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ORIGINAL ARTICLE

Comparative *in vitro* activity of sitafloxacin against bacteremic isolates of carbapenem resistant *Acinetobacter baumannii* complex

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Carbapenem-resistant *Acinetobacter baumannii*;
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Sitafloxacin

Background: The emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) complex has posed a great challenge to clinicians worldwide. Sitafloxacin has been shown to have *in vitro* activity against pathogens resistant to other fluoroquinolones. However, data comparing the anti-CRAB activity of sitafloxacin with that of other antimicrobial agents are limited.

Methods: Genospecies were identified by 16S—23S ribosomal RNA intergenic spacer sequencing. Minimum inhibitory concentrations (MICs) were determined by an agar dilution method. Isolates with sitafloxacin MICs $\leq$2 mg/L were provisionally considered as susceptible to sitafloxacin. The MIC breakpoint for tigecycline susceptibility was 2 mg/L.

Results: A total of 167 CRAB complex blood isolates (146 *A. baumannii*, 7 *Acinetobacter pittii*, and 14 *Acinetobacter nosocomialis*) were collected from January 2009 to December 2011. Around 90% of the *A. baumannii* isolates were resistant to amikacin, cefepime, ceftazidime, piperacillin/tazobactam, ampicillin/sulbactam, ciprofloxacin, and levofloxacin. By contrast, the rate of resistance to colistin, sitafloxacin, and tigecycline was relatively low (0%, 41.1%, and 65.1%, respectively). The MIC_{50} and MIC_{90} of ciprofloxacin, levofloxacin, and sitafloxacin were 128 mg/L and >128 mg/L; 16 mg/L and 64 mg/L; 2 mg/L and 8 mg/L, respectively. Compared with ciprofloxacin and levofloxacin, sitafloxacin had a significantly lower MIC

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($p < 0.001$), and the rate of resistance to sitafloxacin was significantly lower than that to ciprofloxacin (97.9% vs. 41.1%, $p < 0.001$), levofloxacin (97.3% vs. 41.1%, $p < 0.001$), and tigecycline ($p < 0.001$).
Conclusion: Sitafloxacin has acceptable *in vitro* activity against CRAB, even against isolates resistant to other fluoroquinolones. Sitafloxacin may be considered an alternative drug of choice in treating CRAB related infections.

Introduction

The emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) complex has posed a great challenge to clinicians worldwide, complicating the management of nosocomial infections,[1] in particular *A. baumannii* infections.[2] The therapeutic option for CRAB is generally limited to colistin and tigecycline.[2,3] Colistin and tigecycline have been shown to have good *in vitro* activity against *A. baumannii* pneumonia isolates, even CRAB pneumonia isolates.[4,5] However, poor pulmonary penetration[6] and renal toxicity are major concerns of colistin use.[7,8] Meanwhile, the resistance of *A. baumannii* to tigecycline is emerging,[9,10] and the low serum level of tigecycline limits it use in bacteremic patients.[11]

Fluoroquinolones are commonly used antimicrobial agents. They have broad-spectrum activity against both Gram-negative and -positive pathogens.[12] Nowadays, resistance to fluoroquinolones in most nosocomial isolates of *A. baumannii* may be attributed to mutations of the *gyrA* and *parC* genes.[13,14] Fluoroquinolones have thus become a less than ideal treatment for CRAB-related infection. Sitafloxacin has been shown to have good *in vitro* activity against pathogens resistant to other fluoroquinolones.[15,16] The rate of CRAB susceptibility to sitafloxacin was deemed acceptable by one report.[17] Genospecies 2 (*A. baumannii*) has been associated with greater resistance to antimicrobial agents as well as higher mortality rates in bacteremic patients.[18–20] Because Thamlikitkul et al[17] did not identify the genospecies of *A. baumannii* complex in their study, they may have overestimated the susceptibility of *A. baumannii* to sitafloxacin.

