

Short Communication

H-NS enhances phage resistance in *Klebsiella pneumoniae* by suppressing quorum sensing

Yajing Song^{1,2}, Changhui Huang³, Hailong Ou³, and Kai Zhou^{1,4,*}

¹Department of Shenzhen Institute of Respiratory Diseases, Shenzhen People's Hospital (The First Affiliated Hospital, Southern University of Science and Technology; The Second Clinical Medical College, Jinan University), Shenzhen 518020, China, ²Post-doctoral Scientific Research Station of Basic Medicine, Jinan University, Guangzhou 510632, China, ³School of Basic Medical Sciences, Guangxi Medical University, Nanning 530027, China, and ⁴Guangdong Key Laboratory of Regional Immunity and Diseases, Department of Pathogen Biology, Shenzhen University Medical School, Shenzhen 518020, China

*Correspondence address. E-mail: zhouk@mail.sustech.edu.cn

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Abstract

The molecular mechanisms underlying *Klebsiella pneumoniae* (KP) resistance to phages have not been fully elucidated, especially those involving the quorum-sensing (QS) system and the global regulator H-NS. In this work, we investigate the relationship between H-NS and QS in phage resistance by detecting transcriptional variation under phage pressure with RT-qPCR. *LuxS* and *Isr* operon knockout strains are generated via CRISPR editing, and H-NS-overexpressing mutants are constructed using plasmid-based overexpression. We also determine phage susceptibility by measuring the efficiency of plating (EOP). The capsular uronic acid content and phage adsorption efficiency are evaluated. The results show that phage pressure strongly downregulates *luxS* and *Isr* operon gene expressions but upregulates the transcription of H-NS. Knockout of either *luxS*/*Isr* operon genes or overexpression of H-NS results in diminished phage sensitivity and the downregulation of CPS synthase genes. However, these changes do not reduce the capsular uronic acid content or affect phage adsorption rates, suggesting that H-NS-mediated resistance is independent of capsular modulation. In addition, this H-NS-QS regulatory link is conserved between HvKP and CRKP. H-NS enhances phage resistance by suppressing QS through a mechanism independent of capsular polysaccharide modulation, as evidenced by unaltered phage adsorption and statistically insignificant changes in capsular uronic acid. This provides a novel explanation for a resistance mechanism involving intracellular defenses (e.g., abortive infection) and provides possible avenues for improving phage therapy targeting multidrug-resistant *K. pneumoniae*.

Key words *Klebsiella pneumoniae*, quorum sensing, H-NS, phage resistance

Introduction

Klebsiella pneumoniae is a major pathogen of hospital-acquired infections, causing various diseases, including pneumonia, sepsis, and intra-abdominal infections. The global spread of carbapenem-resistant *K. pneumoniae* (CRKP) and highly virulent *K. pneumoniae* (HvKP) has added great challenges to clinical treatment [1,2]. Phage therapy is considered a new means to effectively combat multidrug-resistant bacterial infections because of its advantages of selective killing and less pan-resistance [3,4]. Bacteria have evolved various mechanisms to resist phage infection, such as CRISPR-Cas system-mediated immune defense against phages [5], adsorption blockade due to mutations in receptor proteins [6], and abortive infection [7].

Therefore, bacterial resistance regulatory mechanisms are very diverse, which hinders the effectiveness of phage therapy, and further exploration of bacterial resistance regulatory networks is needed for the effective treatment of bacterial infections.

Quorum sensing (QS) is a control system that consists of sensing population size and population coordination among bacteria through small signaling molecules, which are involved in regulating biofilm production, pathogenesis-related gene expression, and metabolic response [8]. In recent years, QS has also been reported to be involved in regulating the interactions between bacteria and phages; e.g., LasI/LasR can regulate phage infection by affecting the expression of its outer membrane proteins in *Pseudomonas*

aeruginosa [9]. In addition, the QS signaling molecules (CAI-1 and AI-2) of *Vibrio cholerae* can also affect host survival by directly regulating phage lysis-related decisions [10]. However, reports on whether the QS system of KP is involved in the regulation of phage resistance have not yet been reported in the literature, and the molecular mechanism of phage resistance is still unknown.

Histone-like nucleoid structuring protein (H-NS) is a highly conserved global transcriptional regulator in gram-negative bacteria that regulates chromosome structure and gene expression by binding to DNA and plays a central role in the environmental stress response (e.g., osmotic pressure changes and acidic environments) [11]. Recent studies have shown that H-NS can limit bacterial adaptive evolution by inhibiting the lateral transfer of virulence island genes [12], but its functional association with the QS system and its role in phage resistance have not been explored. On the basis of these findings, the present study proposes the scientific hypothesis that H-NS may mediate phage resistance in KPs by modulating key pathways of the QS system and that this mechanism is generalizable across different clinical strains.

In this study, we clarified the key role of H-NS-QS axis signaling in mediating phage resistance through CRISPR gene editing, gene transcript expression analysis, and phenotypic characterization. Our results revealed that (1) H-NS expression is upregulated and the gene expression of the *lsr* operon gene of the QS system is repressed under phage infection stress; (2) inactivation of the QS system or overexpression of H-NS significantly increases phage resistance by a mechanism that is independent of adsorption blockage mediated by capsular polysaccharides; and (3) this mechanism also occurs conservatively in HvKP and CRKP. These results provide new ideas for improving the therapeutic efficacy of phages by targeting and modulating bacterial signaling pathways in clinical applications.

