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Antimicrobial susceptibility of *Acinetobacter baumannii* isolated from hospital patients

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ABSTRACT: The incidence of multidrug-resistant *Acinetobacter baumannii* (MDR *A. baumannii*) is increasing worldwide and is leading to therapeutic problems. We investigated the in vitro activities of cefoperazone/sulbactam, colistin, imipenem, and rifampicin alone and in double combinations against 100 *A. baumannii* isolates from patients at Songklanagarind Hospital in Songkhla Province, Thailand. The E-test method was used to determine antimicrobial susceptibility, the minimal inhibitory concentration (MIC) and for antimicrobial combination testing. *A. baumannii* isolates were susceptible to colistin (97%), cefoperazone/sulbactam (69%), imipenem (45%), and rifampicin (13%). Fifty-nine percent of them were MDR *A. baumannii*. Colistin was superior to cefoperazone/sulbactam, rifampicin and imipenem against MDR *A. baumannii* and the MIC_{50}, MIC_{90} of colistin were 0.75 and 1 μg/ml, respectively. Non-MDR *A. baumannii* isolates were susceptible to cefoperazone/sulbactam (100%), colistin (95%), imipenem (93%) and rifampicin (2%). Combinations of cefoperazone/sulbactam plus colistin or rifampicin, imipenem plus colistin or rifampicin and colistin plus rifampicin showed indifferent effects against most MDR isolates. Of all the antimicrobial combinations tested, cefoperazone/sulbactam plus rifampicin produced the highest percentages (42%) of synergy, partial synergy, and additive results. The activity rate of cefoperazone/sulbactam against MDR *A. baumannii* was higher when combined with rifampicin than colistin. Thus colistin had the greatest activity against most MDR and non-MDR *A. baumannii* isolates among all of the antibiotics tested. Cefoperazone/sulbactam and imipenem showed good activity against non-MDR isolates, and cefoperazone/sulbactam combined with rifampicin may be useful in treating infections caused by MDR isolates.

KEYWORDS: drug combinations, multidrug-resistant *A. baumannii*, colistin, cefoperazone/sulbactam, imipenem, rifampicin

INTRODUCTION

A. baumannii is one of the most important nosocomial pathogens because of its longevity in the hospital environment and ability to resist various antimicrobial agents, such as resistance to broad-spectrum β-lactam antibiotics by β-lactamases production. Changes in penicillin-binding proteins prevent their action resulting in penicillin resistance. Alterations in the structure and number of porin protein result in decreased permeability to antibiotics through the outer membrane of the bacterial cell. The activity of efflux pumps lead to a reduction of antibiotic concentration within the bacterial cell. Resistance to aminoglycoside antibiotics results from aminoglycoside-modifying enzymes, AdeABC efflux pump, and 16S rRNA methylation. Modifications in the structure of DNA gyrase (mutations in the *gyrA* and *parC* genes) reduce the biding affinity of quinolones to the enzyme-DNA complex resulting in fluoroquinolone antibiotics resistance[1]. *A. baumannii* frequently causes ventilator-associated pneumonia, urinary tract infection, meningitis, surgical site infection, and bacteraemia[1].

Multidrug-resistant *A. baumannii* (MDR *A. baumannii*) is resistant to more than two antimicrobial classes such as antipseudomonal cephalosporins, antipseudomonal carbapenems, β-lactam/β-lactamase inhibitors, aminoglycosides, and fluoroquinolones.

The incidence of MDR *A. baumannii* is increasing worldwide, including Europe, North America, Latin America, and Asia[1]. Infections caused by MDR *A. baumannii* are associated with high morbidity rates, especially in immunocompromised patients, patients admitted to intensive care units, and patients treated

with broad-spectrum antibiotics[1,2].

Antimicrobial agents such as imipenem, sulbactam, colistin and rifampicin have been used for *A. baumannii* treatment[1,3]. However, colistin-resistant and imipenem-resistant *A. baumannii* have emerged and these isolates are often multidrug-resistant[1,4,5].

