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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE ANTIMICROBIAL EFFECT OF *N*-ACETYLCYSTEINE AND ITS INTERACTION WITH ANTIBIOTICS AGAINST *ACINETOBACTER BAUMANNII* ISOLATES

N-ASETİL SİSTEİNİN ACINETOBACTER BAUMANNII İZOLATLARINA KARŞI ANTİMİKROBİYAL ETKİSİ VE ANTİBİYOTİKLERLE ETKİLEŞİMİ

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ABSTRACT

Objective: *The objective of this study was to delve into the effect of N-acetylcysteine (NAC) molecule on the minimum inhibitory concentration (MIC) of meropenem, ciprofloxacin, and gentamicin in clinical isolates of Acinetobacter baumannii, aiming to find potential alternatives for the treatment of bacterial infections that are resistant to conventional antibiotics.*
Material and Method: *The study included 25 A. baumannii isolates that were confirmed to be resistant to meropenem, ciprofloxacin, and gentamicin. The susceptibility of antibiotics was re-evaluated in the presence of NAC using the microdilution method. FIC indexes were calculated based on the checkerboard test results to determine the effect of the combination, defined as synergistic or additive.*
Result and Discussion: *The study demonstrated that NAC molecule, when used alongside meropenem, ciprofloxacin, and gentamicin, effectively reduced the MIC values of these antibiotics against Acinetobacteria. Furthermore, NAC molecule exhibited a synergistic effect when combined with meropenem. Additive effects were observed in all isolates for the GEN-NAC and CIP-NAC combinations. In conclusion, the findings suggest that NAC molecule could serve as a new alternative for combined drug therapy, offering a promising approach to treatment.*
Keywords: *Acinetobacter baumannii, ciprofloxacin, gentamicin, meropenem, N-acetylcysteine (NAC)*

ÖZ

Amaç: *Dirençli bakteriyel enfeksiyonların tedavisi için yeni antimikrobiyal bileşiklerin sentezlenmesi çalışmalarının yanısıra, inhibitör moleküllerin antibiyotiklerle kombine kullanılmasına yönelik araştırmalar da yapılmaktadır. Çalışmamızda, NAC molekülünün Acinetobacter baumannii klinik izolatlarında, meropenemin, siprofloksasinin ve gentamisinin minimum inhibitör konsantrasyonu (MİK) üzerine etkisinin saptanması araştırılarak, tedavi için yeni potansiyel alternatifler bulmak amaçlanmıştır.*
Gereç ve Yöntem: *Çalışmamızda kullanılmak üzere meropeneme, siprofloksasine ve gentamisine dirençli olduğu doğrulanan 50 A. baumannii izolatı çalışmaya alınmıştır. Antibiyotiklerin duyarlılığı NAC varlığında yeniden araştırılmıştır. Antimikrobiyal duyarlılık testleri mikrodilüsyon yöntemi ile yapılmıştır. Dama tahtası testi sonuçlarına göre FİK indeksleri hesaplanmış ve kombinasyonun etkisi sinerjik ya da aditif olarak tanımlanmıştır.*
Sonuç ve Tartışma: *Bu çalışmada, NAC molekülünün meropenem, siprofloksasin ve gentamisin ile*

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birlikte kullanıldığında bu antibiyotiklerin Acinetobakterlere karşı MİK değerlerini etkili bir şekilde azalttığı gösterilmiştir. Ayrıca NAC molekülü meropenem ile birlikte kullanıldığında sinerjik etki göstermiştir. GEN-NAC ve CIP-NAC kombinasyonları için tüm izolatlarda aditif etki gözlenmiştir. Sonuç olarak elde edilen bulgular, NAC molekülünün kombine ilaç tedavisine yeni bir alternatif olarak kullanılabileceği ve tedaviye umut verici bir yaklaşım sunabileceği göstermiştir. NAC molekülünün farklı mikroorganizmalar ve antimikrobiyal maddelerle birlikte kullanılmasının da mümkün olabileceği ve ileride yapılacak çalışmalar açısından çalışmadaki bulguların yararlı olduğu düşünülmüştür.