Materials and Methods

Construction of bacterial strains and culture conditions

The two strains used in the present study, HvKP ATCC 43816 (KP43) and CRKP KP47434 (KP47), and the corresponding bacteriophages were obtained from our laboratory stocks. All the strains were subsequently grown in Luria-Bertani (LB) media at 37°C. To construct gene knockout mutants via CRISPR-Cas9, we first cloned spacer DNA targeting each gene into the pSGKP-Km/RF vector. Next, donor templates containing homologous extensions were synthesized and co-transformed with the plasmid into pCasKP-apr by electroporation. The mutants were selected on LB plates containing 5% sucrose at 37°C. The amplified target gene (*hns*) and its promoter were subsequently cloned and inserted into the pACYC184-Cm/RF plasmid, which was subsequently digested with the *Bam*HI enzyme (Thermo Fisher Scientific, Waltham, USA). The recombinant plasmid was introduced into the strain via electronic transformation. The empty vector (pACYC184-RF) was introduced into the same strain as a blank control. PCR and DNA sequencing were used to confirm the final constructs. The primers used are listed in [Supplementary Table S1](#).

RNA extraction and transcription of genes by quantitative real-time PCR (RT-qPCR)

The expression levels of polysaccharide biosynthesis-related genes (*galF*, *wzi*, *wza*, *wzb*, *wcaJ*, *gnd*, *manB*, *udg*, *hns*, *luxs*, and *lsr* operon genes) were compared between the wild-type strain and mutant strains via RT-qPCR. All bacterial cells were grown in LB

broth to the mid-log phase ($OD_{600} \sim 0.8$, approximately 10^8 CFU/mL). Total RNA was isolated using RNAPrep Pure Cell/Bacteria kit (TIANGEN, Beijing, China), and the quality of the RNA samples was examined using the Bioanalyzer 2100 System (Agilent Technologies, Santa Clara, USA). cDNA was subsequently synthesized with a ReverTra Ace qPCR kit (Toyobo, Shanghai, China) according to the manufacturer's instructions. The quantification of gene expression levels was performed by real-time qPCR analysis on a 7900 HT sequence-detection system (ABI, Foster City, USA) with ABI Power SYBR green PCR master mix. The primers used were designed and synthesized by Sangon Biotechnology (Shanghai, China). We used the comparative Ct value method for RT-PCR in this study. The resulting Ct values were normalized using *gapA* as the reference gene, and the relative expression of the indicated genes was calculated by the $2^{-\Delta\Delta Ct}$ method. Then, the final relative gene expression level was normalized to that of the wild-type strain [13]. The detailed primers used are listed in [Supplementary Table S1](#).

Determination of the efficiency of plating (EOP) and the phage adsorption rate

The EOP was determined as previously described [14], with some modifications. Briefly, the selected isolates were grown overnight at 37°C, and 100 μ L of each was plated. The PFU of the phage was enumerated by spotting the serial dilution of the respective phage suspension on the target lawn. The EOP was calculated as the average PFU on target bacteria divided by the average PFU on host bacteria through three independent assays. The plaque plate images were obtained through the following methods: Overnight cultures of strains were inoculated in fresh LB medium until the stationary phase was reached ($OD_{600} \sim 0.8$). Then, 100 μ L of the culture was mixed with 5 mL of melted 1% LB agar medium to prepare double-layered agar plates. The phages were then subjected to 10-fold gradient dilution in buffer, and 5 μ L aliquots were spotted onto a plate and incubated at 37°C. The initial concentrations of bacteriophages are 10^{11} PFU/mL (phage H5) and 10^{10} PFU/mL (phage P7).

A phage adsorption assay was performed as described previously [15], with minor modifications. Briefly, 10 μ L of 1.5×10^8 PFU/mL phages (initial phage titers determined via the double-layer plate method) was added to 2 mL of subcultured end-logarithmic growth bacteria (the concentration of 1×10^9 CFU/mL bacteria calculated via CFU counting), and the mixture was incubated for 10 min at 37°C at 180 r/min. The mixture was centrifuged for 5 min (4°C, 12,000 g), and the supernatant was filtered with a 0.22- μ m filter and diluted by 10-fold serial dilution. The double-layer agar plate method was used to determine the residual phage titer in the supernatant. The phage adsorption rate was calculated as the phage adsorption efficiency = (initial titer of the phage-residual titer in the supernatant/initial titer) $\times 100\%$. The results are reported as the means of 3 independent measurements.