Previous in vitro studies have demonstrated that a combination of antimicrobial agents such as colistin and rifampicin, colistin and imipenem, imipenem and rifampicin, and cefoperazone/sulbactam combined with imipenem produce better activity against *A. baumannii* and MDR *A. baumannii*[6–8]. The combination of antimicrobial agents seems to be an alternative in *A. baumannii* treatment.

The aim of this study was to determine the in vitro activity of cefoperazone/sulbactam, colistin, imipenem, and rifampicin alone and in double combinations against *A. baumannii* isolated from patients at Songklanagarind Hospital in Southern Thailand.

MATERIALS AND METHODS

Bacterial strains

One hundred *A. baumannii* isolates were collected from clinical specimens such as sputum (64), blood (12), urine (8), pus (from leg, 2; pleural empyema, 1; axilla, 2; vagina, 1; percutaneous endoscopic gastrostomy tube, 1; and eye, 1), body fluid (6) and tissue (2) from patients at Songklanagarind Hospital in Songkhla Province, Thailand during the January–July 2010 period. Each isolate was collected from a different patient. Bacterial isolation and identification were performed using standard laboratory methods[9]. This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (EC 53-139-01-7-3-1).

MDR *A. baumannii* detection

Antimicrobial susceptibilities to amikacin (30 µg, AK), cefoperazone/sulbactam (30/75 µg, SPZ), ceftazidime (30 µg, CAZ), ciprofloxacin (5 µg, CIP) and imipenem (10 µg, IMP) (Oxoid, Ltd., Basingstoke, Hampshire, England) were determined by disk diffusion as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. MDR *A. baumannii* was defined as an intermediately-resistant or resistant isolate to more than two of the following five antimicrobial agents: amikacin, cefoperazone/sulbactam, ceftazidime, ciprofloxacin, and imipenem[1].

Antimicrobial susceptibility testing and MIC determination

The E-test was used to determine antimicrobial susceptibility and the minimal inhibitory concentration (MIC) of cefoperazone/sulbactam (0.016–256 µg/ml, CPS), colistin (0.016–256 µg/ml, CO), imipenem (0.002–32 µg/ml, IP), and rifampicin (0.002–32 µg/ml, RI) (AB Biodisk, Solna, Sweden) against *A. baumannii* based on CLSI guidelines[10]. The cefoperazone MIC breakpoint was used for cefoperazone/sulbactam[11]. Rifampicin MICs of $\leqslant$ 2 µg/ml were considered as susceptible, MICs of 4–8 µg/ml were considered as low-level resistant, and MICs of up to 256 µg/ml were considered as high-level resistant[12,13]. *Escherichia coli* (*E. coli*) ATCC 25922 was used as a control strain.

Antimicrobial combination testing

The antimicrobial combination testing was carried out via the E-test method[14]. Briefly, E-test strips of the two antimicrobial agents were placed at an angle of 90° at the MIC of each antimicrobial agent on an inoculated Mueller Hinton agar plate. The plates were incubated at 35 °C for 18–24 h. The inhibition zone of each antimicrobial agent intersecting the E-test strip was interpreted as the MIC in combination. Fractional inhibitory concentrations (FICs) were calculated by dividing the MIC of drug A and B in combination by the MIC of drug A or B alone. The fractional inhibitory concentration index (FICI) was obtained by the sum of the FICs of each drug. The FICI was interpreted as follows: FICI $\leqslant$ 0.5 represented synergy, $0.5 <$ FICI < 1 represented partial synergy, FICI $= 1$ represented additive effects, $1 <$ FICI < 4 represented indifference, and FICI $\geqslant 4$ represented antagonism[14,15].

