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Original Article

In vitro activities of colistin combined with imipenem, tigecycline or cefoperazone-sulbactam against multidrug-resistant Acinetobacter baumannii blood-stream isolates

Oznur Ak[1], Demet Haciseyitoglu[2], Yasemin Cag[3], Serap Gencer[1], Funda Biteker[1], Serdar Ozer[1]

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Abstract

Acinetobacter baumannii has emerged as one of the most important nosocomial pathogens and multi-drug resistant (MDR) isolates are of great concern worldwide. The aim of the present study was to investigate the in vitro synergistic activity of colistin in combination with other antibiotics against MDR *A. baumannii* blood stream isolates. A total of 54 non-duplicate, MDR *A. baumannii* isolates from blood culture specimens obtained between June 2011 and July 2012 were included in the study. In vitro synergistic activity of colistin in combination with imipenem, tigecycline or cefoperazone-sulbactam against study isolates was investigated by Etest superimposing method and the fractional inhibitory concentration (FIC) index was calculated for each antibiotic combination. The most frequent synergistic effect of colistin was found in combination with tigecycline in only 7 isolates (13.0%). All three antibiotics were found to have synergistic effect with colistin in four isolates (7.4%). Of isolates, 46 (85.2%) showed additive effect of colistin in combination with cefoperazone-sulbactam or tigecycline, 45 (83.3%) with imipenem. We found synergistic activity of colistin with other antibiotics in only a small number of isolates. Although Etest method is a practical method to investigate the synergistic activity, in case of choosing empirical treatment, colistin in combination with another antibiotic may be preferred.

Introduction

Acinetobacter baumannii has emerged as one of the most important nosocomial pathogens, especially for patients in intensive care unit (ICU) and for those in burn unit. The resistance of *A. baumannii* to commonly used antibiotics has become a widespread and serious problem in ICUs of Turkey (1). *A. baumannii* has been a more frequent cause of ICU-acquired bloodstream infections. Crude mortality rate of *A. baumannii* bloodstream infection ranges from 16.3% for hospitalized patients and from 34% to 43% for patients admitted to ICUs (2).

Carbapenems are the most active agents against *A. baumannii*; however, the carbapenem-resistant, multidrug resistant (MDR) and pan-drug resistant (PDR) isolates are of great concern worldwide. A limited number of antimicrobial agents, including polymyxin E (colistin), polymyxin B, tigecycline, carbapenems and sulbactam maintain effectiveness against MDR *A. baumannii* (3,4).

Increase in carbapenem-resistant *A. baumannii* isolates makes it difficult to treat these infections. Therefore, combined antibiotics have been used in the treatment; and various in vitro combination therapies are being investigated (5,6). The aim of this study was to investigate the in vitro synergistic activity of colistin in combination with imipenem, tigecycline or cefoperazone-sulbactam determined by the Etest

Author affiliations: *[1]Department of Infectious Diseases and Clinical Microbiology, Dr.Lütfi Kirdar Kartal Training and Research Hospital, Istanbul, Turkey, [2]Department of Microbiology and Clinical Microbiology, Dr.Lütfi Kirdar Kartal Training and Research Hospital, Istanbul, Turkey, [3]Department of Infectious Diseases and Clinical Microbiology, Medeniyet University Goztepe Training ad Research Hospital, Istanbul, Turkey*

Correspondence to: *akoznur@yahoo.com (Oznur Ak)*

method against MDR *A. baumannii* blood stream isolates.

Materials and Methods

Bacterial isolates

A total of 54 non-duplicate, nosocomially acquired, multidrug-resistant *A. baumannii* isolates from blood culture specimens obtained from the Department of Infectious Diseases and Clinical Microbiology Laboratory between June 2011 and July 2012 were included in the study. All isolates were identified using API 20NE (bio-Mérieux, France).