Assessment of the uronic acid content of the capsule

Acidic polysaccharides were extracted and measured to quantify capsular polysaccharides as described previously [16]. Briefly, 500 μ L of the bacterial culture in the log phase was mixed with 100 μ L of 1% Zwittergent 3-14 detergent in 100 mM citric acid, followed by incubation at 50°C for 20 min. The cells were then pelleted (14,000 g for 2 min). Three hundred microliters of the supernatant was aspirated and mixed with 1.2 mL of absolute ethanol, incubated at

4°C for 20 min, and then centrifuged for 5 min at 14,000 *g*. The pellet was dried and resuspended in 200 μ L of distilled water. Then, 1.2 mL of 12.5 mM sodium tetraborate in sulfuric acid was added and incubated for 5 min at 100°C, followed by incubation on ice for 10 min. Thereafter, a 20- μ L volume of 0.15% 3-phenyl phenol in 0.5% NaOH was added. After a 5-min incubation at room temperature, the absorbance at 520 nm was measured. The glucuronic acid content was then determined from a standard curve of glucuronic acid and expressed as μ g/OD units.

Results

Transcriptional regulation of the H-NS and QS systems under phage stress in HvKP and CRKP

To understand the adaptation process of KP in response to phage infection, we extracted RNA for RT-qPCR assay by co-incubating an HvKP type strain, ATCC 43816 (KP43), and a KL64-type CRKP clinical strain (KP47) with the corresponding phage for 1 h. The results of KP43 revealed that the transcript level of the *hns* gene was significantly upregulated by 2.23-fold ($P < 0.001$) under the stress of phage infection, whereas the transcript level of the *luxS* gene was reduced to 36% ($P < 0.001$) of that of the control group, and the transcript levels of the *lsr* operon gene cluster (*lsrK*, *lsrR*, *lsrA*, *lsrC*, *lsrD*, *lsrB*, *lsrF*, and *lsrG*) were downregulated, decreasing to 13%–64% ($P < 0.001$ –0.01) of those of the control group (Figure 1A). The results of KP47 revealed 2.56-fold ($P < 0.001$) upregulation of H-NS gene expression and significant downregulation of the *luxS* gene (35.3%, $P < 0.001$) and *lsr* operon gene transcript levels under phage stress (16.9%–30.6%, $P < 0.001$; Figure 1B). These results indicate that H-NS is negatively correlated with QS system activity under phage infection stress in both HvKP and CRKP.

Effects of gene knockout and H-NS overexpression on phage sensitivity in HvKP and CRKP

Knockout strains of *luxS* and key genes of the *lsr* operon genes (*lsrR*, *lsrK*, *lsrA*, *lsrB*, *lsrC*, and *lsrD*) were successfully constructed by the CRISPR/Cas9 system, and H-NS-overexpressing strains were constructed using the pACYC184 vector. The EOP experiments revealed that the $\Delta luxS$, $\Delta lsrK$, $\Delta lsrA$, $\Delta lsrB$, $\Delta lsrC$, and $\Delta lsrD$ strains of the KP43 knockout strains presented reduced susceptibility to phage, with the EOP being 60%–82% ($P < 0.01$ –0.05) of the wild type. In

contrast, the KP43 $\Delta lsrR$ strain presented an 18% ($P < 0.05$) increase in EOP, potentially due to its role as a transcriptional repressor of the *lsr* operon (Figure 2A). The KP43 H-NS overexpression strain presented increased resistance to the phage, and the EOP was reduced to 69% ($P < 0.01$) of the wild-type strain (Figure 2A). The plaque plate images revealed that the highest detectable dilution decreased from 10^{-8} (WT) to 10^{-6} (mutant), aligning with a 40% EOP drop (Figure 2C). Similarly, KP47 knockout of *luxS* or *lsr* operon genes reduced phage susceptibility (EOP = 68.7%–82.1%, $P < 0.001$ –0.01; Figure 2B), and overexpression of H-NS in the KP47 strain similarly reduced phage infection efficiency (EOP = 73%, $P < 0.001$; Figure 2B). The most significant plaque plate image showed the highest detectable dilutions ranging from 10^{-7} to 10^{-5} in KP47 $\Delta lsrK$ (Figure 2D). In addition, H-NS overexpression significantly suppressed the QS system, decreasing *luxS* transcript levels to 44% ($P < 0.01$), and *lsr* operon gene transcription was downregulated to 24.5%–28.4% ($P < 0.01$ –0.05) of that of the wild type in the HvKP KP43 strain (Figure 3A). Moreover, H-NS overexpression in the CRKP KP47 strain also repressed *luxS*/*lsr* operon gene transcription (32%–65%, $P < 0.01$ –0.05; Figure 3B). These findings demonstrate that H-NS suppresses QS by downregulating *luxS* and *lsr* operon gene expression.

Regulation of capsular polysaccharide synthesis by the H-NS-QS axis in HvKP and CRKP

The genes related to capsular polysaccharide (CPS) synthesis in the knockout and overexpression strains mentioned above were examined by qRT-PCR, and the HvKP strain KP43 $\Delta lsrR$ strain significantly upregulated *galF*, *wzi*, *wza*, *wzb*, *wcaJ*, *gnd*, *manB*, and *udg* gene expression by 2.5–9.87-fold ($P < 0.001$). In contrast, the expression of these genes was downregulated in the KP43 $\Delta luxS$, KP43 $\Delta lsrK$, KP43 $\Delta lsrB$, and KP43 $\Delta lsrC$ knockout strains, with the most significant suppression of *wza* (19%, $P < 0.01$), *wzb* (16%, $P < 0.01$), *wcaJ* (15%, $P < 0.01$), and *udg* (9%, $P < 0.001$) by KP43 $\Delta luxS$, and the most significant inhibition by KP43 $\Delta lsrK$ on *wzi* (70%, $P < 0.05$), KP43 $\Delta lsrB$ on *wzi* (57%, $P < 0.01$), *wzb* and *wcaJ* (63%, $P < 0.01$), and KP43 $\Delta lsrC$ on *wzi* and *wzb* (62%, $P < 0.01$) were also significantly different (Figure 4A). In addition, H-NS overexpression in the HvKP strain KP43 likewise downregulated the expression of CPS synthesis genes, in which the transcript levels of

