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Research Paper

In vitro Activity of Colistin in Combination with Tigecycline against Carbapenem-Resistant *Acinetobacter baumannii* Strains Isolated from Patients with Ventilator-Associated Pneumonia

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Abstract

Objective: This study investigated the minimum inhibitory concentration (MIC) values and *in vitro* activity of colistin in combination with tigecycline against carbapenem-resistant *Acinetobacter baumannii* strains isolated from patients with ventilator-associated pneumonia (VAP) using the E-test method.

Methods: A total of 40 *A. baumannii* strains, identified using the Phoenix Automated Microbiology System (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) by conventional methods, were included in this study. Pulsed-field gel electrophoresis was performed to examine the clonal relationships between isolates. The carbapenem resistance of the strains to colistin and tigecycline was assessed using the E-test method (Liofilchem, Roseto Degli Abruzzi, Italy). The *in vitro* activity of colistin in combination with tigecycline was evaluated using the fractional inhibitor concentration (FIC) index.

Results: While only 1 of 40 *A. baumannii* strains was determined to be colistin resistant, 6 were tigecycline resistant. The MIC_{50}, MIC_{90}, and MIC intervals of the *A. baumannii* strains were 0.19, 1.5, and 0.064–4 μg/ml for colistin and 1, 8, and 0.094–256 μg/ml for tigecycline, respectively. No synergistic effect was observed using the FIC index; 8 strains exhibited an indifferent effect and 32 exhibited an antagonist effect. Three of the six strains that were resistant to tigecycline were indifferent; the remaining three were antagonistic. The colistin-resistant strain also exhibited an antagonist effect.

Conclusion: In contrast to their synergistic effect against carbapenem-resistant *A. baumannii* isolates, colistin and tigecycline were highly antagonistic to carbapenem-resistant *A. baumannii* strains isolated from patients with VAP when the drugs were administered together. Therefore, alternative treatment options should be used during the treatment of VAP attributed to *A. baumannii.*

Key words: *Acinetobacter baumannii*, carbapenem-resistant, colistin, tigecycline, ventilator-associated pneumonia

Introduction

Ventilator-associated pneumonia (VAP) is a type of nosocomial pneumonia that occurs in patients receiving mechanical ventilation. VAP is usually acquired in the hospital setting approximately 48–72 h after mechanical ventilation [1, 2]. Mechanically ventilated patients are unconscious, and there is no

clearance of secretions in the oropharynx. Defense mechanisms are also ineffective in patients with an impaired immune response [2, 3].

An increase in aerobic Gram-negative organisms has been reported in ventilated patients [4], and nosocomial infections can be transmitted by hospitalized patients and health workers [5, 6]. The microbial flora of hospitalized patients are altered markedly within a few days of antibiotic administration, and long hospital stays are associated with the emergence of resistant pathogens [7, 8].

Acinetobacter baumannii is a leading cause of VAP, frequently as a result of ventilator equipment contamination [9–11]. *Acinetobacter baumannii* is increasingly recognized as an important pathogen in both immunocompromised and hospitalized patients infected by contact with contaminated equipment [12, 13]. *Acinetobacter baumannii* infections should be considered in febrile patients with nosocomial infections, particularly in those with an indwelling catheter, wound or immune dysfunction, or who are on ventilators [13].

The treatment of *A. baumannii* infections is complicated by various antibacterial resistance mechanisms against currently available antibiotics. Combination antibiotic therapy is typically used to treat *A. baumannii* infections; however, determining whether agents are synergistic or antagonistic in their effects is important to achieve therapeutic efficacy [14].

This study used the E-test method to assess the minimum inhibitory concentration (MIC) values and *in vitro* efficacy of colistin in combination with tigecycline against carbapenem-resistant strains of *A. baumannii* isolated from patients with VAP.

Materials and Methods

Acinetobacter baumannii strains

This study was approved by the Ethics Committee of Erzincan University (Erzincan, Turkey). A total of 40 strains of *A. baumannii*, cultured from deep tracheal aspirates of patients diagnosed with VAP in our intensive care unit between January 2013 and January 2014, were included. VAP diagnosis is established by Infectious diseases specialists according to the clinics and radiological criteria specified for Ventilator-related pneumonia in The 2005 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guideline [15]. The isolates were identified using the Phoenix Automated Microbiology System (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) by conventional methods (Gram staining, the oxidase test, and reproduction and movement in triple sugar iron medium).

