The Journal of Antibiotics (2014) 67, 677–680

www.nature.com/ja

ORIGINAL ARTICLE

In vitro activities of rifampin, colistin, sulbactam and tigecycline tested alone and in combination against extensively drug-resistant *Acinetobacter baumannii*

Xiaomeng Dong, Fengzhe Chen, Yajun Zhang, Haihong Liu, Yongjuan Liu and Lixian Ma

The aim of this study was to investigate the *in vitro* activities of rifampin, colistin, sulbactam and tigecycline alone and in combination against extensively drug-resistant *Acinetobacter baumannii* (XDR-Ab). Twenty-five XDR-Ab strains were isolated from patients. Broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) for rifampin, colistin, sulbactam and tigecycline against XDR-Ab strains. The checkerboard microdilution method was used to determine the *in vitro* activities of potential therapeutic combinations of these four antimicrobial agents. Accordingly, the fractional inhibitory concentration (FIC) and FIC index (FICI) were calculated for each of the combinations. According to our results, when tested as single drugs, rifampin, colistin or tigecycline had good bacteriostatic activity against XDR-Ab, whereas sulbactam was not as active against XDR-Ab isolates. On the other hand, when tested in combination, the combinations of colistin/rifampin, rifampin/sulbactam, rifampin/tigecycline and sulbactam/tigecycline showed good *in vitro* activities against XDR-Ab isolates. More importantly, these combination regimens could exert addictive or partially synergistic effects at the sub-MIC levels against XDR-Ab strains. Compared with single drugs, most of the combinations of these antimicrobial agents could exert partially synergistic and/or addictive effects, which might provide a better alternative when treating XDR-Ab infections.
The Journal of Antibiotics (2014) **67,** 677–680; doi:10.1038/ja.2014.99; published online 6 August 2014

INTRODUCTION

Acinetobacter baumannii (Ab) is a gram-negative, non-fermenting, aerobic coccobacillus, which could be widely detected in nature as well as in hospitals.[1] In recent years, Ab has attracted much attention because of its ability to acquire resistance to multiple antimicrobial agents and Ab has been defined as multidrug-resistant, extensively drug-resistant (XDR) and pandrug-resistant strains.[2] For years, antimicrobials used in treating multidrug-resistant-Ab infection have been limited to colistin, imipenem and β-lactamase inhibitors. Because imipenem-resistant Ab isolates are being detected more often throughout the world,[3] scientists have been trying hard to develop new antibiotics and have identified minocycline and tigecycline as having Ab activity,[4] both of which are derivatives of tetracycline. However, reports on the resistance of Ab isolates to minocycline as well as tigecycline have challenged monotherapy antibiotic regimens,[5] leading to the emergence of combination antimicrobial therapies.[6] In the present study, we studied the *in vitro* antimicrobial activities of rifampin, colistin, sulbactam and tigecycline alone and in combinations against Ab.

MATERIALS AND METHODS

XDR-Ab strains

A total of 25 XDR-Ab strains were isolated from patients in three hospitals affiliated to Shandong University, from November 2012 to June 2013. Only one strain from each patient was included. VITEK32 microbial analysis instruments were used to obtain these XDR-Ab isolates, of which 21 were from sputum, 2 from blood and 2 from wound. All of the strains were evaluated by the Kirby-Bauer (K-B) method as resistant to multiple antimicrobials, including aztreonam, piperacillin, ticarcillin/clavulanate, imipenem, ceftazidine, ciprofloxacin, gentamicin, amikacin, tobramycin, sulfamethoxazole, ceftriaxone and intermediate of cefoperazone/sulbactam. *Escherichia coli* ATCC25922 was used as a control.

Broth microdilution assay

Mueller-Hinton (MH) powder was purchased from Boshang Biotechnology Company (Shanghai, China), and dissolved according to the manufacturer's instructions. Isolated colonies of Ab strains were maintained in 10 ml fresh MH broth, shaking in a thermo-incubator at 37 °C overnight. When the turbidity matched 0.5 McFarland (1.5×10^8 CFU ml^{-1}), the cultures were diluted to 1:1000 to get final bacterial counts of 1×10^5 CFU ml^{-1}.

Antimicrobial agents (rifampin, colistin, sulbactam and tigecycline) were provided by BioDee Biotechnology Company (Beijing, China). These drugs were dissolved in double distilled water with a final concentration of 5120 μg ml^{-1}, and stored at −20 °C.

