Anti-restriction protein $KlcA_{HS}$ enhances carbapenem resistance

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Received July 9, 2018; Accepted October 29, 2019

DOI: 10.3892/mmr.2019.10884

Abstract. The *$KlcA_{HS}$* gene was previously identified as coexisting with the *bla_{KPC-2}* gene in the backbone region of a series of *bla_{KPC-2}*-harboring plasmids. The purpose of the present study was to determine the association between the *$KlcA_{HS}$* and *bla_{KPC-2}* genes. *$KlcA_{HS}$* deletion and complementation experiments were used to evaluate the association between $KlcA_{HS}$ and carbapenem minimal inhibition concentrations (MICs). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis was used to detect changes in the expression levels of *bla_{KPC-2}* upon knocking out the *$KlcA_{HS}$* gene in a *bla_{KPC-2}*-harboring plasmid. The imipenem MIC of the transformants harboring Δ*$KlcA_{HS}$*pHS10842 was lower (16 μg/ml) than that of the transformants harboring wild-type pHS10842 (32 μg/ml), whereas the kanamycin MIC of the transformants harboring pET24a was lower (1,024 μg/ml) than that of the transformants harboring pET24a-$KlcA_{HS}$ (2,048 μg/ml). The imipenem MICs of the two NM1049 *Escherichia coli* strains carrying plasmids pHS092839 or Δ*$KlcA_{HS}$*pHS092839 exceeded 16 μg/ml, whereas the ertapenem MIC of the host strains harboring Δ*$KlcA_{HS}$*pHS092839 was 4 μg/ml compared with ≥8 μg/ml observed in the host strains carrying pHS092839. The RT-qPCR results demonstrated that the messenger RNA expression levels of *bla_{KPC-2}* in the transformants carrying Δ*$KlcA_{HS}$*pHS092839 were significantly downregulated (P=0.007) compared with those in the transformants carrying pHS092839. These findings revealed that $KlcA_{HS}$ elevated the MIC values of various antibiotics by upregulating the expression levels of *bla_{KPC-2}*. Therefore, *$KlcA_{HS}$* can confer increased resistance to carbapenems in host strains. The survival probability of clinical pathogens may be enhanced by the presence of the *$KlcA_{HS}$* gene in antibiotics used on a large scale.

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Key words: Klebsiella pneumoiae, carbapenem resistance, *$KlcA_{HS}$*, *bla_{KPC-2}* gene expression

Introduction

Klebsiella pneumoniae (K. pneumoniae) isolates with carbapenemases are often resistant to the majority of antibiotics available. Infections caused by *K. pneumoniae* with carbapenemases often increase mortality rates, which range between 23 and 75% (1), and are partially attributed to the lack of efficacious antimicrobial agents (2). Therefore, timely identification of the drug-resistance profile of *K. pneumoniae* isolates and selection of appropriate antimicrobial agents are essential for anti-infective therapy. Usually, antibiotic susceptibility testing is performed using minimal inhibition concentration (MIC) or agar diffusion methods in clinical laboratories. However, the determination of KPC-producing strains remains intractable, as it has been reported that 7-87% of KPC-producing *K. pneumoniae* clinical isolates are susceptible to imipenem or meropenem (3). Therefore, the identification of carbapenem-resistance phenotypes with automatic systems is not always reliable. The majority of influencing factors remain unclear at present, although the majority of mechanisms correlate with the production of carbapenemase (4,5). Numerous factors, including deletions of the upstream genetic environment (6), *bla_{KPC}* copy numbers (7), outer membrane and porins (8-10), biofilm formation (11), the AcrAB-TolC efflux system (12) and transcriptional start site (13), have been identified to affect carbapenem MICs to varying degrees.

