Pharmaceutical Sciences Asia

Pharm Sci Asia 2020; 47 (1), 37-42
DOI:10.29090/psa.2020.01.019.0012

Research Article

Comparative *in vitro* activity of sitafloxacin against multidrug-resistant and carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Thailand

Taniya Paiboonvong[1],
Vipavee Rodjun[2],
Jantana Houngsaitong[1],
Mullika Chomnawang[3],
Preecha Montakantikul[2],
Suvatna Chulavatnatol[2*]

[1] Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
[2] Faculty of Pharmacy, Siam University, Bangkok, Thailand
[3] Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

***Corresponding author:**
suvatna.chu@mahidol.ac.th

KEYWORDS:
Sitafloxacin; Multidrug-resistant *A. baumannii*; Carbapenem-resistant *A. baumannii*

ABSTRACT

The rapid emergence of multidrug-resistant *Acinetobacter baumannii* (MDRAB) is recognized as a significant health problem worldwide, including Thailand. Due to a limitation of treatment option, a new antimicrobial agent has been challenged. Sitafloxacin, a new fluoroquinolone antimicrobial agent, has shown a good *in vitro* activity against MDRAB and carbapenem-resistant *A. baumannii* (CRAB). The aim of this study was to evaluate *in vitro* activity of sitafloxacin and compare that with other antimicrobial agents against *A. baumannii* clinical isolates, focusing on multidrug-resistant *A. baumannii* and carbapenem-resistant *A. baumannii* (MDR-CRAB). All 350 *A. baumannii* clinical isolates were collected from thirteen tertiary care hospitals in all regions of Thailand. The minimum inhibitory concentrations (MICs) were determined by broth microdilution method. To determine rate of susceptibilities, a susceptible isolate with sitafloxacin was considered as MIC values of ≤ 2 µg/mL and ≤ 1 µg/mL. For other antimicrobial agents, MIC breakpoints were considered according to the Clinical and Laboratory Standards Institute (CLSI) 2018. Our study found that 278 clinical isolates were identified as MDR-CRAB. The MIC range, MIC50 and MIC90 of sitafloxacin against MDR-CRAB isolates were ≤ 0.0625-8 µg/mL, 1 µg/mL and 2 µg/mL, respectively. Additionally, almost of the colistin-resistant isolates were susceptible to sitafloxacin (92.86%). Sitafloxacin had a good activity against multidrug-resistant isolates in Thailand. Thus, sitafloxacin can be considered as an alternative choice for treatment of MDRAB and CRAB infections. Further studies are needed to evaluate treatment outcomes.

1. INTRODUCTION

Acinetobacter baumannii is an important cause of nosocomial infections, and becomes a major problem in healthcare settings in Asian countries, including Thailand. The resistance to numerous different drug classes known as multidrug-resistant *A. baumannii* (MDRAB) has rapidly emerged, and associated with a high mortality rate, especially in critically ill patients[1]. Carbapenems have been mainly used for treatment of MDRAB, whereas the resistant rate has been rising over the world. According to the National Antimicrobial Resistance Surveillance

Thailand (NARST) data, carbapenem-resistant *A. baumannii* (CRAB) has rapidly emerged for two decades up to approximately 80% in 2016[2]. Moreover, several studies reported high rates of MDRAB and CRAB from tertiary care hospitals in Thailand[3,4]. Choosing the effective agent for treatment of infections caused by those resistant pathogens is challenging. The therapeutic option for MDRAB and CRAB is mainly limited to colistin as the first-line treatment. However, the limitations of colistin are the issue of renal toxicity and poor tissue penetration. Therefore, new antimicrobial agents are needed for treating resistant *A. baumannii* infections

Sitafloxacin (DU-6859a) is a new broad-spectrum fluoroquinolone antimicrobial agent, showed an active activity against many drug resistant pathogens. Comparing with other fluoroquinolones, sitafloxacin has the lowest inhibitory concentration (IC50) against DNA gyrase and topoisomerase IV[5, 6]. It has been approved for oral formulation and used in Thailand for treatment of urinary tract infections (UTIs) and lower respiratory tract infections (LRIs) since 2011. Moreover, several studies reported a potent activity of sitafloxacin against drug-resistant *A. baumannii*[7-12]. Nevertheless, the activity of sitafloxacin against both MDRAB and CRAB (MDR-CRAB) isolates collected from all regions of Thailand has not been reported. Therefore, this study was conducted to provide an informative data of sitafloxacin against *A. baumannii* clinical isolates at tertiary hospitals in Thailand, focusing on MDR-CRAB.

