii*, from patients admitted in ICUs Tehran hospitals.

Material and Methods: This study was conducted from March 2009 to November 2010, on a total of 91 *Acinetobacter* species isolated from clinical specimens in ICUs. All isolates were subjected to PCR to detect *blaOXA-51*-like gene that is unique to *A. baumannii*. The antimicrobial susceptibility for 13 different antibiotics was tested.

Results: *A. baumannii* (*blaOXA-51*-like gene) was detected in 84 (92.30%) isolates. Resistance rates in *A. baumannii*, were found to be for Imipenem 50 (59.52%), Gentamicin 65 (77.38%), Ciprofloxacin 81 (96.42%), Amikacin 44 (52.38%), Cefotaxime 81 (96.42%), Cefepime 69 (82.14%), Ceftazidim 81 (96.42%), Meropenem 74 (88.09%), Trimethoprim - sulfamethoxazole 78 (92.85%), Aztreonam 82 (97.61%), Colistin and Polymyxin-B 0%. No interpretive criteria have been approved for tigecycline against *Acinetobacter* spp. so; the results were interpreted by the criteria recommended by Jones, and US FDA for *Enterobacteriaceae*. Resistance rates for tigecycline were 3 (3.57%) (Jones criteria) and 19 (22.61%) (FDA criteria).

Conclusions: It is clear that new antimicrobials are needed to treat MDR *A. baumannii*. Polymyxins and tigecycline are among the few antibiotics available to treat infections with these bacteria but little was known about the antimicrobial activity of these agents. The Present study provided valuable information about the effects of the above mentioned drugs that can be used for health policy. It should be noted that there is a need for regular surveillance of bacterial resistance to these antimicrobial agents.

Keywords: *Acinetobacter baumannii*; Colistin; Tigecycline; FDA criteria

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►Implication for health policy/practice/research/medical education:

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1. Background

Acinetobacter baumannii, because of its ability to up regulate or acquire resistance determinants, is considered as one of the most problematic pathogens for health care institutions, it often infects immune compromised patients, especially in intensive care units (1). *A. baumannii* is responsible for a variety of nosocomial infections, including bacteraemia, urinary tract infections, diabetic ulcers, pneumonia, especially in mechanically ventilated patients and intravenous devices infections. Mortality rates range from 19% to 54 % (2). Although carbapenems are generally considered to be the most active antibiotic, Multidrug-resistant (MDR), pan drug-resistant (PDR) and extremely drug-resistant (XDR) strains which are increasingly being reported, lead to an almost complete lack of choices in the treatment of serious infections (3).

The use of unconventional antibiotics such as the polymyxin, rifampicin, and tetracyclines, has been described, but there are little data on in vitro activities of these agents (4). Tigecycline, a new glycylglycyl, was found to have excellent in vitro activity against multidrug resistant isolates. Tigecycline, obtained the license to be used in the United States in 2005, in Latin America and North America over 2005 (United States) and 2006 (Canada), in Europe and Asia/Pacific in 2006, and in the Middle East over 2006 and 2007 (5). Resistance to tetracycline and its derivatives is due to efflux pumps or ribosomal protective mechanisms. This agent is a substrate for AdeABC efflux pump system (6).

Tigecycline resistance is rare, with the exception of *Pseudomonas* species; tetracycline-resistant bacteria with either tetracycline efflux pumps or ribosomal protective mechanisms are sensitive to tigecycline. Broad-spectrum activity of this agent has been reported against Gram-negative bacteria, even extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* (7). Increasing carbapenems resistance among *A. baumannii* isolates has led health personnel to search for new treatment. Many carbapenemase-producing *A. baumannii*, are resistant to all available agents except for polymyxins and tigecycline, but little data have been published on the antimicrobial resistance among isolates of *Acinetobacter* species to these agents (8).

2. Objectives

The current study aimed to determine in vitro activity of tigecycline and colistin against clinical isolates of MDR *A. baumannii*, from patients admitted in ICUs of Tehran hospitals.

3. Materials and Methods

Hospital setting and collection of bacteria: The study was conducted from March 2009 to November 2010, in patients attending in ICUs of two educational hospitals

in Tehran which their capacities varied from 500 to 1000 beds.