Because data comparing antimicrobial susceptibilities between sitafloxacin and other antimicrobial agents against genospecies identified as CRAB are lacking, our study aimed to evaluate and compare the susceptibility of bacteremic patient-derived CRAB complex isolates to sitafloxacin versus other antimicrobial agents especially other fluoroquinolones.

Methods

Hospital setting and bacterial isolates

This study was conducted at the National Taiwan University Hospital (NTUH). NTUH is a 2200-bed teaching hospital located in Taipei, Taiwan. It provides both primary and

Table 1 Antimicrobial susceptibility results of 167 bacteremic carbapenem-resistant *Acinetobacter baumannii* complex isolates

Antimicrobial agent	*A. baumannii* ($n = 146$)				*Acinetobacter pittii* ($n = 7$)		
	MIC (mg/L)			Rate of susceptibility (%)	MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}		Range	MIC_{50}	MIC_{90}
Imipenem	0.25–128	48	128	11.6	0.25–128	64	128
Meropenem	0.25–>128	32	128	3.4	2–>128	32	>128
Ampicillin/sulbactam	0.5–128	16	64	14.4	0.5–64	2	64
Piperacillin/tazobactam	0.06–>128	>128	>128	1.4	16–>128	64	>128
Cefepime	4–>128	128	>128	2.1	64–>128	128	>128
Ceftazidime	8–>128	>128	>128	2.1	32–>128	>128	>128
Amikacin	2–>128	>128	>128	12.3	4–>128	64	>128
Ciprofloxacin	0.25–>128	128	>128	2.1	0.25–128	0.5	128
Levofloxacin	0.125–128	16	64	2.7	0.25–64	0.5	64
Sitafloxacin	0.015–16	2	8	58.9	0.06–4	0.06	4
Colistin	0.5–2	1	2	100	0.5–1	1	1
Tigecycline	0.25–16	4	16	34.9	0.5–4	1	4
Rifampin	1–>128	8	16	33.6	4–16	8	16

MIC = minimum inhibitory concentration; n.a. = not applicable.
[a] Susceptibility rate compared between different genospecies.

tertiary medical care. From January 2009 to December 2011, bacteremic isolates of CRAB complex were prospectively collected at the NTUH. Isolates were cultured from blood in the microbiological laboratory using the Bactec 9240 system (Becton Dickson, Sparks, MD, USA) during the study period.

Bacterial and genospecies identification

Biochemical methods were used to identify isolates of the *A. baumannii* complex.[21] The Vitek-2 bacterial identification system was used to confirm the identity of the isolates and sequencing of the 16S–23S ribosomal RNA (rRNA) gene intergenic spacer (ITS) region was used to identify genospecies as previously described.[22] The identified genospecies were classified as *A. baumannii*, *Acinetobacter pittii*, or *Acinetobacter nosocomialis*. Other genospecies or unidentified genospecies were excluded.

Antimicrobial susceptibility testing

Carbapenem resistance was defined as isolates resistant to imipenem or meropenem as determined using the disc diffusion method described by the Clinical and Laboratory Standards Institute (CLSI).[23]

An agar dilution method was used to determine amikacin, cefepime, ceftazidime, imipenem, meropenem, ampicillin/sulbactam, piperacillin/tazobactam, colistin, ciprofloxacin, levofloxacin, tigecycline, rifampin, and sitafloxacin minimum inhibitory concentrations (MICs) for CRAB complex isolates defined by CLSI criteria.[23] The testing results of tigecycline susceptibility was interpreted by the US Food and Drug Administration breakpoint for *Enterobacteriaceae* susceptibility to tigecycline.[24] The rifampin breakpoints of for *A. baumannii* were as published by the French Society for Microbiology.[25] Isolates with rifampin MICs of $\leq$4 mg/L or tigecycline MICs of $\leq$2 mg/L were considered susceptible. Isolates with sitafloxacin MICs $\leq$2 mg/L were provisionally considered to be susceptible.[26] The testing results of the other antibiotics were interpreted according to the CLSI criteria.