2. MATERIALS AND METHODS

2.1. Hospitals and bacterial isolates

All 350 *A. baumannii* clinical isolates were collected from in-patients of thirteen tertiary care hospitals, Thailand during the period of 2016. Non-duplicated isolates were collected. All isolates were cultured in suitable media and storage before sending to the laboratory of microbiology at Faculty of Pharmacy, Mahidol University. Bacterial glycerol stocks were prepared and stored at -80°C until being tested.

2.2. Antimicrobial susceptibility tests

Broth microdilution method was used to determine minimum inhibitory concentration (MIC) for sitafloxacin and comparators; ciprofloxacin, ceftazidime, imipenem, meropenem, doripenem, sulbactam, and colistin according to recommendation from CLSI 2016[13].

The standard powder of sitafloxacin was provided by Daiichi Sankyo, Thailand. Standard powders of ciprofloxacin, ceftazidime, colistin, and sulbactam were purchased from Tokyo Chemical Industry CO.,LTD. Standard powders of imipenem and meropenem were purchased from Siam Bheasach CO.,LTD, and doripenem was purchased from Shinogal & CO.,LTD. The MIC values were defined as the lowest concentration of an antimicrobial agent to inhibit a visible growth. The MIC50 and the MIC90 were determined as a concentration that inhibited 50% and 90% of isolates, respectively. Since no MIC breakpoint of sitafloxacin has been published by the CLSI, MIC breakpoint ≤ 2 μg/mL and ≤ 1 μg/mL were considered as susceptible to sitafloxacin in previous studies[7,8,12]. Other antimicrobial susceptibilities were interpreted according to breakpoints criteria from CLSI 2018[14].

MDRAB and CRAB were identified by the results of susceptibility by broth microdilution method. MDRAB in this study was defined as an isolate resistant to at least three antimicrobial classes according to the interim standard definitions for multidrug-resistant organism, which was created by the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention[15]. *Escherichia coli* ATCC 25922 was used for quality control strain in this study. The bacterial isolates were grown in Luria-Bertani broth (LB, BD) and cation-adjusted Mueller-Hinton Broth (CAMHB, BD) media. Serial two-fold dilutions of antimicrobials tested for MIC were prepared in CAMHB as described in CLSI guidelines. Bacterial isolates were adjusted for a final inoculum density approximately 5×10^5 CFU/mL using 0.5 McFarland turbidity standard. All 96-well plates were incubated at 37°C in ambient air for 20 hours. The method was performed as the recommendations from CLSI 2016[13].

3. RESULTS AND DISCUSSION

Our study found that 278 clinical isolates were MDR-CRAB. Most of the isolates were from sputum (78.06%) followed by pus (8.99%), urine (5.40%), blood (4.32%), tissue (2.16%), and nasophyrynx (0.72%).

Overall, sitafloxacin showed high rate of susceptibility than those of other comparative agents. Sitafloxacin showed the best activity among comparators against MDR-CRAB isolates when using MIC breakpoint ≤ 2 μg/mL; the susceptibility rate was 90.65%. Secondary to colistin, the susceptibility rate was 64.03% when MIC breakpoint ≤ 1 μg/mL. The MIC range, MIC50 and MIC90 of sitafloxacin were ≤ 0.0625-8 μg/mL, 1 μg/mL and 2 μg/mL, respectively. Additionally, 41 out of 278 isolates (14.75%) were resistant to colistin (MIC ≥ 4 μg/mL), and almost of all isolates (92.86%) were susceptible to sitafloxacin with MICs of ≤ 2 μg/mL (MIC values ranged from < 0.0625-2 μg/mL). We found the high MIC50 (2 μg/mL) and MIC90 (4 μg/mL) of sitafloxacin from isolates of the hospitals in the central region. Antimicrobial susceptibility results and MIC distribution of sitafloxacin categorized by regions in Thailand are presented in Table 1, and Table 2, respectively.

