Activity of Antimicrobial Combinations Against Extensively Drug-Resistant *Acinetobacter baumannii* as Determined by Checkerboard Method and E-test

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ABSTRACT

Objective: Combination therapy is needed to treat extensively drug-resistant (XDR) *Acinetobacter baumannii* infection. Colistin (Col) in combination with another drug is usually used for that purpose. The aim of this study was to determine the activity of antimicrobial combinations against XDR *A. baumannii* using both standard checkerboard (CB) method and E-test. E-test was also evaluated for application in a diagnostic bacteriology laboratory by comparing its efficacy with that of CB method.

Methods: Eighty clinical isolates of XDR *A. baumannii* were used to determine the activity of the following antimicrobial combinations by CB method and E-test: Col+cefoperazone/sulbactam (Cps), Cps+moxifloxacin (Mox), and Col+Mox. Comparison of CB and E-test was also evaluated.

Results: By CB method, Col+Cps yielded a synergistic effect rate (12.5%) higher than those of the other 2 combinations (CpS+Mox 5% and Col+Mox 0%). The majority of test results revealed additivity. Col+Cps, Cps+Mox, and Col+Mox exhibited additive effect against 78.75%, 85.0%, and 87.5% of isolates, respectively. Overall, E-test and CB yielded only 37.5% concordant rates. However, high concordant rates were specifically observed in additive effect of Col+Cps (73.8%) and Cps+Mox (80.4%).

Conclusion: Col+Cps exhibited better activity than the other two combinations against XDR *A. baumannii*. E-test is the method that should be used, but its use is limited to the additive results of Col+Cps and Cps+Mox.

Keywords: Synergy test; XDR *Acinetobacter baumannii*; colistin; cefoperazone/sulbactam; moxifloxacin (Siriraj Med J 2020; 72: 214-218)

INTRODUCTION

Acinetobacter baumannii, which is one of the most troublesome pathogens in clinical settings worldwide, has a very high rate of resistance to a wide variety of antimicrobial agents, including aminoglycosides, fluoroquinolones, broad-spectrum beta-lactams, and carbapenems. Extensively drug-resistant (XDR) isolates are common, and pandrug-resistant (PDR) strains have been reported.[1] Therefore, the use of monotherapy is now limited, antimicrobial combinations for the treatment of *A. baumannii* infection are needed. However, appropriate regimens and suitable methods for the determination of *in vitro* synergy in a routine diagnostic laboratory setting are not yet available.

Among the agents that are effective for treating *A. baumannii* infection, colistin (Col) in combination with another drug is usually used for XDR *A. baumannii* therapy. Moxifloxacin (Mox), a fluoroquinolone, has been reported to have better activity than ciprofloxacin against *Acinetobacter* species.[2] For beta-lactam combined with

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beta-lactamase inhibitor, combination with sulbactam (*i.e.*, cefoperazone/sulbactam, Cps) seems to have better activity against *A. baumannii* than other combinations since sulbactam has direct antimicrobial activity against the organism.[3,4]

To detect the *in vitro* synergy of antimicrobial combinations, time-kill and checkerboard (CB) tests are standard methods that are commonly used.[5] However, these techniques are time-consuming, labor-intensive, and inappropriate for use in a diagnostic bacteriology laboratory. E-test, which is an easy-to-perform method, has been modified to evaluate antimicrobial combination activity. Therefore, the aims of the present study were to determine the activity of the antimicrobial combinations Col+Cps, Cps+Mox, and Col+Mox against XDR *A. baumannii* by using CB method and E-test, and to evaluate E-test for application in routine clinical service by comparing its efficacy with that of CB method. Since CB method and E-test are based on the same testing principle for determining bacteriostatic activity, CB method was selected as the standard test instead of time-kill method (bactericidal activity determination).

MATERIALS AND METHODS

Eighty non-repetitive isolates of XDR *A. baumannii* were collected from a culture collection maintained at the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. They were cultured from various clinical samples (sputum, 56%; blood, 25%; urine, 7.5% and others, 11.5%) from 19 wards during a 6-year period (2006-2011). The isolates were presumptively identified as genus *Acinetobacter* by presence of the following characteristics: gram-negative coccobacilli, catalase-positive, oxidase-negative, glucose-nonfermenter, and ability to grow on MacConkey agar. The ability of isolates to grow at 44°C was further evidence that the isolates were *A. baumannii*. The *A. baumannii* isolates used in this study were resistant to amikacin, gentamicin, cotrimoxazole, ceftazidime, cefotaxime, ceftriazone, cefepime, piperacillin/tazobactam, imipenem, and meropenem, but were susceptible to Col.

Standard powder of antimicrobial agents and E-test strips of Col, Mox, and Cps were kindly provided by Atlantic Pharmaceutical Co., Ltd., Thailand; Bayer Thai Co., Ltd.; and, Pfizer (Thailand) Ltd., respectively. Ethical approval for this study was not required since no human subjects were involved and no patient information was included or reported.