Bacterial isolates: The clinical *Acinetobacter* isolates were identified by conventional testing methods, Gram negative coccobacillary rods that initially appeared in direct smears as Gram positive cocci were found non motile on SIM medium, oxidize negative, non fermentative on TSI and OF mediums (9). *Acinetobacter* isolates were stored in sterile trypticase soy broth with 30% glycerol, before being preserved at -70°C. These isolates had been collected from specimens of affected patients, (i.e. sputum, urine, cerebrospinal fluid, ascitis and pleural effusion). To avoid duplication, when more than one *Acinetobacter* isolates were concurrently found in an individual patient, only one of the isolates was included in the study.

Molecular detection of blaOXA-51-like gene: All isolates were subjected to PCR to detect blaOXA-51-like gene that is unique to *A. baumannii* species (10, 11). DNA was extracted from the isolates by boiling method. Five colonies were mixed in 200 μ l sterile ultrapure water. After centrifugation in 8000 rpm for 4 minutes, supernatant was removed. 200 μ l sterile ultra pure water was added again, followed by boiling for 10 minutes, then cooling in ice for 10 minutes and centrifugation for 3 min at 8000rpm. Supernatant was used as DNA template in PCR experiments. PCR assay was run using the primer blaOXA-51-like (OXA-51-F 5'-TAA TGC TTT GAT CGG CCT TG-3' and OXA-51-R 5'-TGG ATT GCA CTT CAT CTT GG-3'). PCRs were carried out in 25 μ l reaction volumes with 50 ng of extracted DNA, 10 pmol of each primer, and 1.5 U of Taq DNA polymerase in 1x PCR buffer containing 1.5 mM MgCl₂ and 200 μ M of each deoxynucleoside triphosphate. Conditions for the PCR were as follows: 95°C for 4 min, and then 34 cycles at 95°C for 45 s, 52°C for 45 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min (12).

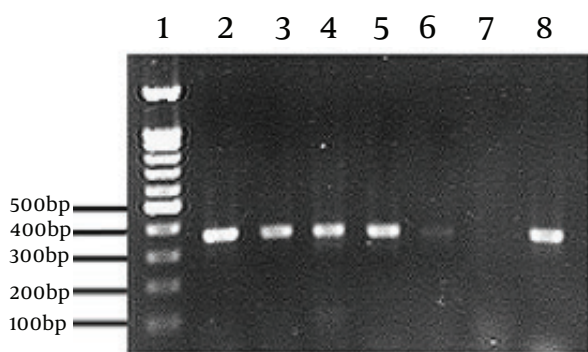
Antimicrobial susceptibility testing: Susceptibility to tigecycline, colistin and other conventional antibiotics was performed by the disk diffusion method as recommended by the clinical and laboratory standards institute (CLSI). Tigecycline (15 μ g), Colistin (10 μ g), Imipenem (10 μ g), Meropenem (10 μ g), Gentamicin (10 μ g), Ciprofloxacin (5 μ g), Amikacin (30 μ g), Cotrimoxazole (25 μ g), Cefepime (30 μ g), Cefotaxime (30 μ g), Aztreonam (30 μ g), Ceftazidime (30 μ g), and Polymyxin B (300U) were obtained from mast Pharmaceutical Inc. U.K. Quality control was performed by testing the susceptibility of *Escherichia coli* ATCC 25922 (13).

4. Results

A total of 91 *Acinetobacter* isolates were collected from patients attending in ICUs of Tehran hospitals. The blaOXA-51-like gene was amplified from genomic DNA to identify *A. baumannii*. 84 isolates (92.30%) that gave a band for bla-

OXA-51-like (353 bp), were identified as *A. baumannii* (Figure 1). The sources of the *A. baumannii* isolates were sputum 1 (1.09%), pus 7 (7.69%), urine 4 (4.39%), and cerebrospinal fluid 3 (3.29%), ear 2 (2.19%) catheter 3 (3.29%),

Figure 1. Detection of blaOXA-51-like Gene (353 bp) by Polymerase Chain Reaction.