Anahtar Kelimeler: *Acinetobacter baumannii, gentamisin, meropenem, N-asetilsistein (NAC), siprofloksasin*

INTRODUCTION

Infections acquired during the process of receiving healthcare that were not present or in the incubation period during admission to the hospital or developed 48-72 hours after hospitalization or within 10 days after leaving the hospital are defined as nosocomial infections [1-2]. *Acinetobacter* spp., non-fermentative, Gram-negative coccobacilli, have emerged as causes of hospital infections in recent years. While the overall mortality rate of patients due to *A. baumannii* infections is 5% in general hospital wards, it can reach 54% in intensive care units [3]. In a multicenter study conducted to investigate nosocomial Gram-negative bacterial infections in intensive care units in Turkey, Acinetobacter spp. were reported as the third most common bacteria among Gram-negative bacilli, and antibiotic resistance rates were also found to be quite high [4]. Acinetobacter spp. have also been identified as causative agents of ventilator-associated pneumonia in intensive care units and have been reported to be the leading cause of hospital-acquired pneumonia [5,6]. In Trakya University Education Application and Research Center, Acinetobacter species are frequently isolated and cause serious problems, especially in intensive care units and surgical services.

Among the Acinetobacter genus, *Acinetobacter baumannii* is the most important species in terms of the infections it causes. *A. baumannii* can be found in nature and in human skin flora or may be isolated from clinical specimens [7-8]. Resistance to antibiotics used in infections caused by these strains has become a significant global health problem, as increasing multi-antibiotic resistance reduces the possibility of treatment [9,10]. Many Acinetobacter species have shown resistance to antibiotics such as quinolones, carbapenems, and cephalosporins. Colistin is currently used as the only treatment option for such incurable cases; however, colistin-resistant strains have also been reported in recent years. Therefore, new treatment strategies need to be investigated for this and other multi-drug resistant bacterial infections [9,11]. The use of combined antibiotics for treatment or the addition of non-antibiotic compounds to the combination are the most preferred strategies in recent years [6]. These combinations have been reported to decrease the MIC values of the antibiotics or inhibit antimicrobial resistance [12].

One of these molecules, N-Acetylcysteine (NAC), has been extensively studied [13,14]. N-acetylcysteine (NAC) is an N-acetylated derivative of the amino acid L-cysteine and is a precursor molecule of glutathione [13-17]. NAC has provided an alternative pharmacological approach in antimicrobial resistance studies [14]. It is stated that the sulfur groups in the structure of NAC are effective in reducing the adhesion of bacteria to the surface and separating bacteria adhered to a surface [15-17].

The antimicrobial activity of NAC has been proposed to be explained by several mechanisms. These include competitive inhibition of cysteine utilization, reaction of NAC sulfhydryl group with bacterial proteins, and disruption of intracellular redox balance through indirect effects on cell metabolism and intracellular signal transduction pathways [18].

To detect the inhibitory, synergistic, or antagonistic effect of compounds in a combination, synergy tests are used, and the checkerboard assay is one of the most frequently used tests [19]. In the checkerboard test, the combination efficacy is tested by comparing the concentrations of drugs on a 96-well plate. The MIC values of the drugs are compared with the MIC values obtained from the combination, and the fractional inhibitory concentration (FIC) is found. Then, the FIC values of the drugs in the combination are summed, and the FIC index is calculated. The FIC value of each antimicrobial agent is obtained by dividing the lowest antimicrobial agent concentration in the well

without growth by the MIC of that agent against the same strain alone [19,20]. In this study, the aim was to determine the effect of the NAC molecule on the MIC value of antibiotics against meropenem, ciprofloxacin, and gentamicin-resistant clinical isolates of *A. baumannii*. For this purpose, FIC indices of meropenem-NAC, ciprofloxacin-NAC, and gentamicin-NAC combinations were calculated using the checkerboard test, and the effect of the combination was defined as synergistic or additive.

MATERIAL AND METHOD

In our study, as quality control strains recommended by CLSI M100-S28 [21] and European Committee on Antimicrobial Susceptibility Testing- EUCAST [22]; *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853, *A. baumannii* NCTC 1342 and 25 *A. baumannii* isolates isolated from various clinical samples sent to Trakya University Health Research and Application Center were used.