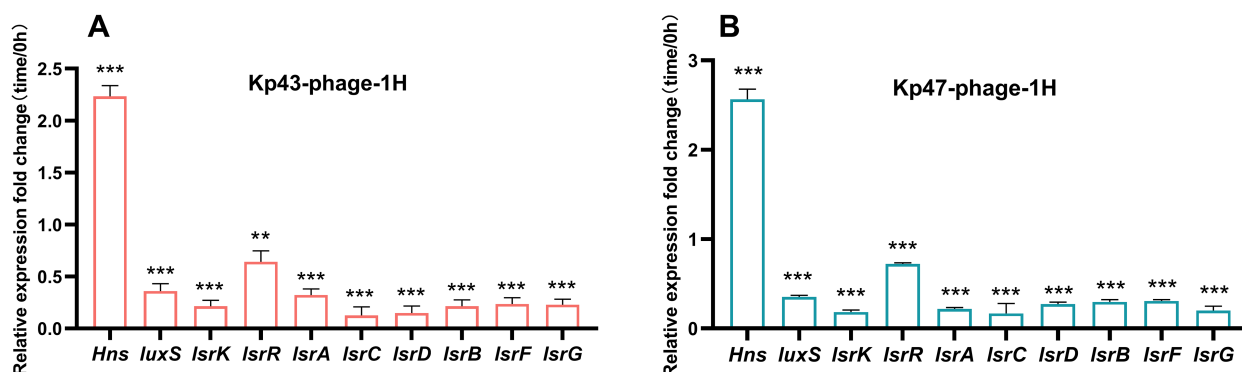


Figure 1. The gene transcription levels of *hns*, *luxS* and *lsr* operon genes (A,B) Under bacteriophage pressure for 1 h, the expressions in a hvKP type strain KP43 (A) and a CRKP type strain KP47 (B) via RT-qPCR. The resulting CT values were normalized using *gapA* as a reference gene. Data are presented as the mean $\pm$ SD from three independent experiments. *P* values were calculated using *t*-tests (** $P < 0.01$, *** $P < 0.001$).