Line 1; 100 bp ladder, line 8; positive control, line 7; negative control, lines 2-6; positive samples

wound 12 (13.18%), ascitis 1 (1.09%) and pleural effusion 58 (63.73%). Resistance rates in *A. baumannii* were found

to be for, Imipenem 50 (59.52%), Gentamicin 65 (77.38%), Ciprofloxacin 81 (96.42%), Amikacin 44(52.38%), Cefotaxime 81 (96.42%), Cefepime 69 (82.14%), Ceftazidim 81 (96.42%), Meropenem 74 (88.09%), Trimethoprim-sulfamethoxazole 78 (92.85%), Aztreonam 82 (97.61%). None of the isolates were resistant to Colistin and Polymyxin B. No interpretive criteria have been approved for tigecycline against *Acinetobacter* species by FDA (4). However, there were the criteria recommended by the US FDA for *Enterobacteriaceae* [Tygacil package insert (S \geq 19mm; I;15-18mm; R \leq 14mm)], and also, Jones in 2005 had recommended the criteria for interpretation of results for *Acinetobacter* species (S \geq 16mm; I;13-15mm; R \leq 12mm) (14). Results of the susceptibility test to tigecycline based on two criteria, are shown in Table 1. A characteristic of tigecycline resistance *A. baumannii* isolated from I.C.U is shown in Table 2.

Table 1. Tigecycline Susceptibility Based on Jones and FDA Criteria

| Criteria | Susceptible, No. (%) | Intermediate, No. (%) | Resistant, No. (%) |
|----------|----------------------|-----------------------|--------------------|
| Jones | 55 (65.47) | 26 (30.95) | 3 (3.57) |
| FDA | 19 (22.61) | 46 (54.76) | 19 (22.61) |

Table 2. Characteristics of Tigecycline Resistance *A. baumannii* Isolated From I.C.U

| Isolate | Hospital | Sex | Site of Isolation | Pattern of Resistance ^a |
|----------------|----------|-----|-------------------|---------------------------------------|
| 1 | A | F | Burn | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 2 ^b | A | F | Catheter | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 3 | B | F | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 4 ^b | B | F | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 5 | B | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 6 ^b | B | F | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 7 | B | F | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 8 | A | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM |
| 9 | B | M | Pus | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, AN |
| 10 | B | M | Urine | MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 11 | B | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ, CTX,ATM, GM,AN |
| 12 | A | M | Pleural effusion | MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM,AN |
| 13 | A | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, AN |
| 14 | B | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM |
| 15 | B | M | Burn | IPM,MEN,CP,CAZ,CPM,CTX,ATM, AN |
| 16 | A | M | Urine | MEN,SXT,CP,CAZ,CPM,CTX,ATM, GM |
| 17 | A | M | Pleural effusion | IPM,MEN,SXT,CP,CAZ,CPM,CTX,ATM |
| 18 | A | F | Burn | MEN,SXT,CP,CAZ,CPM,CTX, GM |
| 19 | A | F | Burn | SXT,CP,CAZ,CPM,CTX, GM |

^a Abbreviations: CAZ, Ceftazidime; CTX, Cefotaxime; CPM, Cefepime; ATM, Aztreonam, IPM, Imipenem; MEM, Meropenem; GM, Gentamicin; AN, Amikacin; SXT, Trimethoprim-sulfamethoxazole; CP, Ciprofloxacin

^b Isolates that was positive according to jones criteria

5. Discussion

Since *A. baumannii* is the most clinically important of the *Acinetobacter* species, the ability to distinguish it rapidly from other members of the genus would be valuable. Since the *bla_{OXA-51}*-like genes are consistently found and are also unique to *A. baumannii* species, their detection could provide a simple method of identifying *A. baumannii* which would be more reliable than biochemical identification (11). In the current study, 92.30% of clinical *Acinetobacter* isolates, gave a band for *bla_{OXA-51}*-like and were identified as *A. baumannii*. In other studies, different levels of *bla_{OXA-51}* were acquired. Therefore, 77.8% in Turkey 2003 (15), 89.41% in the U.K 2006 (11), and 84.37% in Iran 2008, were positive for *bla_{OXA-51}* and identified as *A. baumannii* (12).

The current study indicated an increased prevalence of *A. baumannii*. Explanation of this difference could be that our isolates were collected from ICUs, but in other studies *A. baumannii* was isolated from different wards of hospitals. Another point in the current study was that, 10 (31.25%) isolates from A Hospital, and 52 (26.92%) from B Hospital were MDR with resistance to Ciprofloxacin, Imipenem, Gentamicin, Cefotaxime, Cefepime, Ceftazidim, Amikacin, Aztreonam, Trimethoprim-sulfamethoxazole, and Meropenem.