Table 1. The antimicrobial susceptibility and MIC values of 278 MDR-CRAB isolates

Antimicrobial agents	Susceptibility breakpoints (μg/mL)	MIC (μg/mL)			Susceptibility %
		ranges	MIC50	MIC90	
Colistin	≤ 2	0.0625 - 512	0.5	4	85.25
Sitafloxacin	≤ 1, ≤ 2	≤ 0.0625 - 8	1	2	64.03, 90.65
Ciprofloxacin	≤ 1	0.5 - > 512	64	> 512	1.08
Imipenem	≤ 2	8 - 256	32	128	0
Meropenem	≤ 2	8 - 256	32	64	0
Doripenem	≤ 2	2 - 256	16	64	0.72
Sulbactam	≤ 4	2 - 2048	16	128	8.27
Ceftazidime	≤ 8	16 - > 512	512	> 512	0

Table 2. The MIC distribution of sitafloxacin against MDR-CRAB isolates (n=278)

Hospital Regions	MIC (μg/mL)								
	≤ 0.0625	0.25	0.5	1	2	4	8	MIC_{50}	MIC_{90}
Central* (n=88)	2	3	11	19	39	14	0	2	4
North (n=66)	2	0	24	26	12	2	0	1	2
South (n=40)	2	0	5	21	10	1	1	1	2
East (n=23)	0	2	10	7	3	0	1	0.5	2
Northeast (n=61)	0	2	17	25	10	7	0	1	4
Total (278)	6	7	67	98	74	24	2	1	2

*central region included Bangkok

Our results suggest that sitafloxacin has a good activity against MDR-CRAB. The results were consistent with previous studies that showed a high susceptibility rate of sitafloxacin against *A. baumannii*. The susceptibility rates of sitafloxacin against *A. baumannii* isolated in 2016 and 2012 from Thai patients with UTIs and LRIs were 57.9% and 87.9% and were 66.9% and 94.1% when MIC breakpoint ≤ 1 μg/mL, and ≤ 2 μg/mL, respectively[7,8]. This indicated that sitafloxacin remains active against *A. baumannii* isolated from Thai patients over the past 5 years. However, the MIC90 of *A. baumannii* isolated in 2016 was 4 μg/mL, higher than that in 2012 (the MIC90 was 2 μg/mL)[7,8]. Similar to the study of Thamlikitkul and Tiengrim, the susceptibility of CRAB to sitafloxacin was 91.4% when MIC breakpoint ≤ 2 μg/mL[9]. Another report from Thailand showed a similar result that

the susceptibility rate of sitafloxacin against CRAB using disk diffusion method was 66.32% in which the zone diameters for susceptibility referred to the MIC breakpoint ≤ 1 μg/mL[10]. According to Dong et al, the susceptibility rate of sitafloxacin against 24 extensively drug-resistant (XDR) *A. baumannii* isolates was 91.67% when MIC breakpoint ≤ 2 μg/mL[11]. By contrast from Huang et al, the susceptibility rate of sitafloxacin against bacteremic isolates of CRAB was 58.9% that lower than the rate of our study, and the MIC50 and MIC90 were high as 2 μg/mL and 8 μg/mL, respectively[12]. This may result from those isolates were collected from only single center of teaching hospital, but our study collected the isolates from both teaching and provincial hospitals in all regions of Thailand. Comparing to other agents, sitafloxacin was more active than ceftazidime, ciprofloxacin, and sulbactam in which the resistant rate was 100%, 98.92%, and 91.73%, respectively. Our results were consistent with previous reports that overall susceptibility rate of those agents was lower than 25%[7,8,10,12].

Multiple acquired resistance determinants have been found in MDRAB and CRAB, including transfer of plasmids, transposons, and integrons, encoding a resistant gene to several antimicrobial classes. A mutation in quinolone resistance-determining region (QRDR) of target genes encoding DNA gyrase (gyrA) and topoisomerase IV (parC) has been reported as the main mechanism of quinolone resistance in *A. baumannii*. In addition, the plasmid-mediated quinolone resistance (PMQR) genes have also been found and conferred high level resistance to ciprofloxacin[16,17]. Our study found that almost of all isolates were resistant to ciprofloxacin; MIC values ranged from 4 to > 512 μg/mL, however, all of ciprofloxacin-resistant isolates were susceptible to sitafloxacin. This advantage of sitafloxacin may be a potent activity to inhibit the target enzymes of Gram-negative pathogens; it exhibited the lower IC50 than ciprofloxacin approximately 4-fold against the mutations in type II topoisomerases of *Pseudomonas aeruginosa*[6].