Method

Microdilution Method

Antimicrobial susceptibility tests were conducted in accordance with CLSI-M100-S28 recommendations. In microplates, in cation-adjusted Mueller Hinton broth (CA-MHB), NAC concentrations were between 4096-64 µg/ml. Concentrations of meropenem, ciprofloxacin and gentamicin were prepared in the range of 256-0.25 µg/ml. Bacterial suspensions were prepared at a density of 5 x 10^5 cfu/ml and were added to the wells. Microplates were incubated at 37ºC for 18-24 hours and the lowest drug concentration that inhibited growth was determined as MIC.

Checkerboard Method

In the checkerboard method, serial dilutions of the antibiotics (256-0.25 µg/ml) were dispensed into the first ten wells of the microplate from left to right, and serial dilutions of NAC (4096-64 µg/ml) were dispensed into the first eight-well from top to bottom of another microplate. The contents of the two plates were combined in another microplate. The concentration range of the antibiotics used was determined according to the MIC values. Bacterial inoculum prepared at a density of 5 x 10^5 cfu/ml was added to the wells. Plates were incubated at 37ºC for 18-24 hours. Evaluation of the combination test was performed according to the fractional inhibitory concentration (FIC) index (Figure 1) [5-7].

$FIC\ A = MICA_{combination}/MICA$

$FIC\ B = MICB_{combination}/MICB$

$FIC = FIC\ A + FIC\ B$

$FIC \leq 0{,}5$, synergistic effect

$0{,}5 < FIC \leq 4$, additive effect

$FIC > 4$, antagonistic effect

Figure 1. Calculation of Fractional Inhibitor Concentration (FIC) Values

RESULT AND DISCUSSION

MIC values determined as sensitive if the MIC value was ≤2 µg/ml, and resistant if ≥8µg/ml for meropenem [22]. All isolates were found to be resistant to meropenem. The detected MIC values were over 16 µg/ml.

MIC values obtained as a result of the susceptibility test performed with the microdilution method were determined as sensitive if the MIC value was ≤1 µg/ml, and resistant if ≥4 µg/ml for ciprofloxacin [22]. All isolates were found to be resistant to ciprofloxacin. The detected MIC values were over 32 µg/mL.

MIC values obtained as a result of the susceptibility test performed with the microdilution method were determined as sensitive if the MIC value was ≤4 µg/ml, and resistant if ≥16 µg/ml for gentamicin [22]. All isolates were found to be resistant to gentamicin. The detected MIC values were over 16 µg/ml.

According to the EUCAST guidelines all isolates were determined to be resistant to meropenem and gentamicin and ciprofloxacin. The MIC values were given in Table 1.

Table 1. MIC values of the agents against *A. baumannii* isolates

	MER*(µg/ml)	**GEN**(µg/ml)**	**CIP***(µg/ml)**
2	16	64	64
3	16	256	128
7	16	256	128
8	128	256	128
11	64	256	256
13	8	256	256
14	16	256	256
15	16	64	128
17	32	64	64
19	16	64	64
20	32	256	256
21	256	64	64
24	16	256	128
25	64	256	256
26	32	64	64
28	32	32	32
29	128	64	64
30	32	64	64
31	256	256	256
32	16	64	64
33	16	32	256
35	16	16	128
36	64	64	64
41	64	64	64

*MER: Meropenem,**GEN: Gentamicin,***CIP: Ciprofloxacin

In the presence of NAC, a four-fold or more decrease was found in the MER MIC values to 19 (76%) of 25 *A. baumannii* isolates included in the study (Table 2). Despite the decrease in 4 of 19 isolates, the MIC value were still above the resistance limit value. In the presence of NAC, 8 (32%) of 25 resistant isolates was determined to be susceptible.

In the presence of 2038 mg/l NAC, a four-fold or more decrease was found in the CIP MIC values of 11 (44%) of 25 *A. baumannii* isolates included in the study (Table 3). Although there was a decrease in 8 of 11 isolates, the MIC value is still above the resistance limit value. In the presence of 2038 mg/L NAC 3 (12%) of 25 isolates resistant to GEN became susceptible to GEN.

In the presence of 2038 mg/l NAC, a four-fold or more decrease was found in the CIP MIC values of 4 (16%) of 25 *A. baumannii* isolates included in the study (Table 4). However, the MIC value was still above the resistance limit value in all isolates.

