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In-vitro effects of various antimicrobial combinations against multidrug-resistant *Acinetobacter baumannii* strains.

Rıdvan Güçkan[1*], Semra Kurutepe[2], Hörü Gazi[2], Çetin Kilinç[1]

[1]Laboratory of Microbiology, Amasya Sabuncuoglu Serefeddin University, Training and Research Hospital, Amasya, Turkey.

[2]Celal Bayar University Faculty of Medicine Microbiology Department Manisa,Turkey.

Abstract

In recent years, *Acinetobacter* strains have emerged as one of the most important nosocomial pathogens, especially in patients admitted to an intensive care unit (ICU). The progressively increasing antibiotic resistance against *A. baumannii* is now a major problem in our country as it is throughout the world. This resistance against *A.baumannii* has increased and led clinicians to find alternative antibiotics or antibiotic combinations. In the present study, it is aimed to evaluate the interaction between colistin-rifampicin, colistin-imipenem, tigecycline-rifampicin and tigecycline-imipenem antibiotic combinations using microdilution checkerboard and E-test methods against ten multidrug resistant *A. baumannii* strains. Since *A. baumannii* strains have become frequently observed as an infection factor and since antimicrobial resistance rates have increased, there should be newly developed drugs for better treatments.

In this study, 50 *A. baumannii* strains were isolated from various clinical specimens between June 2005 and September 2009 in Celal Bayar University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology, and in Bacteriology Laboratory. Isolation and identification procedures were performed by using conventional biochemical tests as well as by BBL Crystal GN; N/F ID (Becton Dickinson, USA) or Phoenix 100 BD systems (Becton Dickinson, USA). The antibiotic susceptibilities of strains were investigated by using the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute.

According to our in vitro study results, the checkerboard method, which was used to examine the synergy between colistin-rifampicin and colistin-imipenem, showed 80% synergistic activity. Tigecycline-imipenem combination had lowest synergetic (10%) efficiency and highest antagonistic effect (30%).

The consistency between checkerboard and E-test methods was 52,5% (range 10-70%). Further comparison studies of the E-test synergy technique with the checkerboard and time-kill methods are warranted.

Keywords: *Acinetobacter baumannii*, Synergy, Checkerboard, E-test, Colistin, Tigecycline.

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Introduction

Acinetobacter has been detected as one of the most common nosocomial infections particularly in intensive care unit (ICU) [1,2]. Recently, there has been an increasing resistance against antimicrobial agents that are commonly used for infections caused by *Acinetobacter baumannii* strains and this resistance has given rise to an important health issue in Turkey as it is the case throughout the world. New options of drugs should be searched and established since *A. baumannii* infections have become more and more diverse and the antimicrobial agent resistance has increased [1-3].

There have been new studies which try to develop different treatment protocols because of the increment in the resistance rates of microorganisms. Combination therapies and the production of new antibiotics are some of the focuses of these studies. Antimicrobial drug combinations are used in order to obtain a broad spectrum, prevent the development of resistant strains, minimize the toxicity, and obtain a synergetic effect between two drugs [4,5].

In this study, the aims are to examine the in vitro effect of different antibiotic combinations on *A.baumannii* isolates using synergistic tests such as checkerboard and E-test methods and to assess the compatibility between these two methods.

Materials and Methods

This study was approved by Celal Bayar University, Dean of the Faculty of Medicine, and Ethics Committee with Decision No. 390 in the scientific meeting which was held on 06.18.2009.

This study was conducted in Celal Bayar University, School of Medicine, Department of Medical Microbiology, and Laboratory of Bacteriology. 50 *A.baumannii* strains were isolated from various clinical samples between 2005 and 2009. Only one clinical isolate from each patient was included in the study. In order to evaluate these tests, we isolated 10 *A. baumannii* strains, which were isolated from different patients, which had different sensitivity profiles, and which were resistant against at least three antibiotic groups.