Colistin-resistant *A. baumannii* (CoR-AB) has been reported from various countries over the world, including Thailand[18-23]. Similar to our study, CoR-AB isolates were found in 14.75%. Interestingly, almost of all CoR-AB were susceptible to sitafloxacin (92.86%) probably involved the difference of resistant mechanism. The main mechanism of CoR-AB has been proposed as alteration of lipopolysaccharide (LPS); lipid A modification or loss of LPS[19-21]. Overexpression of efflux pump system has been reported for the mechanism of resistance, and recently found in clinical isolates from Thailand[22,23]. However, sitafloxacin resistance to *A. baumannii* has not been reported as an overexpression by efflux pump system. Although the mobile colistin resistance gene *mcr-1* has been found in gram negative pathogens, especially *E. coli*, the *mcr-1* has not yet been reported on *A. baumannii* clinical isolates[20]. However, *mcr-1*-carrying plasmid could be introduced into *A. baumannii*, and co-localized with multiple plasmid replicon types, which may be involved in resistance to quinolones[24,25]. The limitation of this study is that there is no standard MIC breakpoint criteria for susceptibility established for sitafloxacin. Although synergistic effects of sitafloxacin in combination with colistin, sulbactam, rifampicin, and tigycycline were reported against XDR-*A. baumannii*[11], combinations against MDRAB and CRAB should be investigated to confirm a result of different strains in Thailand.

4. CONCLUSION

Sitafloxacin showed a good *in vitro* activity against MDR-CRAB, including colistin-resistant isolates. Thus, sitafloxacin can be considered as an alternative choice for treatment of infections caused by resistant strains. Further studies are needed to evaluate treatment outcomes.

5. ACKNOWLEDGEMENTS

We would like to thank Daiichi-Sankyo (Thailand) Ltd. for providing sitafloxacin powder. We also thank microbiology staffs from all hospitals for specimrns collection; Taksin Hospital, Klang Hospital, Nakhon Pathom Hospital, Samut Sakhon Hospital, Maharaj Nakorn Chiang Mai Hospital, Buddhachinaraj Hospital, Sawanpracharak Hospital, Surat Thani Hospital, Maharaj Nakhon Si Thammarat Hospital. Chonburi Hospital, Sunpasitthiprasong Hospital, Surin Hospital, and Buriram Hospital.

Conflict of interests

The authors declare no conflicts of interests in this research.

Funding

No funding to declare

Ethical approval

The study protocol was approved by the Institutional

Review Board of the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2017/040.2607).

Article info:

Received February 6, 2019
Received in revised form March 25, 2019
Accepted April 19, 2019