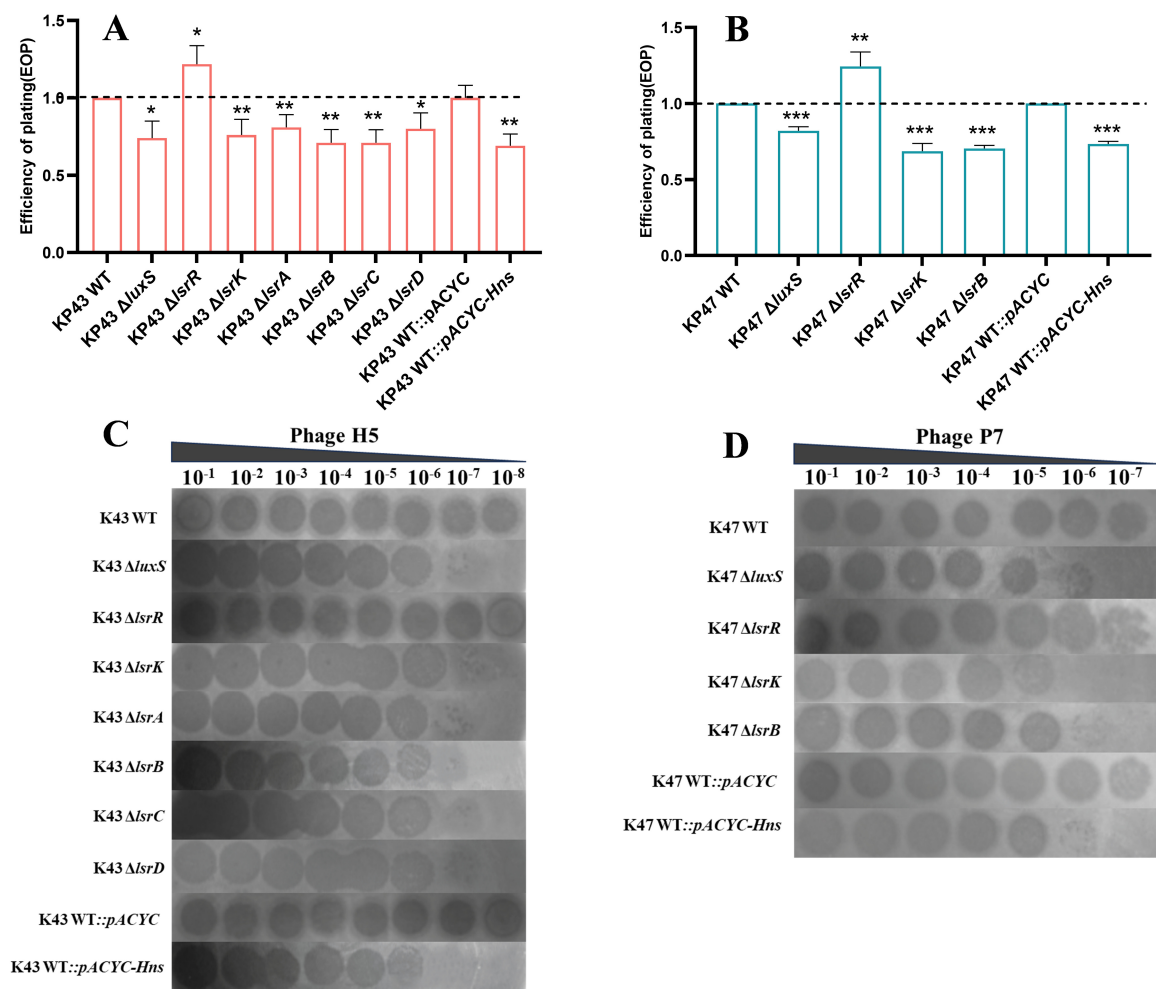


Figure 2. Sensitivity of H-NS overexpressing strains and *luxS*/*lsr* operon gene-deficient strains to bacteriophages (EOP) and the plaque plate images (A,C) hvKP type strain KP43. (B,D) CRKP type strain KP47. Data are presented as the mean $\pm$ SD from three independent experiments. Statistical significance of the differences between the WT control group and the mutant groups was analyzed by using *t*-tests (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

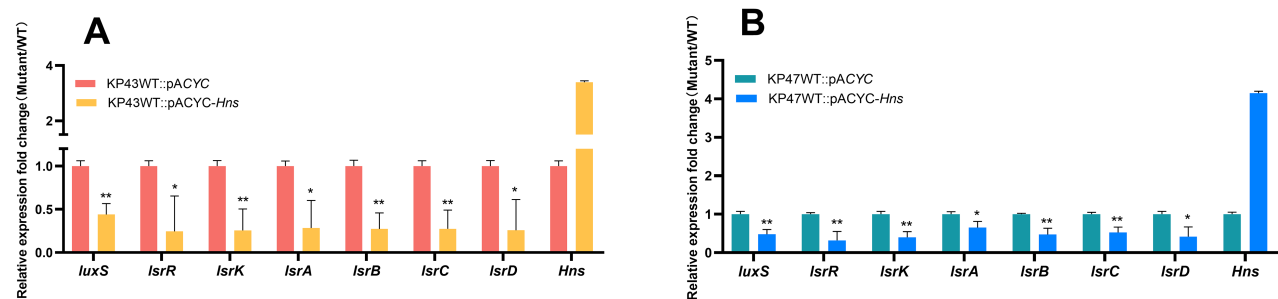


Figure 3. The gene transcription levels of *luxS* and *lsr* operon genes in H-NS overexpressing strains (A) hvKP type strain KP43 via RT-qPCR. (B) CRKP type strain KP47 via RT-qPCR. The resulting Ct values were normalized using *gapA* as a reference gene. Data are presented as the mean $\pm$ SD from three independent experiments. *P* values were calculated using *t*-tests (* $P < 0.05$, ** $P < 0.01$).

the *galF*, *wzi*, *wza*, *wzb*, *wcaJ*, *gnd*, *manB*, and *udg* genes were reduced to 15%–68% ($P < 0.01$ – 0.05) of those of the wild-type strain (Figure 5A). On the other hand, knockout of *luxS* or *lsr* operon genes in the CRKP KP47 strain also downregulated CPS

genes (e.g., *galF* to 45%–67% ($P < 0.01$ – 0.05), *wzi* to 38%–63% ($P < 0.001$ – 0.01) of those in the wild type (Figure 4B). Similarly, overexpression of H-NS in the KP47 strain downregulated the CPS gene transcript levels, whereas the *wza* gene transcript levels were

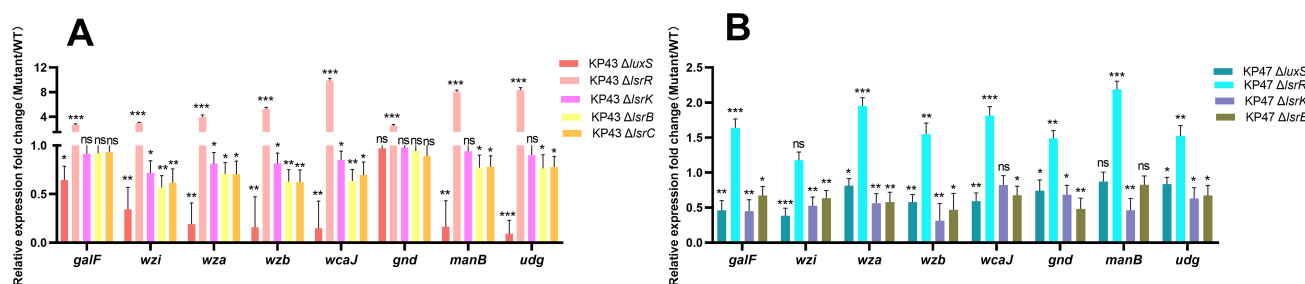


Figure 4. The gene transcription levels of CPS synthesis-related genes in *luxS/lsr* operon gene-deficient strains (A) hvKP type strain KP43 via RT-qPCR. (B) CRKP type strain KP47 via RT-qPCR. The resulting Ct values were normalized using *gapA* as a reference gene. Data are presented as the mean $\pm$ SD from three independent experiments. *P* values were calculated using *t*-tests (ns, no significance; **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

