Short communication: *N*-Acetylcysteine-mediated augmentation of β -lactam antibacterial activity against methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis cases

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ABSTRACT

The present study investigated the effects of *N*-acetylcysteine (NAC) on β -lactam antibacterial activity against 20 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from bovine mastitis. Minimum inhibitory concentrations (MIC) were determined by the E-test method. The presence of 10 mM NAC reduced the MIC of penicillin, ampicillin, oxacillin, cefoxitin, ceftazidime, and cefotaxime to MRSA. Importantly, the MIC of cefoxitin in MRSA in the presence of NAC was lower than the susceptible breakpoint of cefoxitin. The results provide a new way to use current β -lactam antibiotics combined with NAC against MRSA.

Key words: bovine mastitis, methicillin-resistant *Staphylococcus aureus*, *N*-acetylcysteine, resistance

Short Communication

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major drug-resistant pathogen responsible for nosocomial infections in humans and IMI in cattle (Graveland et al., 2011). Of note, MRSA is resistant to most β -lactam antibiotics and multiple other antimicrobial classes currently available for mastitis therapy, which has major implications for current and future treatment options for this pathogen (Vanderhaeghen et al., 2010; Lee et al., 2018). *N*-Acetylcysteine (NAC) is an important modulator of antibiotic activity (Goswami and Jawali, 2010). Our previous study showed that NAC increased the antibacterial activity of penicillin and ampicillin against bovine mastitis *Staphylococcus aureus* (Yang et al., 2016), which were later con-

firmed as methicillin-susceptible *Staphylococcus aureus* (MSSA; data not shown). Here, we report the effect of NAC on β -lactam antibacterial activity against MRSA recovered from bovine mastitis cases.

The 20 MRSA isolates used in this study were isolated from cases of bovine mastitis in China. The MRSA were screened by using ChromID MRSA agar (BioMérieux, Marcy-l'Étoile, France) and confirmed by cefoxitin (30 μ g) disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2013). The effect of NAC (Sigma-Aldrich, Lyon, France) on the susceptibility of MRSA to β -lactam antibiotics (penicillin, ampicillin, oxacillin, cefoxitin, ceftazidime, and cefotaxime) was investigated on Mueller-Hinton agar (Oxoid, Basingstoke, UK) in the presence or absence of 10 mM NAC. To compare the effect of NAC on antibacterial activity of β -lactam antibiotics between MRSA and MSSA, we also tested the effect of NAC on β -lactam antibacterial activity against 5 MSSA strains. The MIC of these antibiotics were determined by the E-test (Liofilchem, Roseto, Italy) method, with MIC ranging from 0.016 to 256 μ g/mL. All experiments were carried out at least in duplicate. *Staphylococcus aureus* ATCC29213 was used as a reference strain for the E-test. Statistical analysis was carried out in SPSS version 19.0 (IBM Corp., Armonk, NY) using T-test.

The results are summarized in Table 1. In the presence of 10 mM NAC, the MIC of all β -lactam antibiotics to MRSA decreased remarkably. Interestingly, the MIC of oxacillin and cefoxitin in MRSA were lower than the corresponding susceptible breakpoint (≥ 2 and ≥ 4 μ g/mL) recommended by CLSI (CLSI, 2013), which means these MRSA strains were sensitive to methicillin in the presence of NAC. In addition, NAC decreased the MIC of penicillins (penicillin, oxacillin, and ampicillin) to $\leq 2.1\%$ in MRSA, a much larger reduction than that in MSSA (5.9% to 50.0%). Similarly, NAC reduced the MIC of cephalosporins (cefoxitin, ceftazidime, and cefo-

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Table 1. Effect of *N*-acetylcysteine (NAC) on β -lactam antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA)