Statistical analysis

The associations between MICs and rates of resistance of different *A. baumannii* complex genospecies or between MICs and resistance to different fluoroquinolones were compared using Kruskal–Wallis one-way analysis of variance (ANOVA), or Fisher's exact test. *Posthoc* analysis using the Mann–Whitney *U* test or Fisher exact test utilized a modified Bonferroni-adjusted α for pair-wise comparisons if the result of the initial Kruskal–Wallis one-way ANOVA or Fisher's exact test was statistically significant. Data were analyzed using Stata software, version 12 (StataCorp, College Station, TX, USA).

Results

The MIC distribution and antimicrobial susceptibility of 167 CRAB complex isolates

We collected 167 CRAB complex isolates, including 146 (87.4%) *A. baumannii*, seven (4.2%) *A. pittii*, and 14 (8.4%) *A. nosocomialis* isolates. All of the isolates were derived from patients with bacteremia. The antimicrobial susceptibility testing results are listed in Table 1. Overall, colistin possessed the best *in vitro* activity with 100%, 61.7%, 40.1%, and 34.1% of the 167 CRAB complex isolates being susceptible to colistin, sitafloxacin, tigecycline, and rifampin, respectively.

Acinetobacter nosocomialis (n = 14)					p[a]		
Rate of susceptibility (%)	MIC (mg/L)			Rate of susceptibility (%)	Overall	*A. baumannii* vs. *A. pittii*	*A. baumannii* vs. *A. nosocomialis*
	Range	MIC_{50}	MIC_{90}				
28.6	0.25–128	8	64	21.4	0.2	0.21	0.39
28.6	16–128	16	128	0	0.04	0.03	0.99
71.4	0.5–64	5	64	50	<0.001	0.002	0.004
14.3	0.015–>128	>128	>128	28.6	<0.001	0.13	0.001
0	2–128	8	128	57.1	<0.001	0.99	<0.001
0	4–>128	8	>128	64.3	<0.001	0.99	<0.001
14.3	2–>128	4	>128	64.3	<0.001	0.99	<0.001
57.1	0.125–>128	0.375	>128	64.3	<0.001	<0.001	<0.001
57.1	0.125–16	0.25	16	78.6	<0.001	<0.001	<0.001
85.7	0.015–4	0.06	4	78.6	0.19	0.24	0.25
100	0.5–2	1	2	100	n.a.	n.a.	n.a.
85.7	0.25–16	1	8	71.4	0.001	0.011	0.01
28.6	1–32	8	16	42.9	0.75	0.99	0.56

The percentage of *A. baumannii* isolates susceptible to colistin, sitafloxacin, tigecycline, and rifampin was higher (100%, 58.9%, 34.9%, and 33.6% respectively; Table 1) than that to amikacin, ceftazidime, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, ciprofloxacin, and levofloxacin (all <15%). The percentage of isolates susceptible to sitafloxacin was significantly higher than that susceptible to tigecycline (58.9% vs. 34.9%, $p < 0.001$).

Compared with *A. pittii* isolates, *A. baumannii* isolates showed higher ampicillin/sulbactam, ciprofloxacin, levofloxacin, and tigecycline resistance (all $p < 0.05$). Compared with *A. nosocomialis* isolates, *A. baumannii* isolates showed higher resistance to all tested antimicrobial agents (all $p < 0.05$) except sitafloxacin and rifampin. The percentage of *A. baumannii*, *A. pittii*, and *A. nosocomialis* isolates susceptible to sitafloxacin was similar (58.9%, 85.7%, and 78.6%, respectively, $p = 0.19$; Table 1).

The MIC distribution and antimicrobial susceptibility of 100 tigecycline-resistant CRAB complex isolates

The drug susceptibility of CRAB and tigecycline-resistant CRAB isolates was of interest. Among 167 CRAB complex isolates, 100 were tigecycline resistant, including 95 *A. baumannii* isolates, one *A. pittii* isolate, and four *A. nosocomialis* isolates. All 100 tigecycline-resistant isolates were susceptible to colistin, which remained the most active agent (100% susceptibility rate). The percentage of the 95 *A. baumannii* isolates susceptible to sitafloxacin and rifampin was 51.6% and 28.4%, respectively, whereas the percentage susceptible to amikacin, ceftazidime, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, ciprofloxacin, and levofloxacin was only around 10% (Table 2).