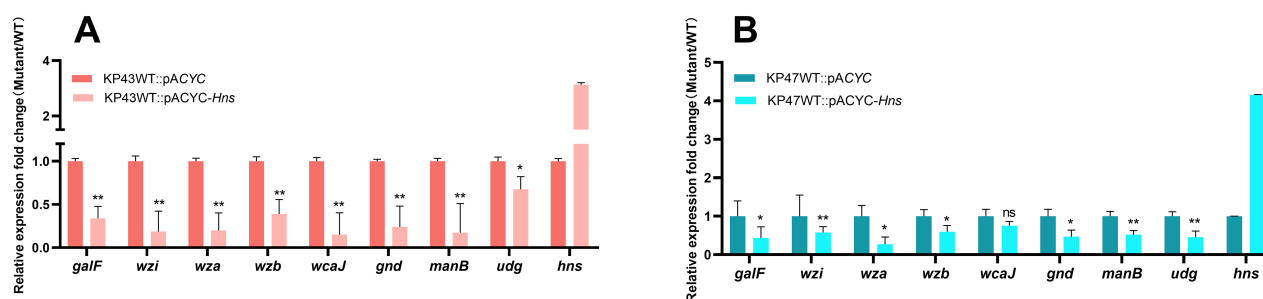


Figure 5. The gene transcription levels of CPS synthesis related genes in H-NS overexpressing strains (A) hvKP type strain KP43 via RT-qPCR. (B) CRKP type strain KP47 via RT-qPCR. The resulting Ct values were normalized using *gapA* as a reference gene. Data are presented as the mean $\pm$ SD from three independent experiments. *P* values were calculated using *t*-tests (ns, no significance; **P* < 0.05, ***P* < 0.01).

reduced to 27% ($P < 0.05$) of those in the wild type (Figure 5B). In summary, H-NS overexpression and the QS system synergistically regulate CPS biosynthesis. Although the gene transcription level was downregulated, the results of the uronic acid test revealed no significant differences (Figure 6A,C), indicating that H-NS-QS-mediated resistance operates independently of capsular modulation.

The uronic acid contents of the H-NS-overexpressing strain and *luxS/lsr* operon gene-deficient strains were determined, and the uronic acid content of the KP43 Δ *lsrR* knockout strains increased by 6.4% ($P = 0.09$), whereas those of the KP43 Δ *luxS*, KP43 Δ *lsrK*, KP43 Δ *lsrB*, and KP43 Δ *lsrC* knockout strains decreased by 4.31% ($P = 0.2$), 2.8% ($P = 0.26$), 3.07% ($P = 0.18$), and 8.02% ($P = 0.06$), respectively. The uronic acid content of H-NS overexpressing the KP43 strain was reduced by 4.89% ($P = 0.38$) (Figure 6A). Moreover, the CRKP KP47 Δ *lsrB* and H-NS overexpression strains presented slight reductions in the capsular uronic acid content of 7.53% ($P = 0.04$) and 2.35% ($P = 0.12$), respectively (Figure 6C).

The effect on the phage adsorption rate was also subsequently determined, and the phage adsorption efficiency of KP43 Δ *lsrR* increased by 1.5% ($P = 0.21 > 0.05$), whereas phage adsorption by the KP43 Δ *luxS*, KP43 Δ *lsrK*, KP43 Δ *lsrB*, and KP43 Δ *lsrC* knockout strains decreased by 1.9% ($P = 0.14$), 1.8% ($P = 0.07$), 2.3% ($P = 0.07$), and 1.5% ($P = 0.13$), respectively. The phage adsorption efficiency of H-NS overexpression in the KP43 strain was reduced by 1.38% ($P = 0.07$) (Figure 6B). Additionally, the phage adsorption efficiency of the CRKP KP47 Δ *lsrB* and H-NS overexpression strains decreased by 13% ($P = 0.03$) and 12.7% ($P = 0.04$), respectively

(Figure 6D). Collectively, the above results indicated that the H-NS overexpression strains or knockout of the *luxS/lsr* operon genes did not affect the phage adsorption efficiency of the strains, suggesting that the H-NS-QS-mediated resistance mechanism is independent of the capsular adsorption pathway.