Strain ¹	MIC ($\mu\text{g/mL}$) for <i>Staph. aureus</i> with NAC treatment ²											
	Penicillin			Ampicillin			Oxacillin			Cefoxitin		
	Control	+ NAC		Control	+ NAC		Control	+ NAC		Control	+ NAC	
R1	64	0.75		48	1		>256	0.094		>256	1	
R2	24	0.25		24	0.38		>256	0.19		>256	0.5	
R3	64	0.5		48	0.75		>256	0.19		>256	0.75	
R4	48	1.0		48	1		>256	0.125		>256	0.5	
R5	64	0.75		64	1		>256	0.125		>256	0.5	
R6	64	0.75		64	1		>256	0.125		>256	0.5	
R7	192	1.0		96	0.75		>256	0.19		>256	0.75	
R8	64	0.75		48	1		>256	0.19		>256	0.75	
R9	96	0.5		64	0.75		>256	0.19		>256	1.5	
R10	96	0.5		64	0.75		>256	0.25		>256	1.5	
R11	48	1.0		64	1		>256	0.38		>256	0.5	
R12	64	0.75		64	0.5		>256	0.19		>256	0.75	
R13	24	0.25		24	0.38		>256	0.38		>256	0.75	
R14	64	1.0		64	0.75		>256	0.19		>256	0.75	
R15	64	0.75		48	0.75		>256	0.125		>256	1	
R16	64	0.75		64	0.75		>256	0.25		>256	0.75	
R17	96	0.5		48	0.75		>256	0.125		>256	0.38	
R18	24	0.38		24	0.5		>256	0.125		>256	0.5	
R19	48	0.25		32	0.5		>256	0.094		>256	0.38	
R20	64	1.0		48	0.75		>256	0.125		>256	0.5	
S1	1	0.064		0.38	0.19		0.19	0.047		3	0.5	
S2	1	0.125		0.75	0.125		0.25	0.064		3	0.5	
S3	0.75	0.125		0.5	0.19		0.25	0.064		1.5	0.38	
S4	0.75	0.094		0.75	0.125		0.25	0.047		2	0.25	
S5	1	0.125		0.75	0.125		0.25	0.094		2	0.38	

¹R = MRSA; S = MSSA.²In each case, the final NAC concentration was 10 mM.

taxime) to 3.1% to 50.0% in MSSA, a smaller decrease than that in MRSA ($\leq 1.6\%$).

The mechanisms of antibiotic susceptibility modulation by NAC are not yet clear. Aslam et al. (2007) found that NAC made the biofilm-associated bacteria more susceptible to tigecycline by degrading the extracellular polysaccharide matrix of biofilm. Rodríguez-Beltrán et al. (2015) reported that NAC-based modulation of imipenem activity is dependent mainly on outer membrane porin D (OprD). However, these theories cannot reasonably explain the contrasting changes in our previous study, in which we reported that NAC reduced the MIC of penicillin and ampicillin but increased the MIC of erythromycin, kanamycin, and ciprofloxacin to *Staph. aureus* (Yang et al., 2016). In the current study, NAC markedly increased β -lactam antibacterial activity against MRSA. Meanwhile, NAC-mediated modulation of MIC in MRSA was higher than that in MSSA. It is known that MRSA and MSSA strains and isolates have different resistant mechanisms to β -lactam antibiotics (Lowy, 2003). Methicillin resistance in *Staph. aureus* is usually conferred by the acquisition of a non-native gene *mecA* encoding an altered penicillin-binding protein (PBP2a) or variant *mecC* encoding PBP2a_{LGA}. Both altered proteins have extremely low affinity for most β -lactam antibiotics (Lee et al., 2018). Thus, we speculate that NAC-based modulation of β -lactam antibacterial activity to MRSA may depend on the methicillin-associated resistance gene (*mecA* or *mecC*) and corresponding products penicillin-binding protein (PBP2a or PBP2a_{LGA}). The specific mechanisms are being explored in our laboratory.

This study provides new perspectives for the use of NAC combined with β -lactam antibiotics against MRSA, but further in vivo studies are required to evaluate the effectiveness, target animal safety, and withholding periods of these combinations.

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