Comparison of the *in vitro* activity of the three fluoroquinolones

Table 3 shows the MIC distributions of three different fluoroquinolones. Of the *A. baumannii* isolates, the MIC_{50}, MIC_{90}, and geometric means of the MICs of ciprofloxacin, levofloxacin, and sitafloxacin were 128 mg/L, >128 mg/L, 96.3 mg/L; 16 mg/L, 64 mg/L, 14.6 mg/L; 2 mg/L, 8 mg/L, and 2.7 mg/L, respectively. Fig. 1 depicts the distribution of ciprofloxacin, levofloxacin, and sitafloxacin MICs in terms of cumulative probability. The MICs of the three fluoroquinolones differed. The sitafloxacin MIC was significantly lower than that of the two comparator fluoroquinolones ($p < 0.001$). The rate of susceptibility to sitafloxacin was significantly higher than that to ciprofloxacin and levofloxacin [2.1% vs. 58.9% ($p < 0.001$) and 2.7% vs. 58.9% ($p < 0.001$), respectively] and the MIC of sitafloxacin was significantly lower than that of the comparator fluoroquinolones in *A. nosocomialis* isolates (ciprofloxacin vs. sitafloxacin, $p = 0.006$; levofloxacin vs. sitafloxacin, $p = 0.02$).

Discussion

Our results indicate that, after colistin, sitafloxacin is the second most effective antibiotic against CRAB complex blood isolates and tigecycline-resistant CRAB blood isolates. Compared with ciprofloxacin and levofloxacin, sitafloxacin had the lowest MIC value and resistance rate.

This is the first study focused on the *in vitro* activity of sitafloxacin against genospecies-identified CRAB complex isolates from bacteremic patients. A similar report from Thailand showed that the MIC range, MIC_{50}, and MIC_{90} values of sitafloxacin for 258 CRAB complex isolates were 0.016–4 mg/L, 1 mg/L, and 2 mg/L, respectively.[17] By using ≤2 mg/L as the MIC breakpoint for susceptibility, 91.4% of these isolates were susceptible to sitafloxacin in that study. The MIC range, MIC_{50}, MIC_{90} in our genospecies-identified CRAB isolates (0.015–16 mg/L, 2 mg/L, 8 mg/L, respectively) were higher and the susceptibility rate was lower (91.4% vs. 58.9%). Because genospecies was not taken into consideration by that study, the rate of susceptibility to sitafloxacin might have been overestimated. In addition, the isolates in our study were all from patients with bacteremia, whereas those reported by Thamlikitkul et al[17] were from patients with lower respiratory tract infection, urinary tract infection, or bacteremia. Therefore the clinical significance of our findings may be different.

Colistin and tigecycline are the main therapeutic options for CRAB complex.[2] Our study confirmed that colistin has excellent *in vitro* activity. As for tigecycline, the susceptibility of *A. baumannii* isolates (rate, 34.9%) was much lower than that of *A. pittii* (85.7%) and *A. nosocomialis* (71.4%). This highlights the importance of MIC confirmation and genospecies identification when treating CRAB infection. Resistance of *A. baumannii* to tigecycline is emerging,[9,10] and simultaneous resistance to colistin, rifampicin, and tigecycline has been reported.[27] Sitafloxacin could be an option in such cases.

