Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2021; 38(4): 471-473
doi: 10.52142/omujecm.38.4.13

Investigation of the effect of *N*-Acetylcysteine on colistin mic values in *Acinetobacter Baumannii* strains isolated from clinical samples

Fahriye EKŞİ , Mehmet ERİNMEZ*

Department of Medical Microbiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Received: 07.03.2021 • **Accepted/Published Online:** 31.03.2021 • **Final Version:** 30.08.2021

Abstract

Acinetobacter baumannii is an opportunistic pathogen which colonize inpatients and cause severe infections, septic shock and death. With emergence of multi-drug resistant gramnegative species and being effective in *A. baumannii* infections, colistin becomes a treatment option again. N-acetylcysteine (NAC), is a mucolitic agent which used commonly in lower respiratory tract infections especially patients who have cronic respiratory disorders like Chronic obstructive pulmonary disease, cystic fibrosis and bronchiectasis. In this study we aim to investigate the effect of NAC, which commonly added in lower respiratory tract infections tratment regime, on MIC values colistin used in *A. baumanni* tratment. Fifty A.*baumannii* isolates were included in the study. The isolates were identified by automated identification system. With broth microdilution method, we investigated and compared the MIC (minimum inhibitory concentration) values of colistin and NAC+Colistin combination. Colistin MIC50 value is 0.25 µg/mL and MİK90 value is 1 µg/mL, NAC+Colistin combination MIC50 value is 0.25 µg/mL and MİK90 value is 1 µg/mL. The screening for the effectiveness of clinical drugs may provide clinical strategy to improve treatment outcomes of *A. baumannii* and reduce hospitalization days.

Keywords: *A.baumannii,* colistin, lower respiratory tract infection, microdilution method, N-acetylcysteine

1. Introduction

The Acinetobacter genus is classified in the *Moraxellaceae* family and consists of bacteria in the morphology of immobile, oxidase-negative, Gram-negative coccobacillus (1). *A. baumannii* is an opportunistic microorganism that can colonize in hospitalized patients and cause serious infections, bacteremia, septic shock, and death (2). These pathogens most frequently cause urinary tract infections and lower respiratory tract infections, especially in patients hospitalized in intensive care units (3). Although the frequency of hospital-acquired lower respiratory tract infections caused by *A. baumannii* varies from country to country, region to region (27-50%), the mortality rate in these infections is between 30-70% (4). Colistin is a lipopeptide antibiotic that belongs to the class of polymyxins, and its use was reduced or completely discontinued due to its nephrotoxic and neurotoxic properties (5). Later, the emergence of multidrug-resistant gram-negative pathogens and its effectiveness in the treatment of *A. baumannii* infections made colistin a treatment option again (5). N-acetylcysteine (NAC) is a mucolytic drug commonly used in lower respiratory tract infections especially in patients with chronic respiratory disorders such as COPD, cystic fibrosis, and bronchiectasis (6). In this study, it was aimed to investigate the effect of NAC, which is frequently added to the treatment of lower respiratory tract infections, on the minimum inhibitory concentration (MIC) values of colistin used in the treatment of *A. baumannii*.

2. Materials and methods

The colistin susceptibility status of 50 *A. baumannii* strains that isolated from clinical samples, including 19 (38%) tracheal aspirate, 15 (30%) blood culture, 7 (14%) wound, 6 (12%) sputum, 2 (4%) CSF, 1 (2%) catheter samples were investigated with Vitek 2 (BioMérieux, Lyon, France) automated system. Of the 50 *A. baumannii* strains investigated, 47 were found to be sensitive to colistin, and 3 to colistin-resistant. Colistin MIC values of these strains with and without N-acetylcysteine using N-Acetyl-L-Cysteine (A7250 Sigma Aldrich, USA) were investigated by the broth microdilution method. Using colistin sulphate (C4461, Sigma Aldrich, USA), between 128 and 0.125 µg / ml concentrations, prepared in double-fold dilutions in microplates according to ISO-standard broth microdilution method (20776-1) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (7) studied according to their recommendations.