Antimicrobial agents and MIC assays

The E-test method was used to determine the MIC values of the *A. baumannii* strains for imipenem, colistin, and tigecycline. A suspension equivalent in density to a 0.5 McFarland opacity standard was prepared for all *A. baumannii* strains, which were cultivated on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) plates (15 cm in diameter), using a sterile swab. After drying the plates, imipenem (0.002-32 µg/mL), colistin (0.064-1024 µg/mL), and tigecycline (0.016-256 µg/mL) strips (Liofilchem, Roseto Degli Abruzzi, Italy) were applied. The plates were then incubated at 37°C for 18–24 h, and the MIC values (the intersection of the E-test strip and ellipse) were read and recorded. Antimicrobial activity of other antibiotics for the *Acinetobacter baumannii* strains was detected with BD Phoenix automated system (Becton Dickinson, USA). The MIC values obtained for imipenem and colistin were evaluated according to the recommendations of the Clinical and Laboratory Standards Institute [16]. Breakpoints for tigecycline susceptibility were derived according to the FDA breakpoint of 2 mg/l [17].

Molecular analysis

The *A. baumannii*-optimized protocol of Durmaz et al. [18] was employed to determine the clonal relationships among the bacterial isolates, which were transferred to blood agar and incubated at 37°C overnight; a suspension of fresh colonies was prepared in 4 ml of cell suspension buffer (100 mM Tris and 100 mM EDTA, pH 8). The bacterial density was adjusted to an absorbance of 1 at 590 nm in a spectrophotometer (Boeco, Hamburg, Germany). DNA agar fragments prepared with bacteria were cut using 30 U of *Apa*I (Promega Corp., Madison, WI, USA). DNA pieces were run in agarose subjected to a 1% pulsed field at 14°C and 6 V/cm² using the CHEF-DR II system (Bio-Rad Laboratories, Hercules, CA, USA) for 20 h. The DNA band profiles were analyzed using GelCompar II (ver. 3.0; Applied Maths, Sint-Martens-Latem, Belgium). Clonal relatedness between strains was established using Tenover's criteria [19].

Synergistic interactions

The synergistic, additive, indifferent, and antagonistic effects of colistin and tigecycline on the *A. baumannii* strains were evaluated using the fractional inhibitory concentration (FIC) index. The FIC value was calculated as the MIC value of the drug alone divided by the MIC value of the drug used in combination with another drug. The FIC index was calculated by summing the FIC values of the drugs used in combination. An FIC index ≤ 0.5 was considered in-

dicative of a synergistic effect; values > 0.5–1 were additive, and values ≥ 2 were antagonistic.

Statistical analysis

Drug effects are described using descriptive statistics (i.e., means ± *SD* and minimum and maximum values) for continuous variables, and numbers and percentages for categorical variables. The SPSS for Windows software package (ver. 13; SPSS Inc., Chicago, IL, USA) was used to perform all analyses. Descriptive statistics were expressed as mean, standard deviation, and minimum-maximum.

Results

A total of 40 patients with VAP who met the inclusion criteria were identified during the study period. Most of these patients were male (22/40, 55%), mean age (56.8 ± 20.7), and prior use of broad-spectrum antibiotics (100%). Many patients also had significant underlying diseases (chronic obstructive pulmonary disease 42.5%, diabetes mellitus 37.5%, and congestive heart failure 20.0% and 85% (34/40) of those patients died during the hospitalization period. The demographic and clinical data of these patients are summarized in Table 1.

Table 1. Basic clinical information of included subjects

Clinical characteristics		Patient group*
Sex		
	Famale	18
	Male	22
Age (Year)		56.8 ± 20.7 (4–85)
Patient Population		
	Medical	24
	Surgical	4
	Trauma	3
	Mixed	9
Ventilation Days		26.4 ± 19.2 (6-73)
ICU Days		28.8 ± 20.6 (6-81)
Total Hospitalization Days		33.3 ± 23.5 (7-93)
Use Broad-Spectrum Antibiotics		40
Death		34

*Data are expressed as number of cases (percentage) or mean±SD (range)

Pulsed-field gel electrophoresis was conducted using 40 *A. baumannii* strains isolated from patients diagnosed with VAP. Of the 40 strains, 6 genotypes were detected (5 clusters and 1 specific profile). The 5 clusters contained a total of 39 strains. The dendrograms for all 40 *A. baumannii* strains isolated from the patients are illustrated in Figure 1.

An MIC value of 32 μg/ml was observed for imipenem using the E-test method. Only 1 (2.5%) of the 40 *A. baumannii* strains was resistant to colistin, compared to 6 (15%) tigecycline-resistant strains. The MIC_{50}, MIC_{90}, and MIC ranges of the *A. baumannii* strains were 0.19, 1.5, and 0.064–4 μg/ml, respectively, for colistin, and 1, 8, and 0.094–256 μg/ml, respectively, for tigecycline. The MIC and strain resistance values are listed in Table 2, and the MIC distributions are given in Figure 2.

