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*Original Article*

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

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**Abstract:** Objectives: To detect the *in vitro* activities of sitafloxacin alone and in combination with rifampin, colistin, sulbactam, and tigecycline against extensively drug-resistant *Acinetobacter baumannii* (XDR-*A. baumannii*). Materials and methods: 24 XDR-*A. baumannii* strains were isolated from patients' specimens. Broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) for sitafloxacin, rifampin, colistin, sulbactam, and tigecycline against XDR-*A. baumannii* strains. The checkerboard microdilution method was used to determine the *in vitro* activities of sitafloxacin combined with the other four antimicrobial agents. Accordingly, the fractional inhibitory concentration (FIC) and FIC index (FICI) were calculated for each of the combinations. Results: According to our results, when tested alone, the rate of susceptibility for sitafloxacin was 91.67% against XDR-*A. baumannii*, followed by colistin 62.5%, and then tigecycline 54.17%, rifampin 41.67%. Sulbactam, with a 16.67% rate of susceptibility was the least effective one. On the other hand, when tested in combination, all those three combinations except tigecycline/sitafloxacin revealed remarkable synergistic effects. Colistin/sitafloxacin showed the highest indifference rate. These combination regimens could exert addictive or partially-synergistic effects at the sub-MIC levels against XDR-*A. baumannii* strains. Conclusion: Sitafloxacin has acceptable in vitro activity against XDR-*A. baumannii* strains as well as tigecycline, rifampin and colistin. Compared with single drugs, most of the combinations of these antimicrobial agents could exert synergistic and/or partially synergistic and/or addictive effects, which might provide a better alternative when treating XDR-*A. baumannii* infections.

**Keywords:** XDR-*A. baumannii*, sitafloxacin, rifampin, colistin, sulbactam, tigecycline, MIC, FICI

## Introduction

During the recent decades, the world has witnessed a dramatic increase in the ability of *A. baumannii*'s resistance toward antimicrobial agents [1]. *A. baumannii* has been defined as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug-resistant (PDR) strains. While XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) [2]. Due to its remarkable potential to acquire antibiotic resistance and to survive in nosocomial environments, *A. baumannii* has become a significant nosocomial infectious agent worldwide [1, 3]. As long as one agent was applied to treat *A. baumannii* infection, the resistance of *A. baumannii* to this agent was developed [4, 5]. Thus, the options of antibiotics for treating *A. baumannii* infections are limited, complicating the management of nosocomial infection. It is urgent for both clinicians and researchers to screen out antimicrobial agents or their combinations to control the spread and infection of *A. baumannii*. Fluoroquinolones which have broad-spectrum activity against both Gram-negative and -positive pathogens are commonly used antimicrobial agents [6]. Nowadays, resistance to fluoroquinolones could be found in most nosocomial isolates of *A. baumannii*. Fluoroquinolones have thus become a less than ideal treatment for *A. baumannii* -related infection. Sitafloxacin, a new fluoroquinolone, has been shown to have good in vitro activity against pathogens resistant to other

**Table 1.** MIC values of sitafloxacin, rifampin, colistin, sulbactam, and tigecyclinetested alone against XDR-Ab isolates

| Antimicrobial agents | MIC range (μg/mL) | $MIC_{50}$ (μg/mL) | $MIC_{90}$ (μg/mL) | Rate of susceptibility (%) |
|---|---|---|---|---|
| Sitafloxacin | 0.125-16 | 1 | 2 | 91.67 |
| Rifampin | 1-32 | 4 | 16 | 41.67 |
| Colistin | 0.5-64 | 2 | 8 | 62.5 |
| Sulbactam | 4-≥128 | 32 | 64 | 16.67 |
| Tigecycline | 0.5-4 | 2 | 4 | 54.17 |

fluoroquinolones [7, 8]. The rate of carbapenem-resistant *A. baumannii* susceptibility to sitafloxacin was deemed acceptable by other reports [9, 10]. Nevertheless data testing the antimicrobial activities of sitafloxacin alone and in combination with other agents against XDR-*A. baumannii* are lacking. In the present study, we studied the *in vitro* antimicrobial activities of sitafloxacin alone and in combination with rifampin, colistin, sulbactam, and tigecycline against XDR-*A. baumannii*.