As the medium cation-adjusted Mueller Hinton Broth (MHB, Merck KGaA, Darmstadt, Germany) was used. The stock solution of colistin antibiotic (512 µg/ml) was dissolved in distilled water, portioned, and stored at -20°C. For management of serious lower respiratory disorders, generally

* Correspondence: mehmeterinmez92@hotmail.com

used therapeutic concentration of NAC ranges between 4 and 10 mM per day (8), therefore a NAC concentration of 10 mM was used in this study. N-Acetyl-L-Cysteine was dissolved in distilled water and the stock solution (10mM, 1.6 mg/ml) was prepared daily when the test was performed (9). Inoculum suspension was added to each well at a final bacterial concentration of $5x10^5$ cfu/ml. In addition, bacterial growth control (MHB+microorganism) for bacteria and sterility control (MHB) for each microdilution plate was studied. Microdilution plates were incubated at 35°C for 24 hours in an oven under aerobic conditions. The results were evaluated according to the breakpoint values determined in EUCAST standards (≤2 µg / ml sensitive and> 2 µg / mL resistant) and the MIC values of N-acetylcysteine-Colistin combination and colistin were compared.

3. Results

The MIC (µg/mL) values of *50 A. baumannii* strains isolated from clinical samples, determined by the broth microdilution method, are shown in Table 1.

Table 1. MIC (µg/mL) values of 50 *A. baumannii* strains isolated from clinical samples determined by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	50	0.125	4	0.55	0.91
NAC+Colistin MIC	50	0.125	8	0.73	1.39

*Sd: Standard deviation

The mean colistin MIC value of the strains was 0.55 ± 0.91 µg/mL, and the mean NAC+Colistin combination MIC value was 0.73±1.39 µg/mL. Colistin MIC50 value of 50 *A. baumannii* strain isolated was determined as 0.25 µg/mL and MIC90 value as 1 µg/mL, NAC+Colistin combination MIC50 value was determined as 0.25 µg/mL and MIC90 value was determined as 1 µg/mL.The MIC (µg/mL) values of 47 *A. baumannii* strains, which are known to be sensitive to colistin, determined by the broth microdilution method are shown in Table 2.

Table 2. MIC (µg / mL) values of 47 *A. baumannii* strains known to be sensitive to colistin by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	47	0.125	1	0.33	0.27
NAC+Colistin MIC	47	0.125	4	0.43	0.60

*Sd: Standard deviation

The mean colistin MIC value was 0.33 ± 0.27 µg/mLand average NAC+Colistin combination MIC value was 0.43± 0.60 µg/mL. Colistin MIC50 value of 47 *A. baumannii* strain known to be sensitive to colistin was determined as 0.25 µg/mL and MIC90 value as 1 µg/mL, MIC value for NAC+Colistin combination MIC value as 0.25 µg/mL and MIC90 value as 1 µg/mL.

Table 3. MIC (µg/mL) values of 3 *A.baumannii* strains known to be colistin-resistant by broth microdilution method

	n	Min	Max	Average	*Sd(±)
Colistin MIC	3	4	4	4	0
NAC+Colistin MIC	3	4	8	5.5	2.3

*Sd: Standard deviation

The MIC (µg/mL) values of 3 *A. baumannii* strains, known to be colistin-resistant, determined by broth microdilution method are shown in Table 3. The mean colistin MIC value of colistin-resistant strains was 4 µg / mL and the mean NAC + Colistin combination MIC value was 5.3 ± 2.3 µg/mL. Colistin MIC50 value of 3 *A. baumannii* strains known to be colistin-resistant was 4 µg/mL and MIC90 value was 4 µg/mL, NAC+Colistin combination MIC50 value was 4 µg/mL and MIC90 value was determined as 8 µg/mL. Colistin and NAC+Colistin combination MIC values of all strains are shown in Table 4.