Discussion

This work reports for the first time the molecular mechanism by which H-NS, a global transcriptional regulator of *K. pneumoniae*, enhances phage resistance by repressing the QS system. The H-NS expression level was upregulated under phage infection conditions and mediated the downregulation of the transcription of *luxS/lsr* operon genes, a cluster of genes that are important in the QS system. Both the knockdown of QS-related genes (*luxS* or *lsr* operon genes) and the *in vitro* overexpression of H-NS significantly increased resistance to phages (50%–80% decrease in EOP values). Although H-NS overexpression and QS deletion reduced the transcript levels of the CPS synthesis genes, they did not significantly alter the capsular uronic acid content or phage adsorption efficiency, suggesting that the mechanism of phage resistance enhancement is unaffected by the classical CPS receptor blockade pathway. The results of this study were validated in both HvKP and CRKP, which may constitute a conserved resistance mechanism across strains of *K. pneumoniae*.

H-NS is a conserved global transcriptional regulator in bacteria, and previous studies have focused mainly on the environmental stress response (osmotic stress, cold stress) and virulence gene silencing (virulence plasmid regulation), e.g., in *Escherichia coli*,

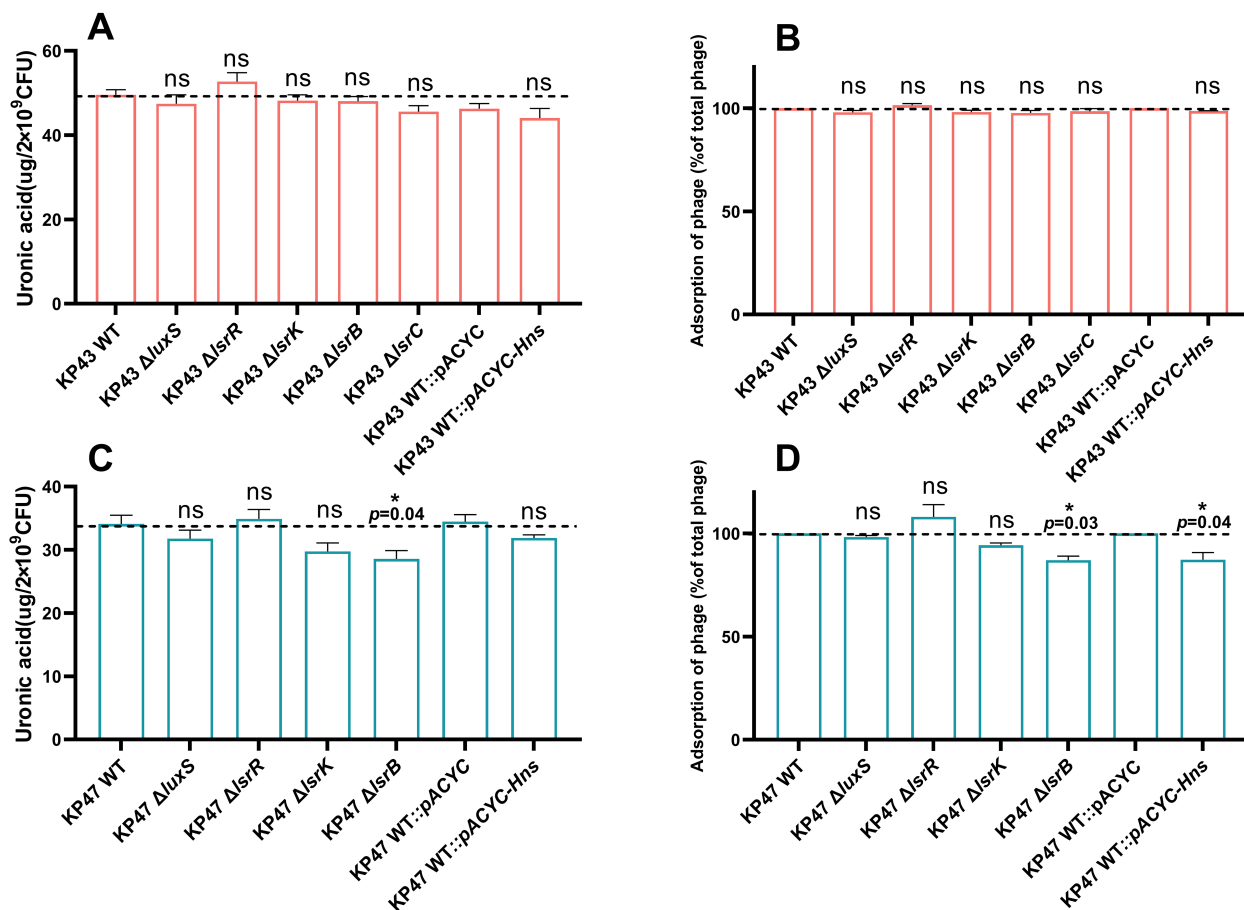


Figure 6. Capsular uronic acid content and phage adsorption efficiency in H-NS overexpression and *luxS/luxR*-deficient strains (A,B) hvKP strain KP43. (C,D) CRKP strain KP47. Data are presented as the mean $\pm$ SD from three independent experiments. *P* values were calculated using *t*-tests (ns, no significant; **P* < 0.05).