In our previous study, a series of *bla_{KPC-2}*-harboring plasmids that possessed a backbone region, in which the *$KlcA_{HS}$* gene coexisted with the *bla_{KPC-2}* gene, were identified (14). Of note, the subscript HS is used in this context to distinguish the KlcA sequence investigated in the present study from other KlcA sequences. *$KlcA_{HS}$* deletion and complementation experiments were performed to investigate the association between carbapenem MICs and the *$KlcA_{HS}$* gene, revealing that the *$KlcA_{HS}$* gene can increase carbapenem MICs by upregulating the gene expression of *bla_{KPC-2}*.

Materials and methods

Strains, plasmids and antibiotics. The pHS10842 and pHS092839 plasmids were extracted from *Escherichia coli*

(*E. coli)* and *K. pneumoiae* clinical isolates, respectively, in the clinical laboratory at Huashan Hospital (Shanghai, China). The pIJ790 plasmid was used as a helper plasmid to generate λ-RED (gam, bet, exo) proteins to knock out the *KlcA*$_{HS}$ gene in pHS092839. The pET24a expression plasmid was used to generate the recombinant plasmid pET24a-KlcA. The *E. coli* BL21 (DE3) strain was selected as a host strain to produce Δ*KlcA*$_{HS}$pHS10842, and the *E. coli* BW25113 strain was selected as a host strain to produce Δ*KlcA*$_{HS}$pHS092839. The *E. coli* strain NM1049 was selected as a host strain to harbor wild-type and mutant pHS092839. This strain, which carries a type I A restriction and modification system (15), was provided by Professor Dryden (University of Edinburgh, UK).

Ampicillin (AMP, 100 μg/ml), chloramphenicol (Cm, 25 μg/ml), kanamycin (Kan, 25 μg/ml) and L-arabinose were all purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), and added to the culture medium as indicated. Mueller-Hinton (MH) agar was obtained from BD Biosciences (Franklin Lakes, NJ, USA). L-arabinose (final concentration, 0.2%) was added into Luria-Bertani (LB) medium (Beijing Lablead Biotech Co., Ltd.) to induce the expression of genes under the control of the pBAD promoter (16). All enzymes used in the present study were purchased from New England BioLabs, Inc.

Construction of the recombinant plasmid pET24a-KlcA$_{HS}$. The *KlcA*$_{HS}$ gene was amplified from the pHS092839 plasmid by polymerase chain reaction (PCR) using the primers P1/P2, in which the *Nde*I and *Eco*RI recognition sites were pre-located and underlined (Table I). PCR was carried out in a 25 μl reaction mixture, which contained 20 ng template DNA, 0.15 U Taq DNA polymerase, 2.0 mM dNTPs, 1X Taq DNA polymerase buffer (Takara Biotechnology Co., Ltd.) and 10 μM primer. DNA amplification was done under the following conditions: 95˚C for 5 min, followed by 30 cycles of 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 30 sec, with a final extension at 72˚C for 5 min. The PCR products obtained, together with the pET24a vector, were digested with *Nde*I and *Eco*RI, purified and ligated with T4 DNA ligase, prior to being transformed into $CaCl_2$-treated competent BL21 (DE3) cells (Sangon Biotech Co., Ltd.). The correct clones were screened using colony PCR. Briefly, a masterbatch was prepared for PCR amplification and placed in the PCR tube. A sterile toothpick for was sued for each colony to remove a small number of bacterial colonies directly from the plate to be tested. The toothpick was placed in the PCR tube with the masterbatch and the bacteria scraped into the PCR mixture. PCR was performed: 94˚C for 5 min, 30 cycles: 94˚C for 30 sec, 55˚C for 30 sec, 72˚C for 1 min and a final extension of 72˚C for 5 min. To ensure no errors in the sequence had been introduced during the PCR amplification step, the 426 bp PCR product of KlcA$_{HS}$ amplification was sequenced using primers P3/P4 (Table I). Each sequencing reaction was performed using 0.5 μl of BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and 1.6 μl of each primer (1 μM) in 10 μl final volume per reaction. Dye-labelled products were sequenced using an ABI 3500 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sequencing chromatograms were edited manually using Sequencher 4.7 software (Gene Codes Corporation).

