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N-Acetylcysteine-Mediated Modulation of Bacterial Antibiotic Susceptibility▽

N-Acetylcysteine (NAC) acts as a precursor for glutathione biosynthesis (1, 2), in addition to performing several other biological functions in mammals and bacteria (1, 5). As per our earlier studies (3, 4), glutathione is an important modulator of antibiotic activity in bacteria; consequently, it is of interest to study the effect of NAC on bacterial antibiotic susceptibility. It becomes even more important since NAC is supplied as a mucolytic agent in combination with antibiotics during treatment of lower respiratory tract infection (1). Here we report the effect of NAC on various antibiotics against different bacterial strains, including opportunistic respiratory pathogens like *Klebsiella* and *Pseudomonas*.

The *Escherichia coli* strains used were same as those in one of our earlier studies (4). *Pseudomonas* and *Klebsiella* strains were obtained from the National Collection of Industrial Microorganisms (NCIM; Pune, India). MICs of antibiotics were determined in Mueller-Hinton agar as outlined by the Clinical Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) (7).

The effect of NAC on bacterial susceptibility to 10 antibiotics belonging to different groups was studied by measuring their MICs in the presence and absence of 10 mM NAC in the medium. Antibiotic susceptibilities of three *Escherichia coli* strains, two *Klebsiella* strains, and one *Pseudomonas* strain (Table 1) were determined for this purpose. As the commonly used therapeutic concentration of NAC ranges between 4 and 10 mM per day, for management of severe respiratory disorders (8), an NAC concentration of 10 mM was chosen for this study. The presence of 10 mM NAC did not affect the growth of these bacterial strains, with the exception of *Klebsiella pneumoniae* (NCIM no. 2883), which failed to grow on a plate containing NAC. Further analysis revealed that even at a concentration as low as 2.5 mM, NAC was inhibitory for growth of the *K. pneumoniae* strain. The effect of NAC on the MICs of the antibiotics for the remaining strains was studied, as described in Table 1. In the case of fluoroquinolones, the MICs of both ciprofloxacin and ofloxacin increased substantially for all *E. coli* and *Klebsiella aerogenes* strains and moderately for *Pseudomonas aeruginosa* in the presence of NAC (Table 1). Similarly, the presence of NAC led to significant increase in MICs of aminoglycosides like streptomycin, kanamycin, and spectinomycin against all the bacterial strains tested. These observations suggest that presence of NAC gives protection against wide variety of the aminoglycoside and fluroquinolone antibiotics. Along the lines of aminoglycosides and fluoroquinolones, NAC provided substantial protection against erythromycin as well, for all of the strains. However NAC did not alter the MICs of chloramphenicol and tetracycline for any bacterial strain, suggesting that the action of these antibiotics is not affected by the presence of NAC. The presence of NAC did not alter the MIC of penicillin against *K. aerogenes* or any of the *E. coli* strains, although it led to a reduction in MIC of penicillin against *P. aeruginosa*. In addition, NAC reduced the MIC of ampicillin for all of the strains except that of *K. aerogenes*. Overall, we could see a more profound augmentation of ampicillin activity in comparison to penicillin by NAC for most of the strains.

We therefore conclude that presence of NAC can either reduce the antibacterial activity of aminoglycosides, fluoroquinolones, and erythromycin or enhance the efficacy of β-lactams against several bacterial strains. Besides, the presence of NAC can be detrimental for bacteria like *K. pneumoniae*. Our present study therefore demonstrates that NAC is an important modulator of antibiotic activity. Although specific mechanisms of antibiotic susceptibility modulation are not yet clear, these findings along with our earlier data (3, 4) suggest that thiols are an important determinant of antibiotic activity against bacteria. This proposition is additionally supported by an earlier independent report in which L-cysteine was shown to be involved in alteration of antibiotic activity against *Clostridium* (6). Our results further suggest that administration of aerosolized NAC as a mucolytic agent during antibiotic therapy of the respiratory tract infection could modulate the outcome of the therapeutic process, depending on the target bacterial pathogen and antibiotic being used for the therapy. There-

TABLE 1. Effect of NAC on susceptibility of diverse bacterial strains to different antibiotics

Antibiotic	MIC (μg/ml) for strain with treatment[a]:									
	E. coli						*P. aeruginosa* NCIM 5029		*K. aerogenes* NCIM 2281	
	MG1655		W3110		DH5α					
	Control	+ NAC	Control	+ NAC	Control	+ NAC	Control	+ NAC	Control	+ NAC
Ciprofloxacin	0.008	0.512	0.004	0.064	0.016	0.512	1.024	2.048	0.016	0.512
Ofloxacin	0.032	1.024	0.008	0.032	0.016	1.024	0.256	2.048	0.064	0.512
Streptomycin	2	32	2	16	1	32	8	64	2	16
Kanamycin	1	32	1	16	0.5	32	64	256	1	16
Spectinomycin	8	256	6	128	4	128	256	512	8	128
Erythromycin	32	1,024	4	512	16	1,024	256	1,024	32	1,024
Chloramphenicol	4	4	1	1	2	2	128	128	1	1
Tetracycline	1	1	0.062	0.062	1	1	8	8	1	1
Ampicillin	4	1	8	2	4	2	1,024	128	32	32
Penicillin	64	64	64	64	32	32	>4,096	2,048	64	64

[a] Antibiotic susceptibility data for *K. pneumoniae* NCIM 2883 are not included in the table because the presence of NAC led to complete growth inhibition of *K. pneumoniae*. In each case, the final NAC concentration was 10 mM.

fore, further clinical studies are required to establish the clear-cut relationship between aerosolized NAC and the antibiotics being used for therapeutic purposes against diverse bacterial pathogens.

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