Table 2. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	MER* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+MER MIC (µg/ml)**	**MER-NAC Combinations (µg/ml)**	**FIC*** Index**	**Interaction type**
2	16	2038	8	8/509	1	Additive
3	16	2038	0.5	16-2038	1.06	Additive
7	16	2038	8	16-509	1.5	Additive
8	128	2038	8	8-509 16-127 32-64	0.31 0.187 0.28	Synergy Synergy Synergy
11	64	2038	16	2-1018 16-509	0.53 0.49	Additive Synergy
13	8	2038	4	4-509	1	Additive
14	16	1018	4	4-509	1.5	Additive
15	16	2038	8	4-1018	0.62	Additive
17	32	2038	4	8-509	0.5	Synergy
19	16	2038	1	1-509	0.31	Synergy
20	32	2038	16	16-64	0.56	Additive
21	256	2038	4	8-254 8-509	0.15 0.28	Synergy Synergy
24	16	1018	4	16-509	1.5	Additive
25	64	2038	4	4-1018	0.56	Additive
26	32	2038	0.5	16-509	0.75	Additive
28	32	2038	4	4-1018	0.62	Additive
29	128	2038	4	4-1018 8-509 16-509 16-127 16-64	0.53 0.31 0.37 0.187 0.031	Additive Synergy Synergy Synergy Synergy
30	32	2038	0.5	0.5-1018 1-1018 8-509	0.51 0.53 0.5	Additive Additive Synergy
31	256	4075	8	16-256	0.31	Synergy
32	16	2038	8	16-509	1.5	Additive
33	16	2038	0.5	8-509	0.75	Additive
35	16	2038	8	8-509	1	Additive
36	64	2038	16	16-1018	0.75	Additive
41	64	2038	1	32-64	0.53	Additive
43	16	2038	1	1-509	0.31	Synergy
A. baumannii NCTC 1342	16	4075	1	1-509	0.31	Synergy

*MER: Meropenem, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

Table 3. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	GEN* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+GEN MIC (µg/ml)**	**GEN-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
2	64	2038	4	4-1018	0.56	Additive
3	256	2038	32	256-1018	1.5	Additive
7	256	2038	16	16-1018	0.56	Additive

Table 3 (*continue*). Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	GEN* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+GEN MIC (µg/ml)**	**GEN-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
8	256	2038	64	64-1018	0.75	Additive
11	256	2038	256	256-1018	1.5	Additive
13	256	2038	64	64-1018	0.75	Additive
14	256	2038	256	256-1018	1.5	Additive
15	64	2038	4	4-1018	0.56	Additive
17	64	2038	64	256-1018	1.5	Additive
19	64	2038	64	256-1018	1.5	Additive
20	256	2038	64	64-1018	0.75	Additive
21	64	2038	2	2-1018	0.53	Additive
24	256	2038	128	128-1018	1	Additive
25	256	2038	256	256-1018	1.5	Additive
26	64	2038	16	32-1018	1	Additive
28	32	2038	32	32-1018	1.5	Additive
29	64	2038	64	32-1018	1	Additive
30	64	2038	64	32-1018	1	Additive
31	256	2038	64	64-1018	0.75	Additive
32	64	2038	32	32-1018	1	Additive
33	32	2038	32	32-1018	1.5	Additive
35	16	2038	8	256-1018	1.5	Additive
36	64	2038	16	256-1018	1.5	Additive
41	64	2038	64	32-1018	1	Additive
43	32	2038	16	16-1018	1	Additive
A. baumannii NCTC 1342	64	2038	64	4-1018	0.56	Additive

*GEN: Gentamicin, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

Table 4. Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	CIP* MIC (µg/ml)	**NAC** MIC (µg/ml)**	**NAC+CIP MIC (µg/ml)**	**CIP-NAC Combinations (µg/ml)**	**FIC** Index**	**Interaction type**
2	64	2038	32	32-1018	1	Additive
3	128	2038	64	64-1018	1	Additive
7	128	2038	32	32-1018	0.75	Additive
8	128	2038	32	32-1018	0.75	Additive
11	256	2038	128	128-1018	1	Additive
13	256	2038	256	128-1018	1	Additive
14	256	2038	256	256-1018	1.5	Additive
15	128	2038	64	64-1018	1	Additive
17	64	2038	64	32-1018	1	Additive
19	64	2038	64	32-1018	1	Additive
20	256	2038	128	128-1018	1	Additive
21	64	2038	64	32-1018	1	Additive
24	128	2038	128	128-1018	1.5	Additive
25	256	2038	256	256-1018	1.5	Additive
26	64	2038	32	32-1018	1	Additive
28	32	2038	32	32-1018	1.5	Additive