CB method and E-test were performed according to published methods.[5,6] *Pseudomonas aeruginosa* ATCC 27853 was used as the control organism. The interpretive criteria for susceptibility were ≤2 µg/ml, susceptible for Col[7]; 16 µg/ml, susceptible for Cps (16/8, cefoperazone/sulbactam)[3]; and, 2 µg/ml, susceptible for Mox. For CB, two-fold dilutions of Col (2 to 0.031 µg/ml), Mox (32 to 0.125 µg/ml), and Cps (256 to 0.25 µg/ml) were used. These dilutions were prepared fresh immediately prior to use. Three pairs of antimicrobial combinations (i.e., Col+Cps, Col+Mox, and Cps+Mox) were tested by both methods. Minimal inhibitory concentrations (MICs) of each drug alone and in combination with other drugs were read after 20-hours of incubation at 35°C. For the isolates with a MIC exceeding the E-test strip detection limit, the next two-fold dilution was used for fractional inhibitory concentration (FIC) calculation. The FIC index (FICI) was defined as the FIC of drug A plus the FIC of drug B. The FIC of each drug was calculated using the MIC of the drug in combination divided by the MIC of that drug alone. The FICIs were interpreted, as follows: synergy = FICI of ≤0.5; additivity = FICI of >0.5 to ≤1; indifference (no interaction) = FICI of >1 to ≤4; and, antagonism = FICI of >4.

RESULTS

Susceptibility to Col, Cps, and Mox of all XDR *A. baumannii* isolates tested is demonstrated in Table 1. All XDR isolates in this study were still susceptible to Col, and the majority (56.25%) of them exhibited MIC at 1 µg/ml. All XDR isolates were found to be resistant to Cps and Mox with MIC ranges of 32 to >256 mg/ml and 4 to 32 µg/ml, respectively. The MIC_{50} and MIC_{90} of Cps were 128 µg/ml and 256 µg/ml, respectively; whereas, those MICs of Mox were 16 µg/ml and 32 µg/ml, respectively.

The *in vitro* activities of antimicrobial combinations among Col+Cps, Cps+Mox, and Col+Mox tested by CB and E-test methods against 80 isolates of XDR *A. baumannii* are shown in Table 2. When tested by CB method, synergistic effect was mostly observed from the combinations of Col+Cps (12.5%) and Cps+Mox (5.0%). There was no significant difference (p>0.05) between the synergy rates of those two combinations. Synergy was not found in any isolate tested with Col+Mox combination. These results indicate that Cps-based combinations were superior to the others. The most common result interpretation was additivity for all three antimicrobial combinations, including Col+Cps (78.8%), Cps+Mox (85%), and Col+Mox (87.5%).

Based on the MIC values of Cps, all 80 isolates studied could be divided into 3 following groups: ≤64, 128, and ≥256 µg/ml (Tables 3 and 4). For Col+Cps combination, the result showed that better synergistic

TABLE 1. Susceptibility results of 80 *Acinetobacter baumannii* isolates to each antimicrobial agent

Antimicrobial agent	MIC range	MIC_{50}	MIC_{90}	% Susceptibility
Col	0.25 - 2	1	1	100
Cps	32 - >256	128	256	0
Mox	4 - 32	16	32	0

Abbreviations: MIC_{50} = minimum inhibitory concentration for 50% of isolates tested; MIC_{90} = minimum inhibitory concentration for 90% of isolates tested; Col = colistin; Cps = cefoperazone-sulbactam; Mox = moxifloxacin

TABLE 2. Results of *in vitro* antimicrobial combination activity determined by checkerboard and E test methods against 80 isolates of *Acinetobacter baumannii*

Interpretation*	Number of isolates (%)								
	Col+Cps			Cps+Mox			Col+Mox		
	CB	E-test	Con**	CB	E-test	Con**	CB	E-test	Con**
S	10	3	0	4	2	0	0	0	0
	(12.5)	(3.7)	(0)	(5)	(2.5)	(0)	(0)	(0)	(0)
A	63	42	31	68	46	37	70	14	7
	(78.8)	(52.5)	(73.8)	(85)	(57.5)	(80.4)	(87.5)	(17.5)	(50)
I	7	35	2	8	32	2	10	66	11
	(8.7)	(43.8)	(28)	(10)	(40)	(6.3)	(12.5)	(82.5)	(16.7)
Total	80	80	33	80	80	39	80	80	18
	(100)	(100)	(41.3)	(100)	(100)	(48.8)	(100)	(100)	(22.5)

Abbreviations: CB = checkerboard method; Con = concordance; Col = colistin; Cps = cefoperazone-sulbactam; Mox = moxifloxacin; S = synergy; A = additivity; I = indifference; *No antagonism detected in this study; **Concordant rate was calculated using the E-test result as the total member.