## Materials and methods

### *XDR-A. baumannii strains*

A total of 24 XDR-*A. baumannii* strains were isolated from clinical specimens in three tertiary hospitals affiliated to Shandong University, from November 2013 to May 2014. Only one strain from each patient was included. VITEK32 microbial analysis instruments were used to obtain these XDR-*A. baumannii* isolates, of which 21 were from sputum, 1 from blood, 1 from cerebrospinal fluid, and 1 from urine. All of the strains were evaluated by Kirby-Bauer (K-B) method as resistant to all other species of antimicrobials, including aztreonam, piperacillin, ticarcillin/clavulanate, meropenem, ceftazidine, ciprofloxacin, levofloxacin, gentamicin, amikacin, tobramycin, sulfamethoxazole, ceftriaxone, but intermediate of or resistant to cefoperazone/sulbactam, susceptible or resistant to tigecycline. *Escherichia coli* ATCC25922 was used as a control.

### *Broth microdilution assay*

Mueller-Hinton (MH) powder was (Boshang Biotechnology, Shanghai, China) 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. Suspensions with a turbidity matched 0.5 McFarland ($1.5\times10^8$ CFU/mL were further diluted to 1:1000 to get final bacterial counts of $1\times10^5$ CFU/mL.

Antimicrobial agents (sitafloxacin, rifampin, colistin, sulbactam, and tigecycline) were provided by BioDee Biotechnology Company (Beijing, China). For preparation, these drugs were dissolved in MH broth and stored at -20°C.

To determine MIC values, broth microdilution method was carried out as described in CLSI [11]. The drug concentrations were 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0 μg/mL. Trays were incubated overnight in ambient air at 37°C. The MIC values were determined by the concentrations of drugs at which the bacterial growth was completely inhibited.

### *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 antimicrobials, as 8×MIC, 4×MIC, 2×MIC, 1×MIC, 0.5×MIC, 0.25×MIC, 0.125×MIC, and 0 μg/mL. Sitafloxacin was added by column, and other agents were added by row. Then the bacterial suspensions was added at $1\times10^5$ CFU/mL, and incubated overnight at 37°C. FICI values were calculated as follows: FICI = MIC (A2)/MIC (A1) + MIC (Sita2)/MIC (Sita1). Where MIC (A2) represented the MIC value of drug A combined with sitafloxacin, while MIC (A1) represented the MIC value of drug A as monotherapy, with the same for sitafloxacin marked as MIC (Sita2) and MIC (Sita1). 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 [12].

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

Furthermore, the MIC values of sitafloxacin when it was combined with 0.25MIC or 0.5MIC another agent were also collected. And average values of those MICs were calculated. The same was done with MIC values of those four agents when they were combined with 0.25MIC or 0.5MIC sitafloxacin.

**Table 2.** Determination of FICI values for sitafloxacin combined with other antibiotics against XDR-Ab isolates

| Combinations \ FICI / Percentage | Synergy (FICI: ≤0.5) | Partial synergy (FICI: 0.5-1) | Addition (FICI: 1) | Indifference (FICI: 1-4) | Antagonism (FICI: ≥4) |
|---|---|---|---|---|---|
| Sulbactam/Sitafloxacin | 12.5% | 41.67% | 25% | 20.83% | 0 |
| Rifampin/Sitafloxacin | 12.5% | 25% | 29.17% | 33.33% | 0 |
| Colistin/Sitafloxacin | 12.5% | 16.67% | 20.83% | 50% | 0 |
| Tigecycline/Sitafloxacin | 0 | 29.17% | 41.67% | 29.17% | 0 |

## Results

*In vitro activities of sitafloxacin, rifampin, colistin, sulbactam, and tigecycline against XDR-A. baumannii strains*