RESULTS

Antimicrobial susceptibility

The antimicrobial susceptibility of 100 *A. baumannii* isolates against four antimicrobial agents is shown in Table 1. Among all the antimicrobial agents tested, 97%, 69%, 45%, and 13% of isolates were susceptible to colistin, cefoperazone/sulbactam, imipenem, and rifampicin, respectively. The MIC_{50} and MIC_{90} values of these four antimicrobial agents showed that most isolates were susceptible to colistin; it had the highest antimicrobial activity. Cefoperazone/sulbactam provided moderate activity, with an MIC_{50} of 12 µg/ml. The MIC_{50} and MIC_{90} of imipenem were higher than the CLSI susceptibility breakpoint, indicating that imipenem had a low antimicrobial activity against these isolates. The MIC_{50} and MIC_{90} of rifampicin were 4 µg/ml and 6 µg/ml, respectively, indicating that *A. baumannii* possesses a low-level resistance against this antimicrobial agent.

Table 1 The MIC_{50} and MIC_{90} values, and the percentages of susceptibility rate of 100 *A. baumannii* isolates against 4 antimicrobial agents.

Antimicrobial agents	*A. baumannii* ($n = 100$)			Antimicrobial susceptibility					
				Non-MDR ($n = 41$)			MDR ($n = 59$)		
	MIC_{50}	MIC_{90}	S (%)	MIC_{50}	MIC_{90}	S (%)	MIC_{50}	MIC_{90}	S (%)
Colistin	0.75	1	97 (97)	1	1.5	39 (95)	0.75	1	58 (98)
Cefoperazone/sulbactam	12	64	69 (69)	1.5	2	41 (100)	24	96	28 (47)
Imipenem	16	> 32	45 (45)	0.19	0.5	38 (93)	> 32	> 32	7 (12)
Rifampicin	4	6	13 (13)	4	8	1 (2)	4	6	12 (20)

MIC breakpoints of cefoperazone/sulbactam: susceptible $\leqslant$ 16 μg/ml, intermediately-resistant 32 μg/ml, resistant $\geqslant$ 64 μg/ml; colistin: susceptible $\leqslant$ 2 μg/ml, resistant $\geqslant$ 4 μg/ml; imipenem: susceptible $\leqslant$ 4 μg/ml, intermediately-resistant 8 μg/ml, resistant $\geqslant$ 16 μg/ml; rifampicin: susceptible $\leqslant$ 2 μg/ml, low-level resistant 4–8 μg/ml and high-level resistant $\geqslant$ 256 μg/ml (CLSI, 2011)

The results of antimicrobial susceptibility tests showed that 59% of isolates were MDR *A. baumannii*. Yet, both MDR and non-MDR *A. baumannii* were susceptible to colistin (98% and 95%, respectively) and had MIC_{50} and MIC_{90} values $<$ 2 μg/ml. All the non-MDR *A. baumannii* were highly susceptible to cefoperazone/sulbactam with MIC_{50} and MIC_{90} values of $\leqslant$ 2 μg/ml, thirty-eight (93%) isolates were susceptible to imipenem and had MIC_{50} and MIC_{90} values of $\leqslant$ 0.5 μg/ml, while only one (2%) isolate was susceptible to rifampicin, having MIC_{50} and MIC_{90} values of 4 μg/ml and 8 μg/ml, respectively. Twenty-eight (47%) of the MDR *A. baumannii* were susceptible to cefoperazone/sulbactam. The corresponding MIC_{50} and MIC_{90} values were 24 μg/ml and 96 μg/ml, respectively. Only seven (12%) isolates were susceptible to imipenem and had MIC_{50} and MIC_{90} values of $>$ 32 μg/ml, and twelve (20%) isolates were susceptible to rifampicin, having MIC_{50} and MIC_{90} values of 4 μg/ml and 6 μg/ml, respectively.

Antimicrobial combination

The effects of double combinations of cefoperazone/-sulbactam, colistin, imipenem and rifampicin against *A. baumannii* isolates are shown in Table 2.

Among all the antimicrobial combinations tested, an indifferent effect was observed in most MDR isolates. Nevertheless, synergy, partial synergy and additive effects could be detected in some isolates. The results indicated that synergy, partial synergy and additive effects were 0–7%, 7–24%, and 0–15%, respectively.