Bacteria were identified at the species level by using BBL Crystal Enteric/Nonfermenter ID Kit (Becton Dickinson, USA) or Phoenix 100 BD system (Becton Dickinson, USA). The sensitivities of A.baumannii strains were determined using disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

The efficiency of colistin-rifampicin, colistin-imipenem, rifampicin-tigecycline, tigecycline-imipenem combinations were examined in 10 *A. baumannii* strains which had multiple drug resistance determined using checkerboard and E-test techniques. The consistency between these two techniques was compared. Evaluations were performed with reference to checkerboard method 6.

Interaction between antibiotic combinations was examined using checkerboard method for each antibiotic and strain whose minimum inhibitory concentration (MIC) values were determined using broth microdilution method. In this study, colistin-imipenem, colistin-rifampicin, imipenem-tigecycline and tigecycline-rifampin combinations were examined.

F*ractional inhibitory concentration* (*FIC*) values of antibiotics were detected using the first horizontal row (A row) for the first antibiotic in the combination and the first vertical row (number 1 column) for the second antibiotic in the combination. The first antibiotic was diluted for four times and each dilution was put in the microdilution plate as starting from the number 1 column till the 8th column. In case of four times dilutions of the second antibiotic, each dilution was put in first 8 rows in the micro dilution plate.

These combinations were also examined using E-test method. FIC index was used in both methods in order to determine the effectiveness of combinations and interactions between antibiotics were recorded as synergy, indifference and antagonism [7]. FIC index was calculated as:

FIC A=MIC value of A in the presence of B/MIC value of A alone

FIC B=MIC value of B in the presence of A/MIC value of B alone

Σ FIC index=FIC A+ FIC B

Evaluation:

Σ FIC index ≤ 0.5 : synergy (4 times decrease)

Σ FIC index ≥ 1 and 4 ≤ : indifference (no interaction)

Σ FIC index >4 : antagonism

Statistical analyses

Sommer' D, Chi-square, Mann-Whitney-U, Student's t-test and Logistic Regression analyses were performed by using SPSS 13.0 software. 'p' values less than 0.05 ($p<0.05$) were accepted as statistically significant.

Statistical analyses of data obtained from the results of in vitro interactions of colistin-imipenem, colistin-rifampicin, imipenem-tigecycline, tigecycline-rifampin combinations by using Fisher's chi-square test with the help of SPSS (SPSS Incorporated, Chicago, USA) program.

Results

In this study, out of 50 *Acinetobacter* strains, 27 (54%) of them were isolated from anesthesia ICU samples, 8 (16%) of them were isolated from pulmonary unit samples, 7 (14%) of them were isolated from neurology unit samples, 4 (8%) of them were isolated from general surgery unit samples, and 4 (8%) of them were isolated from brain surgery unit samples.

Acinetobacter was isolated from blood [39 (78%)], respiratory samples [6 (12%)] or various clinical samples [5 (10%)]. According to the antibiogram results of *A. baumannii* strains that were obtained with the help of disc diffusion method, the maximum resistance was observed against ceftriaxone whereas the minimum resistance was observed against netilmycin. The rate of the resistance against imipenem was 70%.

The efficiency of antibiotic combinations was examined for 10 *A. baumannii* strains which had multi drug resistance. When the microdilution of strains was primarily examined, all strains were found to be colistin and tigecycline sensitive and their MIC_{50} values were detected as 0.07 µg/ml and 0.7 µg/ml; respectively. Out of 10 strains, 8 were found to be imipenem resistant.

When efficiencies of drug combinations were assessed using checkerboard method, it was detected that the highest synergy (80%) was found for combinations of colistin with imipenem and rifampicin (Figure 1). Tigecycline-imipenem pair was detected as a combination which had lowest synergetic (10%) efficiency and highest antagonistic effect (30%) (Table 1).