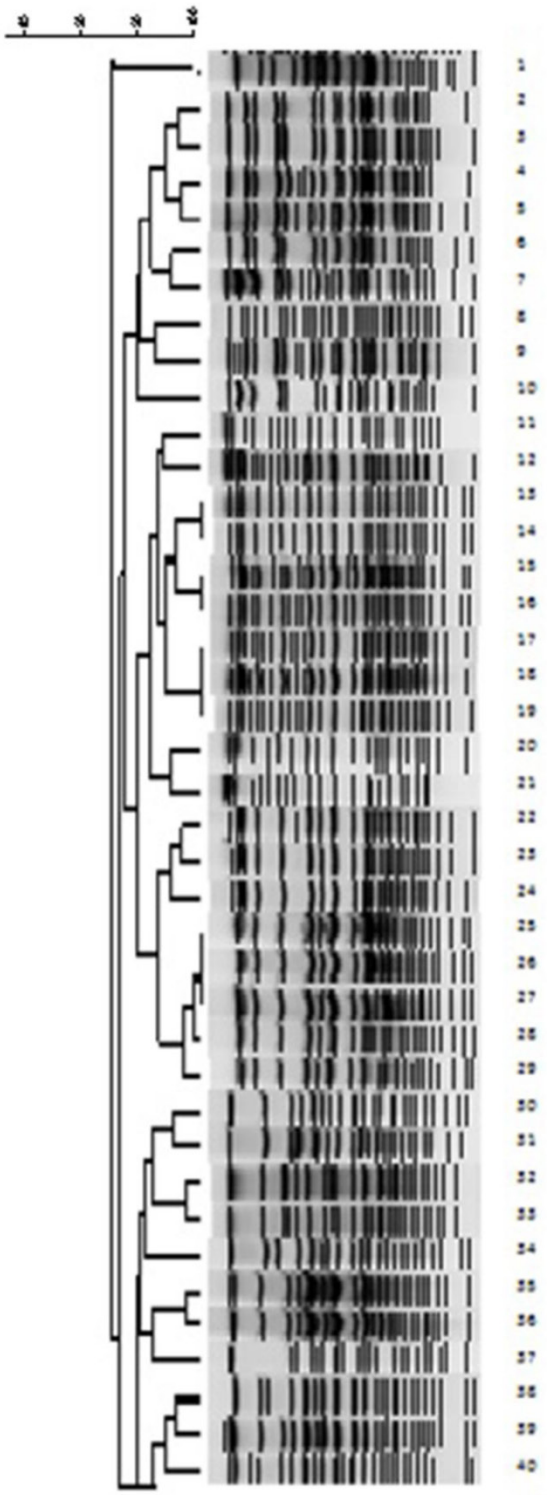

Figure 1. Dendrograms of the 40 carbapenem-resistant *Acinetobacter baumannii* strains isolated from patients with ventilator-associated pneumonia

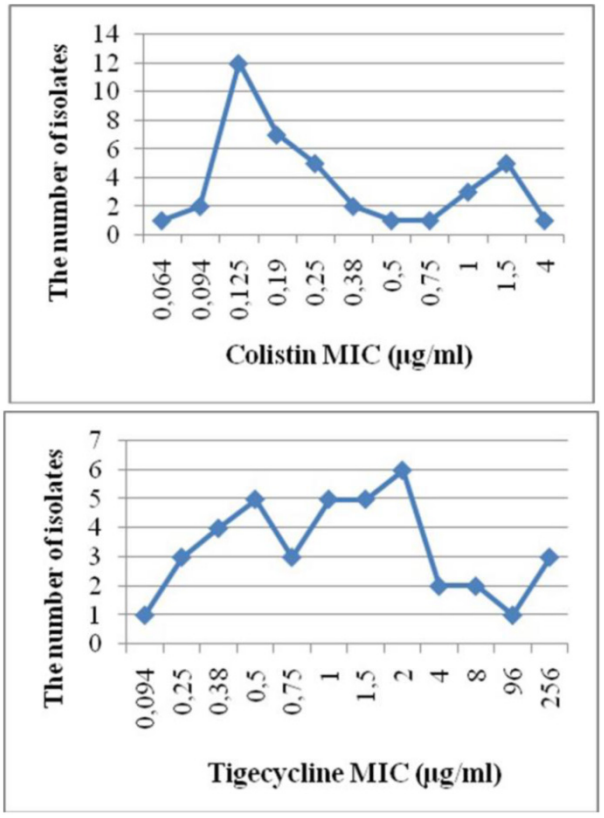

Figure 2. MIC distributions

Table 2. MIC values (mg/ml) and resistance states (n) for the *Acinetobacter baumannii* strains

Antibiotics	MIC ranges	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Colistin	0.064-4	0.19	1.5	39	-	1
Tigecycline	0.094-256	1	8	32	2	6

An evaluation of colistin in combination with tigecycline against the *A. baumannii* strains revealed no synergistic effect based on the FIC index; 8 strains (20%) were indifferent and 32 (80%) exhibited antagonist effects. Three of the six strains that were resistant to tigecycline were indifferent; the remaining three were antagonistic. The colistin-resistant strain exhibited an antagonistic effect. In addition, resistance rates of *A. baumannii* isolated from VAP against different antibiotics are presented in Table 3.