Table 4. Colistin and NAC + Colistin combination MIC values of 50 *A. baumannii* strains isolated from clinical samples

Colistin MIC Values	Strain No.	NAC+Colistin MIC Values	Strain No.
0.125 µg/mL	5,9,10,17, 20,21,22, 23,24,27, 32,36,37, 38,39,40, 41,46	0.125 µg/mL	5,7,8,9,10, 12,14,17,19,2 0,21,22,23, 25,2,29,31, 36,37,38,39,4 0,45
0.25 µg/mL	1,3,4,8, 13,14,15, 16,19,25, 28,33,34,45	0.25 µg/mL	1,3,6, 24,34,45
0.50 µg/mL	6,712, 26,29,31, 33,43,48,50	0.50 µg/mL	2,4,13, 15,16,27, 28,32,35, 42,47,50
1 µg/mL	2,42,44,47, 49	1 µg/mL	41,43,44, 46,48,49
2 µg/mL	18	2 µg/mL	18
4 µg/mL	11, 30	4 µg/mL	30, 33
8 µg/mL	-	8 µg/mL	11

4. Discussion

In some cases, there is a discrepancy between MIC values detected and treatment results; while in-vitro test results indicate susceptibility to antibiotics, *Acinetobacter baumannii*, becomes intrinsically resistant to various antimicrobial agents and immune system products due to biofilm production and eradication of colonization cannot be achieved (10). The use of NAC is considered as an alternative approach in the control of diseases caused by biofilm-producing bacteria in humans. Previous studies have shown that NAC inhibits biofilm formation or disrupts the biofilm structure in various bacteria (11, 12). Pollini et al. (13) showed that the colistin / NAC combination showed synergy against the *A. baumannii* biofilm structure and NAC could reverse the colistin-resistant phenotype of this pathogen.

The chequerboard method, which is the reference method, was not preferred in our study because Rodríguez-Beltrán et al. (14) showed that the antimicrobial effect of NAC was due to the low pH caused by NAC and Landini et al. (15) stated that there was no antimicrobial effect at the concentration ranges used in humans. Instead of different concentrations of NAC, the highest concentration that can be used for humans

was tested in our study. Goswami and Jawali (9) did not observe growth inhibition in their experiments with various bacteria on agar containing the maximum dose of 10mM N-acetyl cysteine in their study based on the 4 and 10mM/day dose range, which is widely used in the treatment of severe respiratory diseases.

When the effect of *N*-acetyl cysteine on the MIC values of various antibiotics was investigated, it was observed that the MIC values of fluoroquinolones and aminoglycosides increased in *E. coli, Klebsiella aerogenes* and *P. aeruginosa strains*, chloramphenicol and tetracycline did not cause a change in MIC values and caused a decrease in the MIC values of penicillin and ampicillin (9). The effect of NAC on the MIC values of antibiotics used in the treatment of different bacteria can be very variable. Therefore, the possible effects on colistin, which is often used as a last resort in treatment, should be well understood.

Zuin et al. (8) reported that the use of high-dose NAC in patients with chronic obstructive pulmonary disease led to an improvement in the clinical condition of the patients and a decrease in inflammation markers. Since it has been shown that NAC can reverse the *A. baumannii* colistin resistance phenotype, the risk of colistin monotherapy resulting in the selection of colistin-resistant strains can be avoided by using the colistin / NAC combination (13). In addition, in our study, by testing the in vitro effect of colistin / NAC combination on *A. baumannii*, it can give an idea about the potential in vivo effects of the inhaled colistin / NAC combination in the next step.

