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Short communication: N-Acetylcysteine-mediated modulation of antibiotic susceptibility of bovine mastitis pathogens

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ABSTRACT

The aim of this study was to investigate the effects of *N*-acetylcysteine (NAC) on antibiotic susceptibility of bovine mastitis pathogens including *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Escherichia coli*, and *Streptococcus agalactiae*. Minimum inhibitory concentrations (MIC) were tested by the agar-based E-test method. The presence of 10 m*M* NAC reduced the MIC of penicillin and ampicillin but enhanced the MIC of erythromycin and ciprofloxacin for all of the strains. In addition, NAC-mediated modulation of MIC of kanamycin, tetracycline, and vancomycin was diverse, depending on the target bacterial pathogen and antibiotic being used. The results suggest that NAC is an important modulator of antibiotic activity against the major bovine mastitis pathogens.

Key words: *N*-acetylcysteine, bovine mastitis, pathogen, antibiotic susceptibility

Short Communication

Bovine mastitis is one of the most costly diseases affecting the dairy industry worldwide (Perreten et al., 2013). Although mastitis can be caused by 137 different microorganisms (Watts, 1988), *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Escherichia coli*, and *Streptococcus agalactiae* are the main etiological agents commonly associated with the disease (Nair et al., 2005). To date, antibiotic therapy is the standard treatment of mastitis. However, the results of this therapy have been disappointing due to the misuse of antibiotics (Pereira et al., 2011; Barrero et al., 2014).

N-Acetylcysteine (**NAC**) is a mucolytic agent that disrupts disulfide bonds in mucus and reduces the viscosity of secretions (El-Feky et al., 2009). Based on these characteristics, NAC has been widely used as an adjuvant in combination with antibiotics during medical treatment of bacterial infectious diseases including chronic bronchitis, vascular catheter-related infection, and urinary tract infection (Marchese et al., 2003; Olofsson et al., 2003; Aslam et al., 2007). However, the effect of NAC on bovine mastitis pathogens has not been studied. The aim of this study was to investigate the effects of NAC on antibiotic susceptibility of *Staph. aureus*, *Strep. dysgalactiae*, *E. coli*, and *Strep. agalactiae* isolated from bovine mastitis cases.

The *Staph. aureus*, *Strep. dysgalactiae*, *E. coli*, and *Strep. agalactiae* strains were isolated from subclinical bovine mastitis in Gansu province in China during 2015. Mastitis infection was confirmed by the California mastitis test. Identification was performed by morphological characterization and biochemical testing as previously described (Cressier and Bissonnette, 2011). Minimum inhibitory concentrations of penicillin, ampicillin, erythromycin, kanamycin, tetracycline, ciprofloxacin, and vancomycin were determined by the E-test (BioMerieux, Marseille, France) method (Liu et al., 2014). Antimicrobial agent concentrations ranged from 0.002 to 32 μg/mL for penicillin and ciprofloxacin, and 0.016 to 256 μg/mL for ampicillin, erythromycin, kanamycin, tetracycline, and vancomycin.

The effects of NAC (Sigma-Aldrich, Lyon, France) on antibiotic susceptibility of pathogens to 7 antibiotics belonging to different groups were studied by measuring their MIC in the presence and absence of 10 m*M* NAC in the medium, respectively. Antibiotic susceptibility of 2 *Staph. aureus* strains (LZ 0215, LZ 84184), 2 *Strep. dysgalactiae* strains (LZ 717, LZ 211), 2 *E. coli* strains (LZ 2552, LZ 282), and 2 *Strep. agalactiae* strains (LZ 17, LZ 21) were determined in this study. The presence of 10 m*M* NAC did not affect the growth of these strains. The experiments were carried out at least twice, and the representative results are mentioned here.