Compared with other fluoroquinolones, sitafloxacin has better *in vitro* activity against Gram-positive cocci,[28,29] *Enterobacteriaceae*,[29,30] other nonfermentative species,[29] and even some quinolone-resistant bacteria.[16,31,32] Our study is the first to report the superior *in vitro* activity of sitafloxacin against CRAB. This advantage of sitafloxacin may be due to its ability to inhibit both DNA gyrase and topoisomerase IV,[33,34] or to overcome plasmid-mediated quinolone resistance.[35] Compared to other fluoroquinolones, sitafloxacin also has a greater affinity for DNA gyrase[36] and inhibits at a lower concentration.[37]

In Taiwan, Chiu et al[38] have investigated mechanisms of quinolone-resistance in *A. baumannii* clinical isolates. They reported that the expression of AdeB, an efflux pump protein, is associated with resistance to ciprofloxacin in *A. baumannii* and that multiple mutations in *gyrA* and *parC* genes also play a role.[38] Because several mechanisms of quinolone-resistance exist in *A. baumannii*, it is unknown whether sitafloxacin could have good clinical efficacy against CRAB. Sitafloxacin is presently available only in oral form, which limits its use in patients with poor gastrointestinal absorption or who are critically ill. However, it still has great potential for patients with less severe diseases, switching from parenteral to oral antibiotics, or

Table 2 Antimicrobial susceptibility results of 100 tigecycline- and carbapenem-resistant *Acinetobacter baumannii* complex isolates

Antimicrobial agent	*A. baumannii* ($n = 95$)				*Acinetobacter pittii* ($n = 1$)		*Acinetobacter nosocomialis* ($n = 4$)		
	MIC (mg/L)				MIC (mg/L)		MIC (mg/L)		
	Range	MIC_{50}	MIC_{90}	Rate of susceptibility (%)	MIC	Rate of susceptibility (%)	Range	MIC_{50}	Rate of susceptibility (%)
Imipenem	0.25–128	64	128	11.6	128	0	0.25–64	48	25
Meropenem	2–128	32	128	2.1	>128	0	16–128	128	0
Ampicillin/sulbactam	0.5–64	16	64	12.6	64	0	0.5–64	48	25
Piperacillin/tazobactam	128–>128	>128	>128	0	>128	0	>128–>128	>128	0
Cefepime	8–>128	128	>128	1.1	>128	0	64–128	96	0
Ceftazidime	64–>128	128	>128	0	>128	0	16–>128	16	0
Amikacin	2–>128	>128	>128	11.6	>128	0	4–64	64	25
Ciprofloxacin	16–>128	128	>128	0	128	0	2–>128	64	0
Levofloxacin	0.25–128	16	64	1.1	64	0	2–16	16	25
Sitafloxacin	1–16	2	8	51.6	4	0	0.5–4	4	25
Colistin	0.5–2	1	1	100	0.5	100	1–2	1	100
Tigecycline	4–16	4	16	0	4	0	8–16	8	0
Rifampin	1–>128	8	16	28.4	16	0	1–16	10	50

MIC = minimum inhibitory concentration.

combination therapy in treating CRAB-related infection. It is worth noting that the resistance rate of CRAB to sitafloxacin (41.1%) is relatively high compared to colistin (0%) in our study. Therefore, empirically using sitafloxacin for CRAB infection without susceptibility test results is not suggested.

Our study has several limitations. First, the number of *A. pittii* and *A. nosocomialis* isolates is small, which might not reflect the true difference between genospecies. Second, there is no standardized MIC breakpoint for susceptibility of *Acinetobacter* species to tigecycline, sitafloxacin, or rifampin. With different MIC cut-off values, the rate of CRAB susceptibility to the above agents might change. Third, our study included only one medical center and had a relatively short duration. Because the epidemiology of CRAB varies widely among institutes and countries, the

Table 3 Distribution of ciprofloxacin, levofloxacin, and sitafloxacin minimum inhibitory concentrations (MICs) among 167 carbapenem-resistant *Acinetobacter baumannii* complex isolates