REFERENCES

1. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, et al. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. Am J Respir Crit Care Med. 2011;184(12):1409-17.
2. National Antimicrobial Resistance Surveillance Center (NARST). Antimicrobial Resistance Surveillance 2000-2016. Available from: http://narst.dmsc.moph.go.th/data/AMR%202000-2016.pdf.[Last cited on 2017 May 2].
3. Werarak P, Waiwarawut J, Tharavichitkul P, Pothirat C, Rungruanghiranya S, Geater SL, et al. *Acinetobacter baumannii* nosocomial pneumonia in tertiary care hospitals in Thailand. J Med Assoc Thai. 2012;95(Suppl 2):S23-S33.
4. Inchai J, Liwsrisakun C, Theerakittikul T, Chaiwarith R, Khositsakulchai W, Pothirat C. Risk factors of multidrug-resistant, extensively drug-resistant and pandrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia in a Medical Intensive Care Unit of university hospital in Thailand. J Infect Chemother. 2015; 21(8):570-4.
5. Nakane T, Iyobe S, Sato K, Mitsuhashi S. *In vitro* antibacterial activity of DU-6859a, a new fluoroquinolone. Antimicrob Agents Chemother.1995;39(12):2822-6.
6. Akasaka T, Tanaka M, Yamaguchi A, Sato K. Type II topoisomerase mutations in fluoroquinolone-resistant clinical strains of *Pseudomonas aeruginosa* isolated in 1998 and 1999: role of target enzyme in mechanism of fluoroquinolone resistance. Antimicrob Agents Chemother. 2001; 45(8):2263-8.
7. Tiengrim S, Phiboonbanakit D, Thunyaharn S, Tantisiriwat W, Santiwatanakul S, Susaengrat W, et al. Comparative *in vitro* activity of sitafloxacin against bacteria isolated from Thai patients with urinary tract infections and lower respiratory tract infections. J Med Assoc Thai. 2012;95 (Suppl 2):S6e17.
8. Tiengrim S, Mootsikapun P, Wonglakorn L, Changpradub D, Thunyaharn S, Tantisiriwat W, et al. Comparative *in vitro* activity of sitafloxacin against bacteria isolated from Thai patients with urinary tract infections and lower respiratory tract infections in 2016. J Med Assoc Thai. 2017;100(10):1061-72.
9. Thamlikitkul V, Tiengrim S. *In vitro* activity of sitafloxacin against carbapenem-resistant *Acinetobacter baumannii*. Int J Antimicrob Agents. 2013;42(3):284-5.
10. Tantisiriwat W, Linasmita P. *In vitro* activity of sitafloxacin and other antibiotics against bacterial isolates from HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University and Samitivej Sukhumvit Hospital. J Med Assoc Thai. 2017;100(4):469-78.
11. Dong X, Chen F, Zhang Y, Liu H, Liu Y, Ma L. *In vitro* activities of sitafloxacin tested alone and in combination with rifampin, colistin, sulbactam, and tigecycline against extensively drug-resistant *Acinetobacter baumannii*. Int J Clin Exp Med. 2015;8(5):8135-40.
12. Huang YS, Wang JT, Sheng WH, Chuang YC, Chang SC. Comparative *in vitro* activity of sitafloxacin against bacteremic isolates of carbapenem resistant *Acinetobacter baumannii* complex. J Microbiol Immunol Infect. 2015; 48(5):545-51.
13. Clinical and Laboratory Standards institute. Performance standards for antimicrobial susceptibility testing: Twenty-sixth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
14. Clinical and Laboratory Standards institute. Performance standards for antimicrobial Susceptibility testing: Twenty-Seventh informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drugresistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.
16. Hujer KM, Hujer AM, Endimiani A, Thomson JM, Adams MD, Goglin M, et al. Rapid determination of quinolone resistance in *Acinetobacter* spp. J Clin Microbiol. 2009;47: 1436-42.
17. Yang H, Hu L, Liu Y, Ye Y, Li J. Detection of the plasmid-mediated quinolone resistance determinants in clinical isolates of *Acinetobacter baumannii* in China. J Chemother. 2016;28(5): 443-5.
18. Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant *Acinetobacter baumannii*: beyond carbapenem resistance. Clin Infect Dis. 2015;60(9):1295-303.
19. Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother. 2012;67(7):1607-15.
20. Jeannot K, Bolard A, Plesiat P. Resistance to polymyxins in Gram-negative organisms. Int J Antimicrob Agents. 2017;49(5):526-35.
21. Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014; 5:643.
22. Ni W, Li Y, Guan J, Zhao J, Cui J, Wang R, et al. Effects of efflux pump inhibitors on colistin resistance in multidrug-resistant Gram-negative bacteria. Antimicrob Agents Chemother. 2016;60(5):3215-8.
23. Lertsrisatit Y, Santimaleeworagun W, Thunyaharn S, Traipattanakul J. *In vitro* activity of colistin mono- and combination therapy against colistin-resistant *Acinetobacter baumannii*, mechanism of resistance, and clinical outcomes of patients infected with colistin-resistant *A. baumannii* at a Thai university hospital. Infect Drug Resist. 2017; 10:437-43.
24. Liu YY, Chandler CE, Leung LM, McElheny CL, Mettus RT, Shanks RMQ, et al. Structural modification of lipopolysaccharide conferred by *mcr-1* in gram-negative ESKAPE pathogens. Antimicrob Agents Chemother. 2017;61(6): e00580-17.
25. Al-Tawfiq JA, Laxminarayan R, Mendelson M. How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? Int J Infect Dis. 2017; 54:77-84.