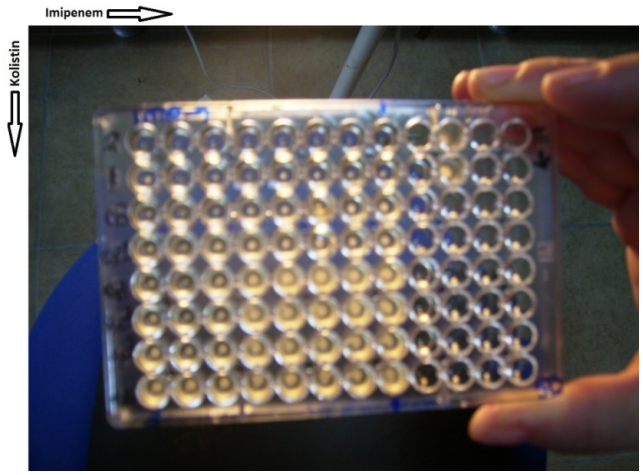

Figure 1: Colistin-imipenem combination and checkerboard method.

Table 1: Drug combination efficiency determined in multi-drug resistant Acinetobacter baumanni strains by checkerboard method (n=10).

Drug combination	Synergy n %	Indifference n %	Antagonism n %
Colistin/imipenem	8 80	2 20	- -
Colistin/rifampicin	8 80	2 20	- -
Tigecycline/imipenem	1 10	6 60	3 30
Tigecycline/rifampicin	6 60	4 40	- -

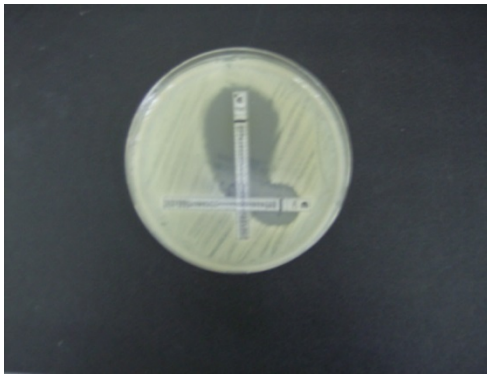

Figure 2: Colistin-imipenem combination and E-test method.

When the consistencies of the techniques (checkerboard and E-test) were examined, the highest sensitivity rate (70%) was detected for tigecycline combinations. Furthermore, in cases of colistin-imipenem combination, 8 of the strains had synergistic interaction with these antibiotics according to the results of checkerboard test whereas they were shown as indifference according to E-test results. The consistency was calculated as 10% (Figure 2, Table 2).

Table 2: The consistency rates between checkerboard and E-test methods (n=10). *The interpretation of the K values specified as: (-0.3) (+0.3): inconsistent +0.3) (+0.5) : weak positive, +0.5 (+0.7): moderate positive, >0.7: strong positive. **Kappa values could not be calculated since the sub efficiency categories of diagnostic tests were not paired.*

Drug combination	Consistency (%)	K	P
Colistin/imipenem	10	0,21	0,03
Colistin/rifampicin	60	**	
Tigecycline/imipenem	70	**	
Tigecycline/rifampicin	70	0,40	0,19

The percentages of liquid values were failed to be compared among combinations due to the fact that the assumption on the expected values of chi-square test did not hold. Therefore, the analysis was performed by colistin and tigecycline combinations. When the efficiencies of the colistin and tigecycline combinations (with imipenem and rifampicin), the combination efficiency of colistin was found two times synergetic compared to tigecycline ($p \leq 0.05$) (Table 3).

Table 3: Combination efficiency of colistin and tigecycline.

Combination	Synergy N %	Indifference N %	Antagonism N %
Colistin+combination	16 80	4 20	0 0
Tigecycline +combination	7 35	10 50	3 15

Discussion

A. baumannii strains have been isolated frequently from nosocomial pathogens for the last 15 years [8]. These strains lead to infections with high mortality and their high resistance against various antibiotics causes a severe issue [9]. Increment in the isolation of multiple drug resistant strains throughout the world as well as in Turkey and increasing resistance against antibiotics decrease the treatment options of clinicians in inpatients who were *suspected of* suffering with *A. baumannii* [10,11]. It is suggested to use antibiotic combinations in order to both ensure the success and prevent the resistance development in the treatment of the infections due to the multi-drug resistant *A. baumanni* strains [12].