Of the antimicrobial combinations tested, cefoperazone/sulbactam plus rifampicin produced the highest percentages (42%) of the total of synergy, partial synergy, and additive results. Only one MDR *A. baumannii* isolate revealed an antagonistic effect for the cefoperazone/sulbactam plus colistin and cefoperazone/sulbactam plus rifampicin combinations.

The percentages of MDR *A. baumannii* isolates susceptible to cefoperazone/sulbactam (47%) increased when it was combined with rifampicin (66%) or colistin (59%). Additionally, the MIC_{50} of cefoperazone/sulbactam against MDR *A. baumannii* isolates (24 μg/ml) was lowered when it was combined with rifampicin (16 μg/ml) or colistin (16 μg/ml). Imipenem combined with rifampicin or colistin and imipenem alone showed poor activity against MDR isolates; the percentages of susceptibility rates were 13%, 13% and 12%, respectively. The MIC_{50} values of imipenem against MDR *A. baumannii* were $>$ 32 μg/ml. When imipenem and rifampicin or imipenem and colistin were used together, the MIC_{50} values of imipenem in combinations were higher than the CLSI susceptibility breakpoint. The results demonstrated that combining imipenem and rifampicin reduced the MIC_{50} of imipenem from $>$ 32 to 24 μg/ml, and the combination of imipenem and colistin lowered the MIC_{50} of imipenem from $>$ 32 to 32 μg/ml (Table 3).

DISCUSSION

The in vitro antimicrobial susceptibility test results of this study reveal that colistin possesses a higher activity rate against *A. baumannii* than cefoperazone/-sulbactam, imipenem, or rifampicin. Similar data obtained from studies from Chiang Mai University Hospital in Northern Thailand indicate that colistin has a higher activity against *A. baumannii* than cefoperazone/sulbactam or imipenem (out of 132 *A. baumannii* isolates, 96%, 77%, and 64% were susceptible to colistin, cefoperazone/sulbactam, and imipenem, respectively)[16]. The result of clinical effectiveness of colistin monotherapy and combination therapy suggested that colistin alone achieves cure and/or

Table 2 Effect of double combinations of cefoperazone/sulbactam (CPS), colistin (CO), imipenem (IP), and rifampicin (RI) against 59 MDR *A. baumannii* isolates.

Antimicrobial combinations	Interpretation (%)					
	Synergy	Partial synergy	Additive effects	Total synergy, partial synergy and additive effects	Indifference	Antagonism
CPS + CO	4 (7)	10 (17)	1 (2)	15 (26)	43 (73)	1 (2)
CPS + RI	2 (3)	14 (24)	9 (15)	25 (42)	33 (56)	1 (2)
IP + CO	3 (5)	6 (10)	0 (0)	9 (15)	50 (85)	0 (0)
IP + RI	1 (2)	4 (7)	2 (3)	7 (12)	52 (88)	0 (0)
CO + RI	0 (0)	4 (7)	2 (3)	6 (10)	53 (90)	0 (0)

Fractional inhibitory concentration index (FICI): synergy, FICI $\leqslant 0.5$; partial synergy, $0.5 <$ FICI < 1; additive effect, FICI $= 1$; indifference, $1 <$ FICI < 4; antagonism, FICI $\geqslant 4$.

Table 3 The MIC_{50} values and the percentages of the susceptibility rate of 59 MDR *A. baumannii* isolates against a single antimicrobial agent and antimicrobial agent combinations.

Antimicrobial agents	MIC_{50} (μg/ml)	S (%)
CPS*	24	28 (47)
CPS*+ CO	16	35 (59)
CPS*+ RI	16	39 (66)
IP*	> 32	7 (12)
IP*+ CO	32	8 (13)
IP*+ RI	24	8 (13)

* Main drug.
MIC breakpoints of cefoperazone/sulbactam: susceptible $\leqslant$ 16 μg/ml and imipenem: susceptible $\leqslant$ 4 μg/ml (CLSI, 2011).

improvement rates ranging from 57% to 78%[17,18], whereas the equivalent rates for combination therapy showed 67% to 74%[18].