To determine MIC values, broth microdilution method was carried out as described in CLSI. The drug concentrations were 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0 μg ml^{-1}. Incubation was at 37 °C for 18–24 h. The MIC values were determined by the concentrations of drugs at which the bacterial growth was completely inhibited.

Department of Infectious Disease, Qilu Hospital, Shandong University, Jinan, Shandong, China
Correspondence: Professor L Ma, Department of Infectious Disease, Qilu Hospital, Shandong University, No.107, Wenhuaxi Road, Jinan, Shandong 250012, China.
E-mail: mlx@sdu.edu.cn
Received 25 February 2014; revised 8 June 2014; accepted 22 June 2014; published online 6 August 2014

Checkerboard microdilution assay

Checkerboard microdilution method was performed in the following way after the MICs of each drug for each strain were determined. Another set of dilution series were prepared for these four antimicrobials, as 8 × MIC, 4 × MIC, 2 × MIC, 1 × MIC, 0.5 × MIC, 0.25 × MIC, 0.125 × MIC and 0 μg ml^{-1}. One drug (A) in a specific combination was added by column, while the other (B) was added by row. Then the bacterial suspensions was added at 1 × 10^5 CFU ml^{-1}, and incubated at 37 °C for 18–24 h. FICI values were calculated as follows:

$$\mathrm{FICI} = \mathrm{MIC}\,(\mathrm{A2})/\mathrm{MIC}\,(\mathrm{A1}) + \mathrm{MIC}\,(\mathrm{B2})/\mathrm{MIC}\,(\mathrm{B1}),$$

where MIC (A2) represented the MIC value of drug A combined with drug B, while MIC (A1) represented the MIC value of drug A as monotherapy, with the same for MIC (B2) and MIC (B1). The FICI values were interpreted as follows: ⩽0.5, synergy; >0.5 to <1, partial synergy; 1, addition; >1 to <4, indifference; and ⩾4, antagonism.[7]

The former steps were carried out three times, average values were recorded as final results.

RESULTS

In vitro activities of rifampin, colistin, sulbactam and tigecycline against pandrug-resistant-Ab strains

MIC profiles for these antimicrobial agents were shown in Table 1.

Our results indicated that, out of total 25 strains analyzed, 22 of them were susceptible to colistin, whereas the rest 3 strains exhibited resistance, according to CLSI 2013 guidelines.[8] CLSI breakpoints were not available for rifampin, tigecycline or sulbactam, used in monotherapy. The breakpoints for rifampin can be referred to that against *Staphylococcus spp*, which are ⩽1, 2 and ⩾4 μgml^{-1}. The breakpoints of ampicilin/sulbactam against *Acinetobacter spp* are ⩽8/4, 16/8 and ⩾32/16 μgml^{-1}. The U.S. Food and Drug Administration recommended tigecycline susceptibility breakpoints for *Enterobacteriaceae* (susceptible ⩽2 g l^{-1}; intermediate 4 g l^{-1}; resistant ⩾8 g l^{-1}) were used as interpretation criteria. These results suggest that, for the single drugs, rifampin, colistin or tigecycline has good inhibitory activity against many XDR-Ab strains, whereas sulbactam alone was not as effective against XDR-Ab.[7]

In vitro activities of rifampin, colistin, sulbactam and tigecycline in combination against XDR-Ab strains

Distribution of FICI values for the therapeutic combinations was shown in Table 2.

Our results indicated that, when co-tested with rifampin, the other three agents showed increased antimicrobial activities. Similar results were obtained when either sulbactam or tigecycline was used in the combinations. Neither the combinations of colistin/sulbactam nor colistin/tigecycline showed the enhanced activity. The results show that combinations of colistin/rifampin, rifampin/sulbactam, rifampin/tigecycline and sulbactam/tigecycline show good *in vitro* activities against XDR-Ab strains.

Table 1 MIC values for rifampin, colistin, sulbactam and tigecycline in monotherapy against XDR-Ab isolates

Antimicrobial agents	*MIC range (μg ml^{-1})*	*MIC$_{50}$ (μg ml^{-1})*	*MIC$_{90}$ (μg ml^{-1})*
Rifampin	0.5–8	2	8
Colistin	2–16	2	8
Sulbactam	4–⩾128	32	64
Tigecycline	1–4	2	2

Abbreviations: MIC, minimum inhibitory concentration; XDR-Ab, extensively drug-resistant *Acinetobacter baumannii*.