Antibiotic susceptibility tests

The antimicrobial susceptibility of isolates to ampicillin-sulbactam (10/10 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), tigecycline (15 µg), cefepime (30 µg), cefoperazone-sulbactam (75/30 µg) and piperacillin-tazobactam (100/10 µg) were tested routinely by Kirby-Bauer disc diffusion test on Mueller-Hinton agar plate and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI 2011) recommendations (7). Breakpoints for cefoperazone-sulbactam and tigecycline were those recommended by the manufacturer. MDR is defined as resistance to at least one agent in at least three group of antibiotics, including aminoglycosides, antipseudomonal carbapenems, antipseudomonal floroquinolones, antipseudomonal penicillins, extended-spectrum cephalosporins, polymyxins and tetracyclines (8).

Synergy studies

In vitro synergistic activity of colistin in combination with imipenem, tigecycline or cefoperazone-sulbactam against study isolates was investigated by Etest superimposing method. Mueller-Hinton agar (MHA) plates were inoculated with McFarland 0,5 suspensions of the isolates. Four MHA plates were used for each combination. All antimicrobial agents were tested alone and in combination. Etest strips (AB BIODISK, Sweden) of study antibiotics were applied to the surface of MHA plates. The strips were removed after incubating for one hour at room temperature. Then a new strip of the other drug was placed over the area of the previously removed strip. Each test was performed in duplicate. The resulting minimum inhibitory concentration (MIC) was read after approximately 20 hours of incubation at 350C. To evaluate the effect of the combinations the fractional inhibitory concentration (FIC) index was calculated for each antibiotic combination according to the following formulas (9,10).

FIC of drug A [FIC (A)] = MIC of drug A when tested in combination with drug B / MIC of drug A alone

FIC of drug B [FIC (B)] = MIC of drug B when tested in combination with drug A / MIC of drug B alone

FIC index = FIC(A) + FIC(B)

Combinations were classified as synergistic if FIC index was ≤0.5; additivite or indifferent for FIC index >0.5 to ≤4; and antagonistic for FIC index >4 (11). The cut offs of resistance for the antibiotics tested were as follows: tigecycline ≥ 8 µg/ml, imipenem ≥ 16 µg/ml, colistimethate ≥ 4 µg/ml, cefoperazone-sulbactam ≥ 64 µg/ml.

Results

A total of 54 MDR A. baumannii blood-stream isolates were studied. Of the isolates, 31 were obtained from burn intensive care unit, 18 from surgical intensive care unit, 3 from the department of internal medicine, one from the department of infectious diseases and one from the department of general surgery. The most common diagnosis for admission was burn (in 57.4% of cases). No concomitant disease was present in 32 patients. No history of antibiotic use was reported in six patients prior to the development of bacteremia. It was noted that most frequently used antibiotics included cephalosporins, and carbapenems before MDR acinetobacter bacteremia. Ten patients had died before detection of growth in blood culture.

MICs of imipenem, colistin, tigecycline, cefoperazone- sulbactam are shown in Table 1. All the isolates were multi-drug resistant but were susceptible to colistin.

In vitro efficacy of combinations with colistin are shown in Table 2. Most of the combinations (more than 83%) had additive effects, while antagonistic effects were seen in less than 4% of isolates. On the other hand, less than 15% of combinations had synergistic effects. The most frequent synergistic effect of colistin was found in combination with tigecycline (14.8%). All three antibiotics were found to have synergistic effect

Table 1. Susceptibility results, and MIC values of antibiotics tested

Antibiotic	MIC (µg/mL)			Susceptibility results(%)	
	%50	%90	Range	Susceptible	Resistant
Colistin	0.25	0.38	0.125–3	100	-
Tigecycline	1.5	4	0.38–8	99	1
Imipenem	>32	>32	4 - >32	-	100
Cefoperazone-sulbactam	>256	>256	16- >256	-	100

with colistin in four isolates (7.4%). No antagonism was found between colistin and tigecycline in any isolates.