Discussion

Acinetobacter baumannii is an opportunistic human pathogen that causes life-threatening nosocomial infections such as VAP, bacteremia, meningitis, and urinary tract and wound infections. The treatment options for infections caused by multi-drug resistant (MDR) *A. baumannii* strains are limited [20]. The treatment of VAP-induced MDR *A. baumannii* infections is particularly problematic [21], with polymyxins prescribed as the agent of last resort [22, 23]. Several researchers have suggested that colistin represents a reliable and effective antibiotic, achieving eradication in approximately two-thirds of *A. baumannii*-induced VAP cases [24]. However, colistin toxicity is a serious problem [25]. Furthermore, given the risk of colistin resistance, its use should be reserved for the most serious indications, including as an agent of last resort for difficult-to-treat infections caused by MDR *A. baumannii*.

Table 3. Antimicrobial activity states for the *Acinetobacter baumannii* strains (*n*:40)

Antibiotics	Susceptible	Intermediate	Resistant
Ampicillin-sulbactam	-	2	38
Cefoperazone-sulbactam	2	3	35
Piperacillin-tazobactam	-	-	40
Aztreonam	-	-	40
Cefepime	-	3	37
Cefotaxime	-	-	40
Ceftazidime	-	2	38
Ciprofloxacin	-	5	35
Levofloxacin	13	3	24
Amikacin	8	1	31
Gentamicin	1	2	37
Tetracycline	14	5	21
Trimethoprim-sulfamethoxazole	8	-	32

Tigecycline is effective against *Acinetobacter* spp. [26, 27], but the rates of resistance continue to increase [28]. Furthermore, several studies have reported that *A. baumannii* treatment may be ineffective in patients with high APACHE II scores and C-reactive protein levels, and in those with low albumin levels [29].

The use of colistin in combination with tigecycline, instead of monotherapy in critically ill patients is now mandatory to reduce clinical failure. The synergistic efficacy of these agents has been demonstrated in a limited number of *in vitro* studies. In a study of MDR *A. baumannii* strains by Ni et al. [30], colistin in combination with tigecycline was associated with a synergistic efficacy of 24.3%, compared to 12% against carbapenem-resistant *A. baumannii* strains in a report by Karaoglan et al. [31].

However, few studies have assessed the efficacy of colistin and tigecycline in patients with *A. baumannii*-induced VAP. We were unable to determine the synergistic efficacy of this combination against carbapenem-resistant *A. baumannii* strains isolated from VAP patients under *in vitro* conditions. However, we observed an antagonistic effect in 80%, and an indifferent effect in 20%, of the strains. In several studies, combination therapy with colistin and tigecycline is considered synergistic at FIC index scores ≤ 0.5, additive at scores of > 0.5–1, indifferent at

scores of 1–4, and antagonistic at scores ≥ 4 [32]. If we interpret our results using these thresholds, 62.5% of our strains exhibited antagonistic effects and 37.5% exhibited indifferent effects. Combination therapy with colistin and tigecycline does not represent a good treatment option in either case.

Colistin exerts a bactericidal effect on *A. baumannii* isolates and other Gram-negative bacteria based on its strong affinity for lipopolysaccharide in the outer membrane [33-36]. Tigecycline, a semi-synthetic tetracycline derivative, inhibits bacterial protein synthesis by reversibly binding to the 30S ribosomal subunit; thus, it exerts a bacteriostatic effect on *A. baumannii* [37-39].

An antagonistic effect has been demonstrated using bacteriostatic and bactericidal drugs in combination [40, 41]; when tigecycline, a bacteriostatic agent, is used in combination with colistin, a bactericidal drug, their effects should be neutralized. Accordingly, we detected a highly antagonistic effect, in contrast to the synergistic effect reported in several other studies.

According to our data, in contrast to their synergistic effect against carbapenem-resistant *A. baumannii* isolates, colistin and tigecycline were highly antagonistic to carbapenem-resistant *A. baumannii* strains isolated from patients with VAP when the drugs were administered together. Therefore, other methods should be used to treat *A. baumannii*-induced VAP. Colistin and tigecycline may exert different pharmacokinetic and pharmacodynamic effects under *in vitro* and *in vivo* conditions; additional experimental and clinical studies are required to investigate this possibility.

Competing Interests

The authors have declared that no competing interest exists.

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