Synergistic effects of the combination regimens against XDR-Ab strains

To further investigate the synergistic effects of the combination regimens, the changes in MICs for each of these antibiotics were calculated when combined with each of the other agents at either 0.25 × or 0.5 × MIC. As shown in Table 3, the average MICs of each agent were decreased when they were used in combination, which was in accordance with the changing trend in FICI values. These results suggest that these combination regimens could exert beneficial effects at the sub-MIC levels against pandrug-resistant-Ab strains.

DISCUSSION

During the past few decades, Ab has changed from an opportunistic pathogen into one of the most common and persistent bacterium capable of causing many kinds of nosocomial infections.[9] Public attention has been focused on this ubiquitous pathogen in recent years.[10,11] The reasons why Ab has attracted so much attention might be that it can stay alive for a long time in the environment,[1] and mostly importantly, it has evolved to be capable of acquiring resistance to multiple antimicrobial agents. Numerous reports worldwide have documented and described increasing evidence concerning the serious infections caused by Ab.[12–14] Ab causes ventilator-associated pneumonia, sepsis, meningitis, skin and soft tissue infection, as well as urinary tract infection, especially in intensive care unit residents whose immunity is usually impaired.[15]

Antimicrobials have been used as monotherapy to treat XDR-Ab. However, when used as monotherapy, resistance eventually occurs. Ab strains isolated from the Asia–Western Pacific region are mostly susceptible to tigecycline,[16] whereas some strains with higher MICs have been recently reported.[5] This has led us to find new ways to deal with this pathogen. Drug combination might be a better choice, and it offers many advantages.[17] First, drugs with different antimicrobial mechanisms may exert synergistic effects and enhance each other's activities. Secondly, combination therapy could reduce the dosages for

Table 2 Determination of FICI values for the therapeutic combinations against XDR-Ab isolates

	FICI				
Combinations	*Synergy (FICI: ⩽0.5)*	*Partial synergy (FICI: 0.5–1)*	*Addition (FICI: 1)*	*Indifference (FICI: 1–4)*	*Antagonism (FICI: ⩾4)*
	Percentage				
Colistin/rifampin	0	56%	36%	8%	0
Colistin/sulbactam	0	32%	24%	44%	0
Colistin/tigecycline	4%	4%	36%	56%	0
Rifampin/sulbactam	4%	32%	40%	24%	0
Rifampin/tigecycline	4%	60%	16%	20%	0
Sulbactam/tigecycline	8%	56%	20%	16%	0

Abbreviations: FICI, fractional inhibitory concentration index; XDR-Ab, extensively drug-resistant *Acinetobacter baumannii*.

Table 3 Synergistic effects of the combination regimens against XDR-Ab isolates

Colistin combined with	*Rifampin*		*Tigecycline*		*Sulbactam*	
	0.25× MIC	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*
MIC for colistin ($\mu g\,ml^{-1}$) (original MIC = 4 $\mu g\,ml^{-1}$)	0.83	0.45	1.66	0.26	1.74	1.21
Rifampin combined with	*Tigecycline*		*Colistin*		*Sulbactam*	
	0.25× MIC	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*
MIC for rifampin ($\mu g\,ml^{-1}$) (original MIC = 3.38 $\mu g\,ml^{-1}$)	0.72	0.20	0.67	0.25	1.95	1.72
Sulbactam combined with	*Tigecycline*		*Colistin*		*Rifampin*	
	0.25× MIC	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*
MIC for sulbactam ($\mu g\,ml^{-1}$) (original MIC = 36.43 $\mu g\,ml^{-1}$)	22.43	2.24	24.29	8.05	28.21	25.26
Tigecycline combined with	*Rifampin*		*Sulbactam*		*Colistin*	
	0.25× MIC	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*	*0.25× MIC*	*0.5× MIC*
MIC for tigecycline ($\mu g\,ml^{-1}$) (original MIC = 2.15 $\mu g\,ml^{-1}$)	0.54	0.42	0.70	0.57	0.84	0.58

Abbreviations: MIC, minimum inhibitory concentration; XDR-Ab, extensively drug-resistant *Acinetobacter baumannii*.

each agent, meanwhile reducing the drug toxicity. Moreover, combination therapy shows a much wider antimicrobial spectrum, and superinfection can be avoided for long-term diseases, including Ab infections.