The effects of NAC on antibiotic susceptibility of *Staph. aureus* and *Strep. dysgalactiae* are shown in Table 1, and *E. coli* and *Strep. agalactiae* are shown in Table 2. In the case of β-lactam antibiotics, the MIC of both penicillin and ampicillin decreased for all of the strains in the presence of NAC. Conversely, NAC increased the MIC of erythromycin and ciprofloxacin for all of the strains. It also led to reduction in MIC of

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Table 1. Effect of *N*-acetylcysteine (NAC[1]) on susceptibility of *Staphylococcus aureus* and *Streptococcus dysgalactiae* to different antibiotics, as measured by MIC (μg/mL)

	Staph. aureus				*Strep. dysgalactiae*			
	LZ 0215		LZ 84184		LZ 717		LZ 211	
Antibiotic	Control	+NAC	Control	+NAC	Control	+NAC	Control	+NAC
Penicillin	1.5	0.38	0.5	0.25	1.5	0.75	0.016	0.004
Ampicillin	2	0.25	0.75	0.5	0.75	0.25	0.032	<0.016
Erythromycin	0.094	3	0.064	8	0.75	3	0.032	0.064
Kanamycin	3	12	1.5	8	32	2	128	48
Tetracycline	0.75	0.5	0.5	0.25	>256	96	0.25	0.047
Ciprofloxacin	0.19	0.25	1	1.5	0.5	1.5	0.75	1
Vancomycin	1.5	1.5	1.5	1.5	2	2	0.75	0.75

[1]In each case, the final NAC concentration was 10 m*M*.

tetracycline for all of the strains except that of *E. coli*. Similarly, the MIC of kanamycin decreased for *Strep. agalactiae* and *Strep. dysgalactiae* strains but increased for *Staph. aureus* and *E. coli* strains in the presence of NAC. In addition, changes in vancomycin MIC were not observed in *Staph. aureus*, *Strep. dysgalactiae*, and *E. coli* strains at the presence of NAC, although it resulted in a reduction in MIC of vancomycin against *Strep. agalactiae*.

The presence of NAC enhances the efficacy of β-lactams antibiotics against all of the strains, while it gives protection against erythromycin and ciprofloxacin. These data are similar to those reported by Goswami and Jawali (2010). However, in our study, it is interesting that NAC reduced the antibacterial activity of kanamycin against pathogens belonging to *Streptococcus* spp. alone. And for tetracycline, the presence of NAC decreased the antibacterial activity against gram-positive bacteria but enhanced that against gram-negative bacteria. In addition, NAC gives protection against vancomycin for *Strep. agalactiae* alone. These findings suggest that the effects of NAC on bacterial antibiotic susceptibility are significantly associated with bacterial species, shape, and structure. It was reported that NAC in combination with fosfomycin and tigecycline displayed opposite effects on bacterial biofilm formation (Marchese et al., 2003; Aslam et al., 2007). The bacteria enclosed in biofilm became 10 to 1,000 times more tolerant to antibiotics than equivalent planktonic cultures (Mah and O'Toole, 2001). In this study, it is possible that NAC-mediated modulation of antibiotic susceptibility against the pathogens may be mediated by biofilm-forming ability, and the specific mechanisms are being explored in our laboratory.

In conclusion, the present study indicates that NAC is an important modulator of antibiotic activity against the major bovine mastitis pathogens. A combination of β-lactam antibiotics and NAC is recommended during antibiotic therapy of bovine mastitis caused by these major pathogens.

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Table 2. Effect of *N*-acetylcysteine (NAC[1]) on susceptibility of *Escherichia coli* and *Streptococcus agalactiae* to different antibiotics, as measured by MIC (μg/mL)

	E. coli				*Strep. agalactiae*			
	LZ 2552		LZ 282		LZ 17		LZ 21	
Antibiotic	Control	+NAC	Control	+NAC	Control	+NAC	Control	+NAC
Penicillin	>32	32	>32	12	0.047	0.016	0.032	0.016
Ampicillin	2	1.5	3	1	0.094	<0.016	0.064	<0.016
Erythromycin	16	>256	48	>256	0.032	0.125	0.047	0. 25
Kanamycin	0.75	1.5	3	25	>256	24	>256	32
Tetracycline	3	8	3	8	48	32	96	32
Ciprofloxacin	0.004	0.012	0.004	0.012	0.5	1.5	0.5	1.5
Vancomycin	>256	>256	>256	>256	1.5	0.75	1	0.5

[1]In each case, the final NAC concentration was 10 m*M*.

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