MIC profiles for these five antimicrobial agents were shown in **Table 1**. Isolates with sitafloxacin MICs ≤2 mg/L were provisionally considered as susceptible to sitafloxacin [10]. According to CLSI 2013 guidelines, the breakpoints for colistin were as follows: susceptible ≤2 μg/mL, resistant ≥4 μg/mL [11]. 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 μg/mL, 2 μg/mL, ≥ 4 μg/mL. The breakpoints of ampicilin/sulbactam against *Acinetobacter spp* are ≤8/4 μg/mL, 16/8 μg/mL, ≥32/16 μg/mL. The U.S. Food and Drug Administration (FDA) recommended tigecycline susceptibility breakpoints for *Enterobacteriaceae* (susceptible ≤2 g/L; intermediate 4 g/L; resistant ≥8 g/L) were used as interpretation criteria. These results suggest that, for the single drugs, sitafloxacin showed the most efficient antimicrobial activity among other agents. The bacteriostatic activity of rifampin, colistin, and tigecycline against XDR-*A. baumannii* strains were less effective but still efficient. Sulbactam was not as effective as others.

*In vitro activities of sitafloxacin in combination with rifampin, colistin, sulbactam, and tigecycline against XDR-A. baumannii strains*

Distribution of FICI values for those four combinations was shown in **Table 2**.

Our results estimated that all those three combinations except tigecycline/sitafloxacin revealed remarkable synergistic effects. Colistin/sitafloxacin showed the highest indifference rate, followed by rifampin/sitafloxacin and tigecycline/sitafloxacin. Sulbactam/sitafloxacin revealed the least indifference effects. The results show that when combined with sitafloxacin, those three agents sulbactam, rifampin as well as tigecycline exert good *in vitro* activities against XDR-*A. baumannii* strains. The combination of colistin/sitafloxacin showed none of that enhanced activity.

*Synergistic effects of the combination regimens against XDR-A. baumannii strains*

To further investigate the synergistic effects of the combination regimens, the changes in MICs for sitafloxacin were calculated when combined with each of the other four agents at either 0.25× or 0.5×MIC. In accordance with the changing trend in FICI values, as shown in **Table 3**, the average MICs of sitafloxacin were decreased when used in combination with others.

The changes in average MICs for rifampin, colistin, sulbactam, and tigecycline when combined with sitafloxacin at 0.25× or 0.5×MIC were calculated in **Table 4**.

These results suggest that those combination regimens could exert beneficial effects at the sub-MIC levels against XDR-*A. baumannii* strains.

## Discussion

Antibiotic-resistant gram-negative bacilli (GNB) are increasingly common causes of health care-associated infections [13] and *A. baumannii* is one of those pathogens. *A. baumannii* is a gram-negative, non-fermenting, aerobic coccobacillus. It could be widely detected in nature as well as in hospital environment [14]. *A. baumannii* causes ventilator-associated-pneumonia, sepsis, meningitis, skin and soft tissue infection, as well as urinary tract infection, especially in immunocompromised residents in intensive care unit (ICU) [15], which are associated with higher mortality rates, longer hospitalizations, and increased health care expenditures [16, 17]. Due to limited therapeutic options, effective treatment for extremely drug-resistant (XDR) GNB infections is challenging

**Table 3.** The decrease of average MIC value of sitafloxacin in combination regimens against XDR-Ab isolates

| Sitafloxacin combined with | Sulbactam | | Rifampin | | Colistin | | Tigecycline | |
|---|---|---|---|---|---|---|---|---|
| | 0.25×MIC | 0.5×MIC | 0.25×MIC | 0.5×MIC | 0.25×MIC | 0.5×MIC | 0.25×MIC | 0.5×MIC |
| MIC for sitafloxacin (μg/mL) (Original MIC = 1.86 μg/mL) | 1 | 0.76 | 1.37 | 0.78 | 1.05 | 1.04 | 1.82 | 1.15 |