Our results suggest that the combination regimens analyzed either exhibit synergistic, partially synergistic, additive or indifferent effects, rather than antagonism. Rifampin appears to be a good companion drug, when co-tested with sulbactam, colistin and tigecycline, respectively. The combination of sulbactam/tigecycline also achieves an improved antimicrobial activity. Some antimicrobial agents may be used as monotherapies against XDR-Ab, such as colistin, tigecycline and β-lactams. However, the activities of these monotherapies are lost quickly, and resistance can be induced. Therefore, when considering the treatment of XDR-Ab infection, combination therapy is proposed to be a better alternative. Our study provides evidence that the combinations of colistin/rifampin, rifampin/sulbactam, rifampin/tigecycline and sulbactam/tigecycline could be used in treating XDR-Ab infections. In contrast to our *in vitro* results, a recent prospective, randomized study that compared colistin vs colistin plus rifampin showed no clinical benefits of adding rifampin.[6] This discordance implies that an *in vitro* experiment is not necessarily correlated with clinical efficacy. This could result because of the discordance of redistribution of different agents in target tissues. Our studies demonstrate that compounds could be screened to find new combinations that could be synergistic *in vivo*. To make the selection process efficient, *in vitro* pharmacodynamic assays that can test actual *in vivo* concentrations may be helpful in determining the doses required. More detailed mechanism studies and clinical trials are still needed to support the practical use of these combination therapies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by the 2012 Innovation Fund (Free Exploration Grant type I, grant No. 26010172611152) of Shandong University.

1 Playford, E. G., Craig, J. C. & Iredell, J. R. Carbapenem-resistant Acinetobacter baumannii in intensive care unit patients: Risk factors for acquisition, infection and consequences. *J. Hosp. Infect.* **65,** 204–211 (2007).
2 Magiorakos, A.-P. *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **18,** 268–281 (2012).
3 Corbella, X. *et al.* Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant Acinetobacter baumannii. *J. Clin. Microbiol.* **38,** 4086–4095 (2000).
4 Olson, M. W. *et al.* Functional, biophysical, and structural bases for antibacterial activity of tigecycline. *Antimicrob. Agents. Chemother.* **50,** 2156–2166 (2006).
5 Chen, Q. *et al.* Decreased susceptibility to tigecycline in Acinetobacter baumannii mediated by a mutation in trm encoding SAM-dependent methyltransferase. *J. Antimicrob. Chemother.* **69,** 72–76 (2014).
6 Simsek, F. *et al.* Colistin against colistin-only-susceptible Acinetobacter baumannii-related infections: Monotherapy or combination therapy? *Indian. J. Med. Microbiol.* **30,** 448–452 (2012).
7 Ji, J. *et al.* In vitro activity of sulbactam in combination with imipenem, meropenem, panipenem or cefoperazone against clinical isolates of Acinetobacter baumannii. *Int. J. Antimicrob. Agents.* **41,** 400–401 (2013).
8 Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement* (Document M100-S23CLSI, Wayne, PA, 2013).
9 Lee, K., Yong, D., Jeong, S. H. & Chong, Y. Multidrug-resistant Acinetobacter spp.: increasingly problematic nosocomial pathogens. *Yonsei. Med. J.* **52,** 879–891 (2011).
10 Dijkshoorn, L, Nemec, A. & Seifert, H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. *Nat. Rev. Microbiol.* **5,** 939–951 (2007).
11 Peleg, A. Y., Seifert, H. & Paterson, D. L. Acinetobacter baumannii: emergence of a successful pathogen. *Clin. Microbiol. Rev.* **21,** 538–582 (2008).
12 Karah, N. *et al.* Emergence of OXA-carbapenemase- and 16S rRNA methylase-producing international clones of Acinetobacter baumannii in Norway. *J. Med. Microbiol.* **60,** 515–521 (2011).
13 Karah, N., Sundsfjord, A., Towner, K. & Samuelsen, O. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. *Drug Resist. Updat.* **15,** 237–247 (2012).

14 Stietz, M. S. *et al.* Acinetobacter baumannii extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III. *Infect. Genet. Evol.* **14,** 294–301 (2013).
15 Martins, N. *et al.* Imported and intensive Care unit-born Acinetobacter baumannii clonal complexes: one-year prospective cohort study Q3 in intensive care patients. *Microb. Drug Resist.* **19,** 216–223 (2013).
16 Farrell, D. J., Turnidge, J. D., Bell, J., Sader, H. S. & Jones, R. N. The in vitro evaluation of tigecycline tested against pathogens isolated in eight countries in the Asia-Western Pacific region (2008). *J. Infect.* **60,** 440–451 (2010).
17 Shin, J. A. *et al.* Clinical Outcomes of Tigecycline in the Treatment of Multidrug-Resistant Acinetobacter baumannii Infection. *Yonsei. Med. J.* **53,** 974–984 (2012).