Antibiotics and mucolytic agents are used together in the treatment of lower respiratory tract infections in patients with chronic respiratory tract problems and nosocomial pneumonia in patients with a prolonged hospital stay. Landini et al. (15) showed in their study that the MIC values of colistin at two different NAC concentrations (10mM and 50mM) did not change, our results are consistent because it covers the concentration (10mM) we used in our study. In different studies, N-Acetylcysteine, a mucolytic agent, has been shown to have synergistic or antagonistic effects for different antibiotics. In our study, it has been shown that the MIC values of colistin, which is an important treatment option in gram-negative bacterial infections with multiple drug resistance, increase the mean MIC values in combination with N-acetylcysteine. It can be useful in developing clinical strategies.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

At “5. Ulusal Klinik Mikrobiyoloji Kongresi” congress was presented as a poster presentation in 2019.

References

1. Garrity GM, Bell JA LT. Taxonomic Outline of The Procaryotes. In: Begery’s Manual of Systematic Bacteriology. 2nd ed. New York: Springer-Verlag; 2004. p. 103.
2. Winn J, Stephen A, William J, Elmer K, Gary P SP. Koneman’s Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Washington: Lippincott Williams and Wilkins; 2006. 353-355 p.
3. Bergogne-Berezin E. Importance of Acinetobacter sp. In: Acinetobacter Biology and Pathogenesis. Paris: Springer; 2008. p. 1-85.
4. Seifert H, Strate A, Pulverer G. Nosocomial bacteremia due to Acinetobacter baumannii. Clinical features, epidemiology, and predictors of mortality. Medicine (Baltimore). 1995;74(6):340-349.
5. 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):1607-1615.
6. Blasi F, Page C, Rossolini GM, Pallecchi L, Matera MG, Rogliani P, et al. The effect of N-acetylcysteine on biofilms: Implications for the treatment of respiratory tract infections. Respir Med. 2016; 117:190-197.
7. EUCAST reading guide for broth microdilution. 2021. Available from:https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/MIC_testing/Reading_guide BMD_v_3.0_2021.pdf
8. Zuin R, Palamidese A, Negrin R, Catozzo L, Scarda A, Balbinot M. High-dose N-acetylcysteine in patients with exacerbations of chronic obstructive pulmonary disease. Clin Drug Investig. 2005;25(6):401-408.
9. Goswami M, Jawali N. N-acetylcysteine-mediated modulation of bacterial antibiotic susceptibility. Antimicrob Agents Chemother. 2010;54(8):3529-3530.
10. Kim HA, Ryu SY, Seo I, Suh SI, Suh MH, Baek WK. Biofilm Formation and Colistin Susceptibility of Acinetobacter baumannii Isolated from Korean Nosocomial Samples. Microb Drug Resist. 2015;21(4):452-457.
11. Marchese A, Bozzolasco M, Gualco L, Debbia EA, Schito GC, Schito AM. Effect of fosfomycin alone and in combination with N-acetylcysteine on E. coli biofilms. Int J Antimicrob Agents. 2003;22 Suppl 2:95-100.
12. Pérez-Giraldo C, Rodríguez-Benito A, Morán FJ, Hurtado C, Blanco MT, Gómez-García AC. Influence of N-acetylcysteine on the formation of biofilm by Staphylococcus epidermidis. J Antimicrob Chemother. 1997;39(5):643-646.
13. Pollini S, Boncompagni S, Di Maggio T, Di Pilato V, Spanu T, Fiori B, et al. In vitro synergism of colistin in combination with N-acetylcysteine against Acinetobacter baumannii grown in planktonic phase and in biofilms. J Antimicrob Chemother. 2018 Sep 1;73(9):2388-2395.
14. Rodríguez-Beltrán J, Cabot G, Valencia EY, Costas C, Bou G, Oliver A, et al. N-acetylcysteine selectively antagonizes the activity of imipenem in Pseudomonas aeruginosa by an OprD-mediated mechanism. Antimicrob Agents Chemother. 2015;59(6):3246-3251.
15. Landini G, Di Maggio T, Sergio F, Docquier JD, Rossolini GM, Pallecchi L. Effect of High N-Acetylcysteine Concentrations on Antibiotic Activity against a Large Collection of Respiratory Pathogens. Antimicrob Agents Chemother. 2016;60(12): 7513-7517.