Colistin monotherapy has shown the problem of nephrotoxicity, neurotoxicity, colistin-resistance, and heteroresistance among gram-negative bacterial populations[19–21]. Nephrotoxicity is a major concern when colistin is administered in patients with a history of chronic renal failure[17]. Colistin combination therapy is being strongly recommended against monotherapy due to selection of heteroresistant strains during prolonged colistin therapy[22] and heteroresistance strains were reported to be correlated with endemic infections in ICU[23]. Thus combination therapy might be beneficial for clinicians to prevent the emergence of resistance during therapy, decreased nephrotoxicity, especially if therapeutic options are limited.

The antimicrobial resistance studies of *A. baumannii* in Thailand during the 2000–2005 period revealed a 46–56% resistance rate by MDR *A. baumannii* isolates[24]. Our results indicated that 59% of *A. baumannii* isolates were MDR. Furthermore, both MDR and non-MDR *A. baumannii* isolates remained highly susceptible to colistin. Various reports from many countries, such as China, Greece, and Turkey have shown 21.5–84% of *A. baumannii* isolates during 1996–2009 were MDR *A. baumannii* and 94–100% of these isolates were susceptible to colistin[11,25,26]. Ninety-eight percent of MDR *A. baumannii* isolates were susceptible to colistin and had MIC_{50} and MIC_{90} values of 0.75 and 1 μg/ml, which were similar to those of previous studies from Dizbay[7,8,25]. This indicates that colistin was effective antimicrobial agent against MDR *A. baumannii*.

Imipenem and cefoperazone/sulbactam have generally been used in *A. baumannii* treatment[4,24]. Our data also affirm that cefoperazone/sulbactam and imipenem are good choices for the eradication of non-MDR *A. baumannii* isolates.

Moreover, rifampicin has been proposed as an alternative antimicrobial agent for the treatment of MDR *A. baumannii*, based on outcomes of in vitro studies[2,7,8] and in vivo infection models[12,27]. A previous study has shown that 64% of MDR *A. baumannii* were susceptible to rifampicin[7]. In contrast, our results show that rifampicin has low antimicrobial activity against most MDR and non-MDR *A. baumannii*. As with our findings, the study by Giamarellos-Bourboulis et al reported that 15% of MDR *A. baumannii* were susceptible to rifampicin, having MIC_{50} and MIC_{90} values higher than 2 μg/ml[3].

The combination antimicrobial therapy has been used as an alternative in MDR *A. baumannii* treatment. The presence of synergy, partial synergy, and additive effect could potentially reduce toxicity and improve outcomes for patients with difficult-to- treat infections[28]. Forty-seven of 59 MDR *A. baumannii* isolates in this study (80%) were resistance to rifampicin (45 isolates had MIC ranges 3–8 μg/ml

and 2 isolates had MICs $>$ 32 μg/ml). The synergy, partial synergy, and additive results could be detected in 28 isolates in the case of susceptible (1 isolate) and resistance to rifampicin (27 isolates).

We found that 1 of 12 MDR *A. baumannii* isolates was susceptible to rifampicin revealed synergy result for cefoperazone/sulbactam plus rifampicin and imipenem plus rifampicin. Twenty-seven of 45 MDR *A. baumannii* isolates were resistance to rifampicin (MIC ranges 3–8 μg/ml); 16 isolates revealed synergy, partial synergy, and additive results for cefoperazone/sulbactam plus rifampicin, 5 isolates revealed partial synergy and additive results for imipenem plus rifampicin, 5 isolates revealed partial synergy and additive results for cefoperazone/sulbactam plus rifampicin and colistin plus rifampicin, 1 isolate revealed partial synergy and additive results for cefoperazone/sulbactam plus rifampicin, imipenem plus rifampicin and colistin plus rifampicin. Although Rifampicin is a hydrophobic antibiotic, its negative charge and large molecular size cause it to be unable to effectively penetrate through the outer membrane of *A. baumannii* alone[5]. Other antibiotics which combine with rifampicin may be related to substantial changes in the outer membrane of *A. baumannii* isolates; thus enhancing the ability of rifampicin to penetrate into the cell[29]. The effectiveness of rifampicin in antimicrobial combinations, however, could depend on the degree of sensitivity and specific resistance mechanisms of individual *A. baumannii* isolates[30].

