Inhibiting peptidoglycan hydrolase alleviates MRSA pneumonia through autolysin-mediated MDP-NOD2 pathway

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ZJ-2 inhibited PG cleavage and MDP production in MRSA USA300

The inhibitory effect of **ZJ-2** on PG hydrolysis was verified by detecting the content of free PG and MDP in the supernatant. The results showed that PG and MDP content in the supernatant of WT after 1/4MIC **ZJ-2** incubation hardly changed, while, those after 1/2MIC **ZJ-2** incubation decreased. Conversely, PG and MDP content in the supernatant of Δ*atlA* after **ZJ-2** incubation didn't change (Figure S1A and B). In all, **ZJ-2** could inhibit PG hydrolysis and reduce MDP production by down-regulating the expression of autolysin-related genes.

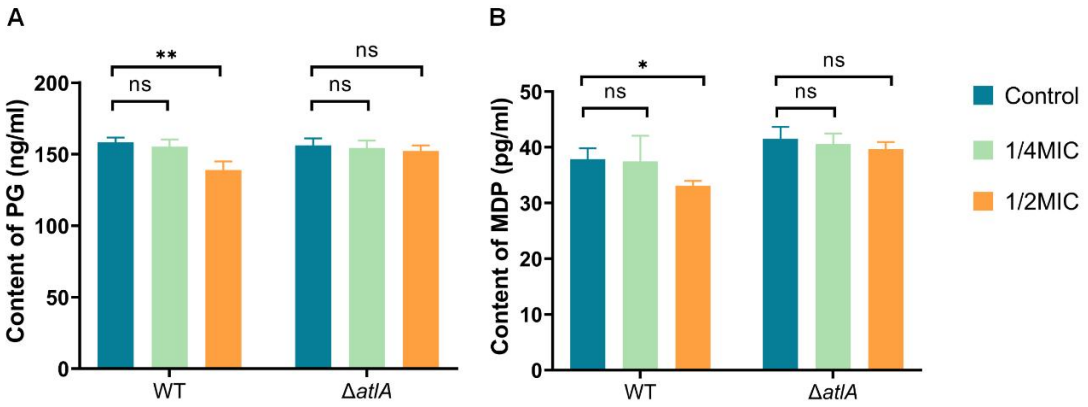

Figure S1 The effect of **ZJ-2** on MRSA USA300 PG (A) and MDP (B) content was examined by ELISA kit. All samples were analyzed in three replicates. $^{**}P < 0.01$, $^{*}P < 0.05$, when compared with the control.

Effect of ZJ-2 on inflammatory cells in the blood

The therapeutic effect of **ZJ-2** on MRSA infection was evaluated by detecting the number of inflammatory cells in the blood of mice. As shown in Figure S2, WBC, NEUT and LY were elevated in the blood of WT and Δ*atlA* infection mice. After treatment with **ZJ-2** (or VAN), whether WT or Δ*atlA*, these cells were all reduced. Notably, the number of inflammatory cells in WT-infected mice exhibited a greater reduction than those in Δ*atlA*-infected mice. This further indicated that **ZJ-2** reduced inflammatory responses of MRSA infection by inhibiting autolysin-mediated PG hydrolysis.

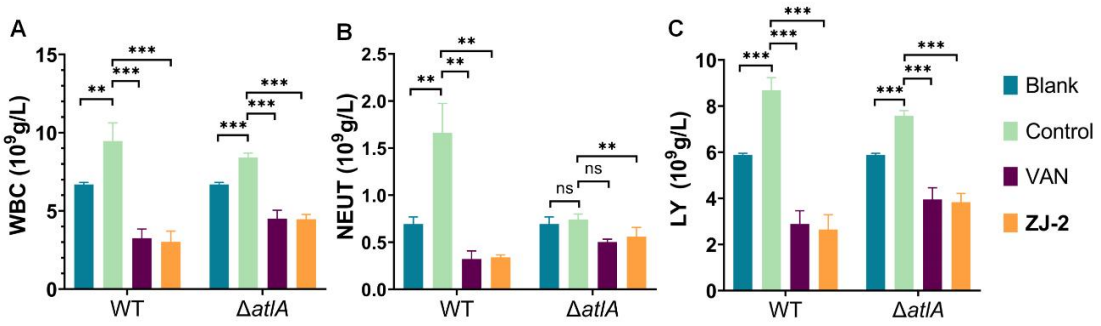

Figure S2 The effect of **ZJ-2** on WBC (A), LY (B) and NEUT (C) was investigated by measuring the hematology of venous blood (n = 3). ***P < 0.001, **P <0.01, when compared with the control.

Effect of ZJ-2 on the levels of cytokine

ELISA (Figure S3) were used to detect the levels of the inflammatory factors IL-6 and TNF-α in mouse alveolar lavage fluid and blood. Both **ZJ-2** and VAN treatment decreased the levels of inflammatory factors, but the levels in Δ*atlA* showed a more significant decline than those in WT. This suggested that **ZJ-2** can decrease the levels of relative inflammatory cytokines by inhibiting MDP-NOD2 pathway.

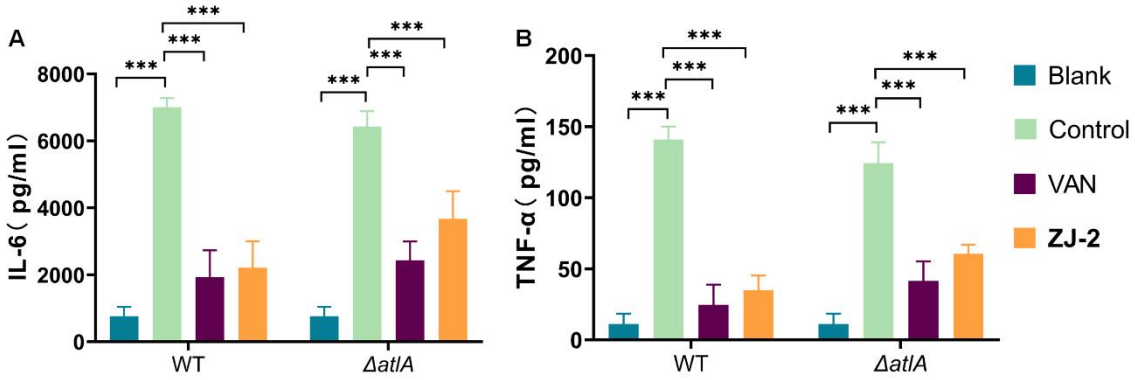

Figure S3 ELISA detected the effect of **ZJ-2** on IL-6 (A) and TNF-α (B) of mouse alveolar lavage fluid (n = 5). Values represent means ± SD; ***$P < 0.001$, when compared with the control.