where the repression of exogenous gene (virulence island, etc.) expression by H-NS maintains bacterial genome stability [17]. In this study, H-NS was found to be functionally linked to the QS system in *K. pneumoniae*, revealing that H-NS enhances phage resistance by reducing the QS effect. The involvement of the QS system in phage-host studies has yielded a number of results; for example, it has been suggested that QS regulates the formation of bacterial biofilms, the presence of which affects phage dispersal [18], whereas others have suggested that the QS signaling molecule (AI-2) serves as an “alarm” that signals the initiation of phage infection [19]. However, in the present study, H-NS was found to function in association with the QS system in *K. pneumoniae*, revealing that H-NS enhances phage resistance by decreasing the QS effect. We noted a seemingly paradoxical observation that EOP was affected by *lsrR* deficiency and H-NS overexpression. This observation may not actually be paradoxical because there is a QS regulatory circuit and a dual role of H-NS. The *lsrR* gene encodes a transcription repressor of the *lsr* operon, which mediates the state of the *lsr* operon when AI-2 import and processing are under low-cell-density conditions. Consequently, $\Delta lsrR$ maintains *lsr* operon transcription disinhibition to increase QS activation. This hyper-QS state is associated with increased expression of CPS genes (Figure 4)

and increased phage susceptibility (Figure 2). In contrast, H-NS represses both *luxS* (AI-2 synthase) and the whole *lsr* operon (including *lsrR*), which induces two types of inhibition: signal depletion (*luxS* repression reduces AI-2 production) and signal reception blockade (*lsr* operon downregulation impairs AI-2 import). The net effect is full QS quenching, which overcomes any fortuitous *lsrR* repression effects. This also accounts for the H-NS overexpression phenotypic effect, mirroring that of a $\Delta luxS/luxR$ (EOP reduction), rather than a $\Delta lsrR$. This dominance architecture is designed for robust QS quenching despite the repressive activity of *lsrR* and illustrates that H-NS acts as a master regulator that overrules subordinate circuit elements. The organization of these types of layered control may be an evolutionary response to place importance on phage defense as opposed to density-dependent processes during the acute phase of infection. In addition, we also reported that the reduction in the QS system enhanced phage resistance, which is different from the reported decrease in phage susceptibility due to *LasR* deletion in *Pseudomonas aeruginosa* [9]. This difference in mechanism may be related to strain specificity. Another possibility is that the QS system of *K. pneumoniae* may indirectly affect phage replication efficiency by regulating metabolic pathways [20,21] (e.g., energy supply) rather than directly

participating in the phage-phage adsorption process.

Classically, it is generally believed that the phage resistance of *K. pneumoniae* is caused mainly by the synthesis or modification of bacterial capsule polysaccharides (CPSs), such as the formation of receptor shielding or adsorption blockade [22]. However, the results of the present study showed that the H-NS-QS axis did not significantly affect the amount of the capsular uronic acid content or the adsorption efficiency of the phage; although it suppressed CPS gene expression, this observation can be explained through two interrelated biological mechanisms: (1) assembly CPS biosynthesis involves multi-enzyme complexes. Key synthases (e.g., Wzi, Wza) exist in functional reserve pools. Our observed 30%–80% transcript reduction (Figures 4 and 5) may not deplete these enzymes below critical thresholds, and residual enzymes can increase catalytic efficiency to compensate for reduced expression; (2) As CPS is essential for virulence, *K. pneumoniae* may employ safeguard mechanisms (evident in the upregulation of the CPS genes of *ΔlsrR*; Figure 4A). This finding reinforces our finding that H-NS-QS mediates resistance through non-capsular mechanisms (e.g., intracellular defenses), not CPS modulation, as evidenced by unaltered phage adsorption and statistically insignificant changes in capsular uronic acid, which provides a new way of thinking for the study of the mechanism of phage-host interactions.

We hypothesize that H-NS can alleviate its transcriptional repression of the abortive infection (Abi) system by inhibiting the QS system. This mechanism is due mainly to the ability of the Abi protein to mediate host programmed cell death or inhibit phage DNA replication to prevent infection spread [23], e.g., the *Lactococcus lactis* AbiK protein affects phage genome integration through reverse transcriptase activity [24]. In this study, H-NS-overexpressing strains presented decreased phage sensitivity but no change in phage adsorption efficiency. This result is highly compatible with the mechanism of action of Abi proteins, and future screening for candidate Abi effectors (e.g., the toxin-antitoxin system) via proteomic screening is needed. Another viewpoint is that the QS system can modulate bacterial metabolism (e.g., carbon utilization and energy synthesis), and phage replication is highly dependent on bacterial metabolic resources [25]. H-NS-mediated QS inhibition may inhibit the raw materials required for phage genome replication by downregulating the expression of relevant metabolic enzymes (e.g., ATP synthase and nucleotide synthases), which may explain the positive correlation between H-NS-mediated QS inhibition and a positive decrease in EOP independent of capsular polysaccharide synthesis.

From a translational perspective, targeting the H-NS or QS system may provide new strategies to improve the clinical application of phage therapy, e.g., synthesizing small-molecule inhibitors of H-NS in conjunction with phages to overcome the barrier of phage resistance [26] or synthesizing engineered phages loaded with QS activation molecules [27,28] (e.g., AI-2 analogues) to achieve the reversal of H-NS-mediated immune escape. Moreover, the generalization of this mechanism to CRKP suggests that it can be used as a common target for the treatment of pan-resistant bacteria.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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