Previously, the efficacy of rifampicin in association with antimicrobial agent was demonstrated even in the case of strains susceptible and resistance to rifampicin[3,7]. In colistin combined with rifampicin, Timurkaynak et al found that four MDR *A. baumannii* isolates (3 of 4 isolates were susceptible to rifampicin and 1 of 4 isolates were low-level resistant to rifampicin) exhibited synergy effect and one MDR *A. baumannii* isolates was resistance to rifampicin showed partial synergy effect[7]. The study of Giamarellos-Bourboulis et al[3] using the time-kill method showed 85% of MDR *A. baumannii* isolates were resistant to rifampicin, having MIC ranges of 2–64 μg/ml. The combination of colistin and rifampicin was a synergy effect against 15% of MDR *A. baumannii* isolates[3].

Combinations of rifampicin with ampicillin/sulbactam, imipenem with colistin or rifampicin, and colistin with rifampicin have shown promising results against MDR *A. baumannii*[6–8]. Recently, Ozseven et al found that the combination of imipenem plus rifampicin produced a 73% rate of synergy effect against 34 MDR *A. baumannii* isolates evaluated by the chequerboard microdilution method[31]. Likewise, Pongpech et al reported that the synergy effect of imipenem plus colistin and sulbactam plus colistin against 30 MDR *A. baumannii* isolates by the chequerboard microdilution panel method were 100% and 53%, respectively[32]. Furthermore, the study of Timurkaynak et al reported the synergy effect of colistin plus rifampicin against 4 MDR *A. baumannii* isolates by the chequerboard method at 100%[7]. In this study, all the antimicrobial combinations tested by the E-test method yielded a predominantly indifferent effect.

Our findings indicate that the synergy effects of cefoperazone/sulbactam combined with colistin or rifampicin, imipenem combined with colistin or rifampicin, and colistin combined with rifampicin were lower than the results of previous studies investigating the same in vitro synergy effect by the chequerboard method[7,31,32]. However, the effects of antimicrobial combinations against organisms could depend on individual strains and evaluation methods[6]. Nevertheless, our study demonstrated that the activity of cefoperazone/sulbactam against MDR *A. baumannii* could be improved by combining it with rifampicin. This suggests that cefoperazone/sulbactam plus rifampicin may be a reasonable choice in the treatment of infections by these organisms. *A. baumannii* is the most common bacteria causing nosocomial pneumonia. Although MDR *A. baumannii* is highly susceptible to colistin, the clinical response rate to this antibiotic is only 25–62% due to low colistin levels in lungs tissue[33]. In addition, the main adverse effects of colistin are nephrotoxic and neurotoxic. The incidences of renal toxicity and neurotoxicity are 7–69%[20,21] and 7%[34], respectively.

We suggest that, in patients suffering from MDR *A. baumannii* nosocomial pneumonia that is not responsive to colistin, the combination of cefoperazone/sulbactam plus rifampicin might be beneficial. However, the combination of cefoperazone/sulbactam with rifampicin needs to be studied on a larger scale than that afforded by this investigation.

We did not use the standard testing method (chequerboard method and time-kill test) for evaluated the in vitro synergy activity of antimicrobial combination against *A. baumannii* isolates. The synergy results in this study may be insensitive to the E-test method when compared with the chequerboard and time-kill test. The agreement between chequerboard method and E-test was 63% and between time-kill test and E-test method was 72%, the minor disagreements occurred when synergy was observed using either the chequerboard or time-kill test while additive or

indifference was observed using the E-test method[22]. In a combination therapy for *A. baumannii* isolates, therefore, interpretation of results requires caution in empirical therapy.

In conclusion, the findings of this study indicate that colistin has the best activity against *A. baumannii*, whereas cefoperazone/sulbactam and imipenem are good choices for the treatment of non-MDR *A. baumannii* infections.

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