Discussion

Antibiotics used in combination with colistin for *A. baumannii* blood-stream infections in our hospital was chosen to investigate the synergistic activity among them. Different methods, including the checkerboard, time-kill, and Etest have been used in studies of synergy. In the study of White et al. the concordance of Etest and checkerboard methods has been reported to be 63-75% and 44-88%, respectively, when the time-kill method was accepted as reference method (12).

In the study by Tan et al. extensively drug-resistant (XDR) A. baumannii isolates were tested for the presence of in vitro synergy to polymyxin B plus rifampin, polymyxin B plus tigecycline, and tigecycline plus rifampin combinations by three methods. However, little agreement between different methods was demonstrated and only one strain had synergy between polymyxin B and rifampin using the Etest method (13). Unfortunately, the optimal in vitro testing method that best corresponds with the clinical outcome of infections remains to be ascertained. Since Etest method is more practical, it is mostly preferred for in vitro synergy tests.

Sands et al. tested synergy between polymyxin and tigecycline in 19 isolates by Etest and found no synergy (5). Kiratisin et al. determined synergy most frequently between cefoperazone-sulbactam and carbapenem (doripenem, imipenem and meropenem) with the Etest in a total of 40 *A. baumannii* isolates, 25 of which were MDR(6). Haddad et al. reported that synergy was present between imipenem and colistin (40%; n=4), and imipenem and amikacin (30%; n= 3) in 10 carbapenem-resistant *A. baumannii* strains using the Etest method(14). Özseven et al. investigated synergistic combinations using the checkerboard method in 34 Acinetobacter isolates from intensive care unit and found synergistic results for the following combinations: meropenem plus sulbactam 94.1%, imipenem plus sulbactam 88%, imipenem plus rifampicin 73.5%, imipenem plus cefoperazone-sulbactam 70.6%, and imipenem plus polymyxin 38.2%, with no antagonism (15).

Bacteremia induced by MDR and pandrug-resistant *A. baumannii* is a serious cause of mortality. Even if colistin alone is effective in the treatment of these infections, combined antibiotics are preferentially used in order to reduce development of resistance, to provide rapid bactericidal effect and to lower mortality. Several in vitro studies demonstrated that synergy was reported to be present between imipenem plus sulbactam, imipenem plus rifampicin, imipenem plus polymyxin (5,16,17). In our study, we investigated synergy by Etest method because it is easy to use and to be cost-effective. Eight isolates (14.8%) had similar synergy rates of colistin with tigecycline, seven isolates (13.0%) had synergy of colistin with imipenem and cefoperazone-sulbactam. It is thought that synergy investigation will contribute to treatment options in isolates in an attempt to better devise treatment planning of future studies. Therefore,

Table 2. The interactions of antimicrobial combinations tested.

Combination	Synergy (FIC index ≤0,5) n (%)	Additivite/Indifferent (FIC index >0.5 - ≤4) n (%)	Antagonism (FIC index > 4) n (%)
Colistin+Imipenem	7 (13,0)	45 (83,3)	2 (3,7)
Colistin+Cefoperazone-sulbactam	7 (13,0)	46 (85,2)	1 (1,8)
Colistin+Tigecycline	8 (14,8)	46 (85,2)	0

because it is more practical than the other methods, the Etest method may be preferred. Use of combined antibiotics identified to have in vitro synergy will be very important in the treatment efficacy of serious infections caused by resistant Acinetobacter spp., such as bacteremia, pneumonia, and meningitis and in terms of reducing drug side effects and mortality.

As a conclusion, we found synergistic activity of colistin with other study antibiotics in only a small number of isolates. However, it is more important to find antagonistic activity of colistin with others in only less than 4% of isolates. Although Etest method is a practical method to investigate the synergistic activity, in case of choosing empirical treatment, colistin in combination with another antibiotic may be preferred. Further studies comparing in vitro and in vivo efficacies of combinations will conclude this debate.

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