Knockout of the KlcA$_{HS}$ gene in pHS10842. The *KlcA*$_{HS}$ gene in pHS10842 (accession no. KP125892) was knocked out using the modified protocol described by Zhang *et al* (17). The primers P5/P6 were designed based on the adjacent sequences of the two outer extremes of *KlcA*$_{HS}$ with Primer Premier 6.22 software (Premier Biosoft International, Palo Alto, CA, USA), and were used to amplify the entire sequence of pHS10842 with the exception of *KlcA*$_{HS}$ (Fig. 1). A High-Fidelity PCR kit was used to amplify the target DNA fragments (Takara Biotechnology Co., Ltd., Dalian, China). The PCR product was subjected to electrophoresis, and the target band was recovered and purified using the E.Z.N.A® Gel Extraction kit (Omega Bio-Tek, Inc., Norcross, GA, USA). The product had a 41-bp homology region at each terminus. The linear DNA was cyclized into ΔKlcA$_{HS}$pHS10842 using the HieffClone One Step Cloning kit (Shanghai Yeasen Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions, and transformed into BL21 (DE3) competent cells. The correct clones were screened by primer P7/P8 (Table I) and colony PCR protocol in the above steps. Inoculated into 3 ml fresh LB supplemented with AMP and incubated overnight at 37˚C. The mutant pHS10842 was obtained.

Knockout of the KlcA$_{HS}$ gene in pHS092839. The *KlcA*$_{HS}$ gene in pHS092839 (accession no. KF724506) was knocked out according to the protocol described by Derbise *et al* (18). Briefly, a four-step PCR procedure was used to generate a Kan cassette with homologous regions flanking the *KlcA*$_{HS}$ sequence in pHS092839. The specific four-step PCR program was: First, using the plasmid pHS092839 as a template, the 407- and 463-bp regions flanking the *KlcA*$_{HS}$ gene were amplified with the primer pairs P9/P10 and P13/P14 (Table I), respectively, with the following amplification cycles: 94˚C for 5 min, 30 cycles: 94˚C for 30 sec, 55˚C for 30 sec, 72˚C for 1 min and a final extension of 72˚C for 5 min. The 20-bp homologous region at each end of the Kan cassette was pre-designed at the 5' end of the primers P10 and P13. Second, the Kan cassette was amplified with the pET24a plasmid as a template using primers P11/P12 (Table I), the PCR reaction procedure for this step was the same as the previous step. The PCR products had a homologous region of 20 bp at their 5' end, with the upstream and downstream DNA fragments, respectively. In this manner, a 40-bp homologous region was formed at each extreme of the Kan cassette with the upstream and downstream DNA fragments, respectively. Third, three separate DNA fragments were integrated into the entire Kan cassette using a series of PCRs. The entire Kan cassette produced in the last step had two homologous regions flanking the *KlcA*$_{HS}$ sequence in pHS092839. Subsequently, the target fragments were recovered and purified with the E.Z.N.A.® Gel Extraction kit. In total, 200 ng purified linear PCR fragments were transformed into electro-competent *E. coli* BW25113 cells carrying pIJ790 (Cm^R) and the desired pHS092839 (AMP^R). The shocked cells were incubated in 0.95 ml LB at 37˚C for 1 h and plated onto LB plates ($3x10^6$/plate) containing AMP and Kan. The transformants were streaked onto LB plates containing Cm to confirm the loss of temperature-sensitive pIJ790 (Cm^R) during incubation at 37˚C. The transformants (Cm^S AMP^R Kan^R) were selected, inoculated into 3 ml LB supplemented with AMP and Kan, and incubated overnight. The mutant

Table I. Primers used in the present study.

