For the last decade, chemical control of bacterial virulence has received considerable attention. Ajoene, a sulfur-rich molecule from garlic has been shown to reduce expression of key quorum sensing regulated virulence factors in the opportunistic pathogen Pseudomonas aeruginosa. Here we show that the repressing effect of ajoene on quorum sensing occurs by inhibition of small regulatory RNAs (sRNA) in P. aeruginosa as well as in Staphylococcus aureus, another important human pathogen that employs quorum sensing to control virulence gene expression. Using various reporter constructs, we found that ajoene lowered expression of the sRNAs RsmY and RsmZ in P. aeruginosa and the small dual-function regulatory RNA, RNAIII in S. aureus, that controls expression of key virulence factors. We confirmed the modulation of RNAIII by RNA sequencing and found that the expression of many QS regulated genes encoding virulence factors such as hemolysins and proteases were lowered in the presence of ajoene in S. aureus. Importantly, our findings show that sRNAs across bacterial species potentially may qualify as targets of anti-virulence therapy and that ajoene could be a lead structure in search of broad-spectrum compounds transcending the Gram negative-positive borderline.