Table 4 (*continue*). Concentrations of the compounds alone and in combination against *A. baumannii* isolates and the interactions between the combined agents

	CIP* MIC (µg/ml)	NAC** MIC (µg/ml)	NAC+CIP MIC (µg/ml)	CIP-NAC Combinations (µg/ml)	FIC** Index	Interaction type
29	64	2038	64	32-1018	1	Additive
30	64	2038	64	32-1018	1	Additive
31	256	2038	128	128-1018	1	Additive
32	64	2038	4	4-1018	0.56	Additive
33	256	2038	256	256-1018	1.5	Additive
35	128	2038	64	64-1018	1	Additive
36	64	2038	64	64-1018	1.5	Additive
41	64	2038	32	32-1018	1	Additive
43	128	2038	16	16-1018	0.62	Additive
A. baumannii NCTC 1342	64	2038	32	32-1018	1	Additive

*CIP: Ciprofloxacin, **NAC: N-acetylcysteine, ***FIC: Fractional inhibitory concentration

While a synergistic effect was detected between NAC and MER in 9 isolates, an additive effect was observed in other isolates. When GEN-NAC and CIP-NAC combinations were examined, additive effects were observed in all isolates.

Studies conducted in recent years focus on synthesizing new antimicrobial compounds while addressing the problem of resistance. However, in addition to synthesizing new antimicrobial compounds, research on inhibiting resistance is also gaining importance. Combined use of inhibitory compounds and antibiotics can be considered as an alternative treatment method. Based on this idea, in our study, which aimed to determine the effect of NAC on the MIC value of antibiotics and to calculate the concentration of CIP + NAC, MER + NAC and GEN + NAC, which eliminates antibiotic resistance, it was shown that the combination of MER + NAC increased the sensitivity of MER.

De angelis et al. [23] studied the combination of NAC with various antibiotics against a total of 30 isolates, 15 of which are carbapenem-resistant *K. pneumoniae* and *15 A. baumannii* isolates. It was shown that the MIC values of the antibiotics alone decreased with the addition of NAC. The combination of meropenem+NAC was noted to show synergism in all strains tested. However, the combination of Rifampin+ NAC was shown to be synergistic in 3/15 (20%) strains. On the other hand, it was stated that no synergism was found in the combinations of Tigecycline+NAC and Colistin+NAC. In our study, synergy was observed between MER-NAC combinations in 9 of 25 isolates.

Pollini et al. [24], synergistic activity of Colistin+NAC combinations was investigated against 16 *A. baumannii isolates*, 9 of which were colistin-sensitive (MIC range 0.5-1 mg/l) and 7 colistin-resistant (MIC range 16-256 mg/l) isolates. In the study combinations of 8000 mg/l NAC and 2 mg/l colistin showed synergy in colistin-resistant strains, while no synergism was observed in colistin-susceptible strains. In addition, synergistic activity of 8 mg/l colistin and 8000 mg/l NAC combination has been demonstrated in all strains (colistin-resistant and colistin-sensitive).

Goswami et al. determinated [25]; The combination of NAC with ampicillin against various bacteria has been investigated. In most isolates, the combination of ampicillin + NAC has been shown to significantly decrease the MIC of ampicillin alone. In contrast, Moon et al. [26] found that the antibiotic susceptibility of *Prevotella intermedia* was not affected by NAC, and ampicillin activity was reduced in the presence of NAC.

Likewise, Rodriguez-Bertram et al. [27] investigated combinations of various antibiotics with NAC against *E. coli*, *P. aeruginosa*, and *A. baumannii*. While an additive effect was observed in the combination of NAC + antibiotics against *P. aeruginosa* isolates, less effect was noted with the combination of NAC + meropenem. Nevertheless, some isolates susceptible to imipenem have been shown to become resistant after combined use with NAC.

Landini et al. [28], investigated the activity of NAC alone and with various antibiotics against

Gram positive and Gram negative bacteria. They determined no synergistic effect with the combination of NAC and antibiotics against isolates. Besides, the effect of carbapenems on isolates decreased after being combined with NAC. This situation was observed more in imipenem than in meropenem.