When colistin was in combination with rifampicin, 8 of 10 stains (80%) had synergetic interaction with this combination of antibiotics. Hogg et al.[13] performed a study in which they used checkerboard method with their isolates, they detected synergetic association between antibiotics in 11 of the 13 strains. Timurkaynak et al. [14] showed that all strains (in 25 of the strains, 100%) had synergetic interaction with antibiotics. According to the study of Giamarellos et al. [15] in which they used time-kill technique, they indicated in 2001 that 39 strains showed (66.7%) synergy in the 24th hours for colistin-rifampicin combinations. Tripodi et al. [16] also used time-kill technique and they showed the 100% synergy of 9 isolates with colistin-rifampicin combinations in 2007. In Turkey, there was a 60% synergy using E-test for colistin-rifampicin combinations in *A.baumannii* strains isolated from 25 different

patients [17]. As a result, our results are consistent with all of these findings, and it has been decided that colistin-rifampicin combinations are efficient and convenient for the treatment of *A.baumannii* infection.

There was a synergy in 8 strains (80%) for colistin-imipenem combinations when we used checkerboard method. According to the results of study performed by Pongpech et al. [18] in 2010 in Thailand, they determined that colistin-imipenem combinations were 100% synergistic when they applied the same technique to 30 isolates. In a study which was conducted by Haddad et al. [5] between 1999 and 2003 in New York with 10 *A.baumannii* isolates that were resistant against all routinely used antibiotics, they used E-test method and detected a 50% synergy for colistin-imipenem combinations. High synergy rates detected in bacterial strains including imipenem resistant strains in all of these studies and particularly in our study let us think that colistin-imipenem combinations are efficient enough for the treatment.

There was a synergy in 6 strains (60%) for the tigecycline-rifampicin combination when we used checkerboard technique. Petersen et al. [19] detected a synergy only in one strain out of 9 strains for tigecycline-rifampicin combination in 2005 using checkerboard method. Dizbay et al. [17] specified that they detected 12% synergy for tigecycline-rifampicin combination using E-test method. In our study, we detected higher synergy rates in this combination compared to other studies.

There was a 10% synergy in one strain for tigecycline-imipenem combinations. Principe et al. [20] performed a study with 22 isolates using checkerboard technique, and they showed 8.3% synergy for tigecycline-imipenem combination. To sum up, tigecycline-imipenem combinations are not effective option for the treatment.

The rate of synergistic interaction is greater in the colistin combinations compared to the tigesiklin combinations; this result was statistically significant ($P<0.05$).

E-test method was also used in addition to checkerboard method in order to examine the antibiotic combinations and the consistency between these methods.

When the consistency of efficiencies which were detected by using E-test method was also examined using checkerboard method, the mean consistency was found to be 52.5% (10%-70%). Bonapace et al. [21] compared the consistency between E-test and checkerboard methods in 10 *A. baumanii* isolates for trovafloxacin or tobramycin-cefepime or piperacillin. They found the consistency between time kill method with E-test and checkerboard methods as 72% and 51%, respectively.

Conclusively, multi-drug resistance (MDR) *A.baumanii* strains are important problems in hospitals and treatment options are limited. Due to high synergy rates that were determined for colistin-imipenem, colistin-rifampicin and tigecycline-rifampicin combinations, it has been thought that these combinations can be efficiently used in the treatment of MDR *A.baumanii* infections. However, it should be noted that some antimicrobial agents in combination with colistin lead to decrease in the activity and create the antagonistic effect. Further studies should be performed since in vitro experiences are limited for colistin and tigecycline. Since *A. baumannii* strains are resistant against majority of antibiotics, surveillance results should be considered when we select empirical antibiotic therapy that will be applied to patients in critical condition.

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*Correspondance to:

Ridvan Güçkan

Laboratory of Microbiology

Amasya Sabuncuoglu Serefeddin University

Training and Research Hospital

Amasya

Turkey