**Table 4.** The decrease of average MIC values of sulbactam, rifampin, colistin andtigecycline when combined with sitafloxacin against XDR-Ab isolates

| Combined with Sitafloxacin (μg/mL) | 0×MIC | 0.25×MIC | 0.5×MIC |
|---|---|---|---|
| MIC for Sulbactam | 29.54 | 22.25 | 12.79 |
| MIC for Rifampin | 5 | 3.82 | 3.31 |
| MIC for Colistin | 5.44 | 4.16 | 2.05 |
| MIC for Tigecycline | 2.71 | 2.20 | 1.73 |

[18]. For decades, scientists and clinicians tried desperately in new drug-discovering and old antibiotics reviving to face the growing problem of drug resistance [8, 19, 20]. However, as a matter of experience, when used as monotherapy, resistance eventually occurs [21]. In case resistance to one agent happens, combination therapy is suggested especially in dealing with *A. baumannii* infection, which has evolved to be capable of acquiring fast resistance to multiple antimicrobial agents. Drug combination provides many advantages [22]. In the first place, different sorts of drugs may gain enhancement over antibiotic activities when combined. Secondly, chances are much less for bacteria to develop resistance simultaneously to drugs with different antimicrobial mechanisms. Moreover, combination therapy could reduce the dosages for each agent, meanwhile reducing the drug toxicity. Last but not the least, combination therapy shows a much wider antimicrobial spectrum, and for long-term diseases like *A. baumannii* infections superinfection can be avoided.

Although an in vitro experiment is not necessarily correlated with clinical efficacy [23], which may be the result of the metabolism of the agents and the discordant redistribution of different agents in target tissues, our studies demonstrate that compounds could be screened in vitro to find new combinations that could be synergistic in vivo.

According to our results, for the single drugs, sitafloxacin has good in vitro activity against XDR-*A. baumannii*, which was in accordance with the results of others [9,10]. The bacteriostatic activity of rifampin, colistin, and tigecycline against XDR-*A. baumannii* strains were acceptable. Nevertheless, there was a decline of susceptibility for XDR-*A. baumannii* to the former three agents comparing to our results of 2013. While sulbactam alone was not so effective as others as before [24]. However, sulbactam revealed the best synergistic effects, referring to combination with sitafloxacin. The reason why colistin/sitafloxacin failed to show such remarkable synergistic effects as others may be as follows, colistin is a cationic lipopeptide, preserving a molecular weight around 2801 [25]. When it interacts with the LPS of the outer membrane of Gram-negative bacteria and competitively displaces divalent cations [26], this giant molecule may block the entrance of other agents.

In conclusion, sitafloxacin has acceptable antimicrobial activity against XDR-*A. baumannii* strains. Besides, sitafloxacin, in combinations with rifampin, colistin, sulbactam, and tigecycline, could exert synergistic and/or partially synergistic and/or addictive effects, which might provide a better alternative when treating XDR-*A. baumannii* infections. More impressively, these combination regimens could exert addictive or partially-synergistic effects at the sub-MIC levels against XDR-*A. baumannii* strains.

Pharmacodynamics is another significant factor associating with the antimicrobial activity. Pharmacodynamic exposure for antimicrobials is expressed relative to the MIC by the amount of time that free (ie. microbiologically active) drug concentrations remain greater than the MIC (*f* T > MIC) or by the ratios of AUC/MIC or Cmax/MIC [27]. Killing activity for β-lactam agents is considered to be time dependent; efficacy does not increase with concentrations

>2 to 4 times the MIC, but with the duration of time that concentrations remain above the MIC. In contrast, fluoroquinolones and aminoglycosides depend on overall drug exposure as defined by Cmax/MIC or AUC/MIC, respectively, to maximize bactericidal activity. Conventional dosing strategies have been manipulated to optimize pharmacodynamic parameters and thus preserve and enhance the utility of these antibiotics [28].

Studies on more detailed mechanisms, phamacodynamics as well as clinical trials need to be carried out before the clinical application of these combination recipes.

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## Disclosure of conflict of interest

None.

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