Outbreaks of multidrug and pan drug-resistant isolates are increasingly being reported (14). Different definitions of the terms multidrug-resistant (MDR) and pan drug-resistant (PDR) *A. baumannii* have been used in the literature, but the majority, define MDR as resistant to three or more antimicrobial classes or resistant to one key antimicrobial agent and pan drug as 'resistant to all antibiotic classes available for empirical treatment (16). Initial reports about multi-resistant, carbapenem-resistant *A. baumannii* infections were in the New York hospital in 1991 (17). In the United States, in 1998, 1999, and 2000, among isolates from ICU patients, 11.6%, 15.4%, and 26.5% were MDR, respectively (18).

Since then, carbapenem-resistant *A. baumannii* have been reported from other parts of the world, such as Spain, Belgium, Brazil, Cuba, England, France, Hong Kong, Kuwait, Singapore, and Argentina (17). Shiraz study, also reported 16.7% imipenem-resistant *Acinetobacter* strains, in ICUs (19), which is close to the rate (17.1%) reported in a European study in 2006 (20). In the study conducted in Tehran Hospitals, high rates of resistance to imipenem (52.5%), meropenem (52.5%), and cefotaxime (92.5%) were observed. Polymyxin B and tigecycline showed good antimicrobial activities (91.2% sensitivity) (20).

In the current study, pan drug-resistant isolates in both hospitals have been isolated. Furthermore, the sensitivity rates of isolates to tigecycline by FDA criteria were below 30% (22.61%), and the susceptibility rates of these isolates to imipenem were below 30%. Clonal spreading of imipenem and tigecycline-resistant isolates could be

a cause of this phenomenon, and molecular typing study is needed to define this phenomenon. The SENTRY Antimicrobial Surveillance Program lists *Acinetobacter* spp. as the eighth most common organism (4.0%) isolated from intensive-care unit patients.

It is clear that new drugs are required to be replaced for the treatment of MDR and PDR *A. baumannii*. Tigecycline was found to be active in 86.7–93.3% of *Acinetobacter* species in different studies (20, 21). In the current study, the susceptibility rates to tigecycline, according to Jones criteria, were 55 (65.47%), and based on FDA criteria were 19 (22.61%). This result is in agreement with those obtained in the European countries (21, 22). Similar findings have also been reported from Turkey, where tigecycline has been found the most effective antibiotic against MDR strains of *Acinetobacter*, including the ones producing metallo beta lactamase (23).

Unfortunately, as Table 2 shows, most of tigecycline resistance strains also show resistance to other antibiotic including carbapenems. It indicates that, limitation in therapeutic options is serious and strict infection control policy is necessary to be implemented in the hospitals. The most important issue to evaluate tigecycline against *A. baumannii* is that FDA has not provided any proprietary tigecycline breakpoints for *Acinetobacter* species (7). Data obtained from the current, and other studies indicated increased levels of intermediate and resistant results according to FDA criteria for *Acinetobacter*, therefore, this measure may not be suitable for *Acinetobacter*.

In a Taiwanese study the rates of susceptible, intermediate and resistant isolates by the disk diffusion method using Jones criteria, were 88.3%, 9.9% and 1.8% respectively, and by the US FDA criteria 44.0%, 51.7% and 4.3%, respectively (24). In Argentina, using FDA- breakpoints, resistance rate to tigecycline was 26% but the same resistance rate using Jones breakpoint was 3%. (25). Since the most probable resistance mechanism for tigecycline in *A. baumannii* is the presence of an efflux pump the possible explanation for tigecycline resistance in this study could be that exhibition to multiple antibiotics in the hospitals, highly activates the efflux systems of these strains.

100% of the isolates in the current study were susceptible to colistin and polymyxin B. Susceptibility to colistin was reported as 91.2–100% in various studies (8, 20, 26), and it seems to be a good option in the treatment of MDR *A. baumannii*, but adverse reactions, has limited use of this agent. Colistin-resistant isolates have been recently identified in several Gram-negative species, such as *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* (3). In conclusion, the broad-spectrum in vitro activity of tigecycline and colistin may make them suitable candidates to be used in the empiric treatment of serious infections. Of course, there is a need to establish a severe hospital infection control policy and continuous surveillance of bac-

terial resistance to antimicrobial agents' should be also measured.

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ICUs of Tehran hospitals.

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Authors' Contributions

None declared.

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