TABLE 3. Results of colistin plus cefoperazone-sulbactam activity against each group of *Acinetobacter baumannii* based on cefoperazone-sulbactam minimum inhibitory concentration

Cps MIC (µg/ml)	Number of isolates	% S	% A	% S+A
≤64	22	9.09	72.73	81.82
128	48	10.42	83.33	93.75
≥256	10	30	70	100

Abbreviations: Cps = cefoperazone-sulbactam; MIC = minimum inhibitory concentration; % S = percent synergy; % A = percent additivity; %S+A = percent synergy plus additivity

TABLE 4. Results of cefoperazone-sulbactam plus moxifloxacin activity against each group of *Acinetobacter baumannii* based on cefoperazone-sulbactam minimum inhibitory concentration

Cps MIC (μg/ml)	Number of isolates	% S	% A	% S+A
≤64	22	9.09	59.09	68.18
128	48	2.08	95.83	97.91
≥256	10	10	90	100

Abbreviations: Cps = cefoperazone-sulbactam; MIC = minimum inhibitory concentration; % S = percent synergy; % A = percent additivity; % S+A = percent synergy plus additivity

effect was obtained from the higher Cps MIC values (Table 3). However, this event was not observed for the Cps+Mox combination (Table 4).

Results obtained from E-test exhibited lower activity than from CB method for all antimicrobial combinations (Table 2). Synergy rates observed from Col+Cps and Cps+Mox by E-test were only 3.7% and 2.5%, respectively. Antagonism was not detected in any antimicrobial combination tested.

Correlation of the results generated by CB method and E-test is also shown in Table 2. Concordant results were commonly observed for the additive effect across all 3 combinations. High additivity concordance was only observed in Col+Cps (73.8%) and Cps+Mox (80.4%). Unfortunately, low concordance of results was observed overall (41.3%, 48.8%, and 22.5% from Col+Cps, Cps+Mox, and Col+Mox, respectively).

DISCUSSION

Col, which is a drug that was first released for clinical use in 1959 is effective for many kinds of bacteria, including *A. baumannii*. However, clinical use of this agent is limited by its toxicity (nephrotoxicity and neurotoxicity), and this led to the introduction of newer and less toxic agents, such as carbapenems. However, *A. baumannii* continues to develop increasing resistance to these newer effective drugs. Importantly, the majority of XDR strains are still susceptible to Col, but these organisms exhibit rather high MICs that are close to the MIC breakpoint (≤2 μg/ml, susceptible). In the present study, approximately 56% and 6% of isolates had Col MICs of 1 and 2 μg/ml, respectively. High Col MIC values of *A. baumannii* isolates were also reported by another groups of investigators.[8,9] Since Col is considered a last-resort option for treatment of resistant bacterial infection, and Col resistance genes have been reported to spread both vertically and horizontally[10], this drug should be used with both care and caution. It has been recommended that Col should be used in combination with another antimicrobial agent to obtain pharmacological or synergistic effect, lower dose-related toxicity, and lower resistance development rate.

Sulbactam is a member of the serine β-lactamase inhibitor family, and it is unable to inhibit any carbapenemases. The activity of sulbactam against *A. baumannii* clinical isolates was found to be mediated via inhibition of the penicillin-binding proteins (PBPs) PBP1 and PBP3, with very low frequency of resistance.[4] Additionally, the outer membrane of *A. baumannii* appeared to allow good sulbactam uptake, thereby promoting antibacterial activity. However, the pbp3 mutant could result in a high level of resistance to sulbactam. Sulbactam in combination with other antimicrobial agent was shown to exhibit less significant effect than Cps (cefoperazone-sulbactam)-based combination against *A. baumannii*. Cps was included in this study; however, all isolates tested were found to be resistant to the drug with high MIC_{90} (256 μg/ml) (Table 1).

For CB method, Col+Cps was found to exhibit slightly higher *in vitro* activity than the activity observed from the other two combinations against XDR *A. baumannii*. E-test, which is an easy-to-perform method, was found to generate results that largely did not agree with those from CB method. However, E-test usually yielded lower activity of antimicrobial combination than CB, which suggests a low possibility of a very major error result from E-test. Similar results were also reported previously.[11,12] Specifically – due to the generally low concordance rate, E-test may be limited to the additive result of Col+Cps and Cps+Mox.

Many studies[3,13-17] in the *in vitro* activity of Col combined with another agent against *A. baumannii* have

been published. Examples of agents used in combination with Col include carbapenems, sulbactam, fosfomycin, tigecycline, rifampicin, minocycline, lipopeptides, and glycopeptides. Among those combinations, Col+meropenem or Col+imipenem was shown to demonstrate superior activity. To date, up to 90% of *A. baumannii* isolates were found to be resistant to carbapenems. Moreover, the synergistic activity was found to depend on the level of resistance to carbapenems. If the strains were resistant to a high level of those drugs, synergism was not demonstrated.[15] This finding is different from the result observed in the present study. We found the synergy rate from Col+Cps to be increased at a high level of Cps resistance (Table 3). A similar result was obtained from the combination between tigecycline and Cps studied by Li, *et al*.[3] They found the synergistic and additive effects of tigecycline+Cps to be increased with higher tigecycline MIC values. However, the Cps+Mox combination in this study did not demonstrate either of those characteristics. These results indicate different effects of resistance levels on synergy rates depending on each antimicrobial combination. Moreover, it seems that antimicrobial synergy testing may need to be assessed on an isolate-by-isolate basis, and a clinical trial is also needed. In addition and importantly, an effective control measure must be implemented whenever XDR or PDR *A. baumannii* is detected.

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