	MIC (mg/L)				Rate of susceptibility (%)	p[a]		
	Range	MIC_{50}	MIC_{90}	Geometric mean		Overall	Ciprofloxacin vs. sitafloxacin	Levofloxacin vs. sitafloxacin
A. baumannii complex ($n = 167$)								
Ciprofloxacin	0.125–>128	128	>128	59.64	9.6			
Levofloxacin	0.125–128	8	64	10.26	11.4	<0.001	<0.001	<0.001
Sitafloxacin	0.015–16	2	8	1.92	61.7			
A. baumannii ($n = 146$)								
Ciprofloxacin	0.25–>128	128	>128	96.3	2.1			
Levofloxacin	0.125–128	16	64	14.6	2.7	<0.001	<0.001	<0.001
Sitafloxacin	0.015–16	2	8	2.7	58.9			
Acinetobacter pittii ($n = 7$)								
Ciprofloxacin	0.25–128	0.5	128	3	57.1			
Levofloxacin	0.25–64	0.5	64	1.8	57.1	0.60	0.56	0.56
Sitafloxacin	0.06–4	0.06	4	0.3	85.7			
Acinetobacter nosocomialis ($n = 14$)								
Ciprofloxacin	0.125–>128	0.375	>128	1.8	64.3			
Levofloxacin	0.125–16	0.25	16	0.64	78.6	0.74	0.68	0.99
Sitafloxacin	0.015–4	0.06	4	0.1	78.6			

[a] Rate of susceptibility rate compared between different genospecies.

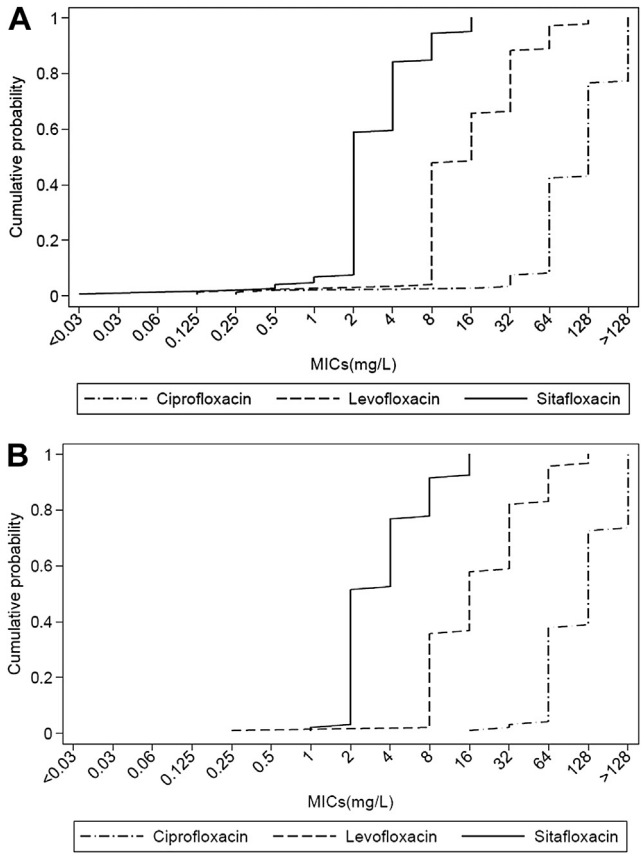

Figure 1. Cumulative plot of the distribution of ciprofloxacin, levofloxacin, and sitafloxacin MICs for (A) 146 carbapenem-resistant *Acinetobacter baumannii* isolates, and (B) 95 tigecycline-resistant *A. baumannii* isolates.

results of this single-center study might not be generalizable, nor could it reflect the detailed resistant pattern in Taiwan. Ideally, CRAB complex isolates from multiple centers with a longer study period would be more representative. Last, the mechanisms responsible for differences in MIC distribution or resistance rate among different fluoroquinolones are not examined in the present study, and warrant further investigation.

In conclusion, sitafloxacin has an acceptable *in vitro* activity against CRAB complex isolates, even isolates resistant to tigecycline or other fluoroquinolones and genospecies identified as *A. baumannii*. Because the nephrotoxicity of colistin is a concern, sitafloxacin can be considered an alternative drug of choice for CRAB-related infections. However, further studies are needed to determine the clinical efficacy of sitafloxacin in treating CRAB-related infections.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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