Çetinkaya et al. [29] detected a four-fold or more reduction in CIP MIC values in the presence of 25 mg/l PAβN in 15.5% of 58 *A. baumannii* isolates. Kaynak Onurdağ et al. [30] also reported a four-fold or more reduction in CIP MIC values in the presence of 25 mg/l PAβN in 43.28% of CIP-resistant *A. baumannii* isolates.

Kuyucuklu et al. [31], reported that 41 ampicillin-resistant *staphylococcus* isolates became susceptible to ampicillin in the presence of appropriate NAC concentrations for 60.98% of the isolates. Likewise, they found a 2-32-fold decrease in vancomycin MIC values in the combination of vancomycin and NAC. According to these results, they observed that the NAC molecule, which has no antimicrobial effect and therefore has a very high MIC value on its own, is an effective chemical agent against staphylococcal isolates, together with ampicillin and vancomycin and reduces the MIC values of antibiotics.

In our study, in the presence of 2038 mg/l NAC, 4 (16%) of the 25 *A. baumannii* isolates included in the study had a four-fold or greater decrease in CIP MIC values. When using NAC as an antimucolytic in adults, the serum concentration dose is 400-600 mg per day. However, 140 mg/kg is used as a loading dose in paracetamol poisoning and the toxic dose is quite high. In our study, the NAC concentration in all antibiotic-NAC combinations was between 2038-64 mg/l, well below the toxic dose [32].

Similar to this study, it was reported in different studies that the combined effect of NAC with different antibiotics (rifampicin, tigecycline, ciprofloxacin) against bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *S. aureus*) caused a decrease in the MIC values of antibiotics [33].

Soudeiha *et al*. [34] in 2017 showed that there was only additive effect and there was no synergism when evaluating combination of colistin and meropenem in *Acinetobacter baumanni.*

It was determined that NAC molecule showed the predicted effect on MIC values of antibiotics when used together with antibiotics. It is thought that the use of NAC molecule together with antibiotics will facilitate the treatment of infections caused by microorganisms that are especially difficult to treat and are resistant to antibiotics. For this reason, based on the observed effect of the combination of MER + NAC in Acinetobacter infections, it can be concluded that NAC may serve as a new alternative for combined drug therapy.

In summary, it was determined that i) NAC molecule, when used together with meropenem, ciprofloxacin, gentamicin, decreased the MIC values of these antibiotics affecting Acinetobacteria, and ii) NAC molecule had a synergistic effect when used together with meropenem. Based on the results obtained, the potential of using NAC in combination with other microorganisms and antimicrobial agents for novel treatment approaches is considered promising.

Some mechanisms have been proposed to understand the antimicrobial activity of NAC. These are: competitive inhibition of cysteine utilization, reaction of the NAC sulfhydryl group with bacterial proteins, indirect effects of disruption of intracellular redox balance on cell metabolism and intracellular signal transduction pathways [18]. In our previous study, it was stated quinolone resistance determined in 73 *A. baumannii* isolates are related to that mutations in the *gyrA, gyrB, parC* genes [35]. Also in one of our studies which studied with the same clinical isolates; it has been found that overexpression of adeA, adeB and adeC genes which are related to efflux pump resistance and it was shown that inhibiting the resistance by an efflux pump inhibitor reduces the MIC values of ciprofloxacin [36]. There are also other studies conducted with different antibiotics and inhibitors in which antimicrobial resistance is associated with the expression levels in *adeABC* genes [37].

It is thought that the mechanism that effects resistance inhibition determined in this study may be due to these previous mechanisms. For this reason we think that this study should be improved with the previous molecular studies including NAC.

AUTHOR CONTRIBUTIONS

Concept: A.S.S.; Design: A.S.S.; Control: A.S.S., S.Ö.; Sources: A.S.S.; Materials: A.S.S., S.Ö.;

Data Collection and/or Processing: A.S.S.; Analysis and/or Interpretation: A.S.S.; Literature Review: A.S.S.; Manuscript Writing: A.S.S.; Critical Review: A.S.S., S.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

Approval was received from T.R. Trakya University Faculty of Medicine Scientific Research Ethics Committee on 29.04.2015 with the approval number TÜTF-BAEK-2015/55-08/10.

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