No.	Primer name	Sequence (5'-3')	Product (bp)
P1	KlcAHS-F	ACGGTGTCATATGATGCAAACAGAACTTAA	
P2	KlcAHS-R	GCTAGAATTCCTAGTCTATTGCGGCCAAG	426
P3	T7 primer F	TAATACGACTCACTATAGG	
P4	T7 terminator R	GCTAGTTATTGCTCAGCGG	
P5	FP	GTCAATACCGGAGAACTCCGCAACAGAAAGCCCCGGTGATG	
P6	RP	CATCACCGGGGCTTTCTGTTGCGGAGTTCTCCGGTATTGAC	
P7	CS-F	CCGCTTGACACAATAGGC	
P8	CS-R	ATCGCCGTTCCTCACTAC	
P9	Upstream F	GAGAACTCCGATGATCCAC	
P10	Upstream R	CCTACATACCTCGCTCTGTTGCATCATCGGAGTTCTC	407
P11	Kan-F	GAGAACTCCGATGATGCAACAGAGCGAGGTATGTAGG	
P12	Kan-R	TGTTGCTAGTCTATTGCGGCAAATATGTATCCGCTCATG	463
P13	Downstream F	CATGAGCGGATACATATTTGCCGCAATAGACTAGCAACA	
P14	Downstream R	CCACTGAGAGGCTTACTAAG	1,184
P15	KPC-2F	TTGCTGGACTTTGTTGAG	
P16	KPC-2R	CTATCTCTTCGTTGCCATC	106
P17	16S-F	CGTATTCACCGTGGCATTCT	
P18	16S-R	GAGCAAGCGGACCTCATAA	110

The underlined nucleotide sequences shown in P1 and P2 denote the NdeI and EcoRI recognition sites. F, forward; R, reverse.

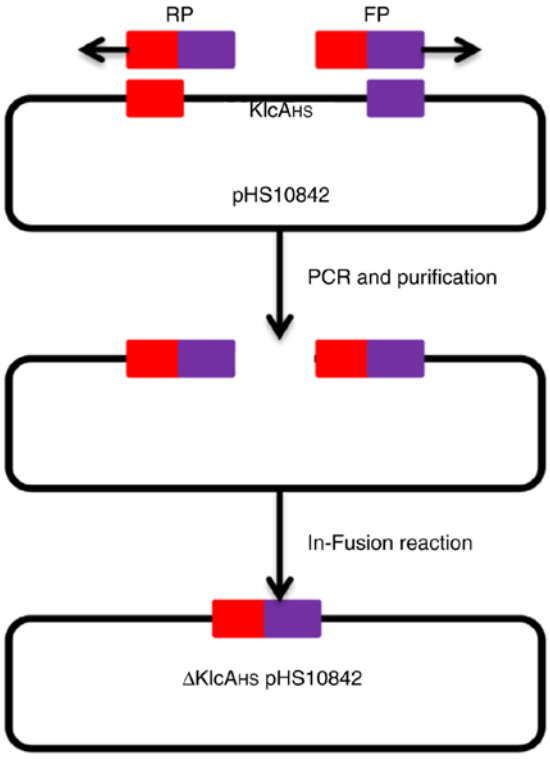

Figure 1. Schematic principle of constructing the mutant pHS10842. The PCR product was obtained with the primers FP/RP and purified. The two homologous regions located at the ends of the purified linear PCR product facilitated its cyclization into mutant pHS10842 *in vitro* using an appropriate kit.

Δ*KlcA*$_{HS}$pHS092839 was extracted and confirmed by DNA sequencing with primers P9/P14 (Fig. 2).

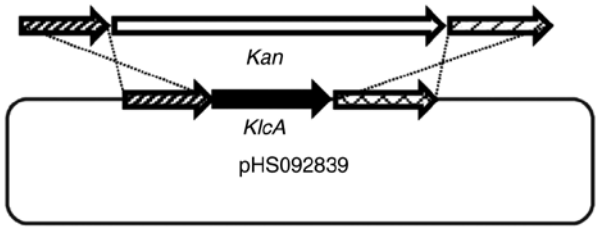

Figure 2. Strategies for replacing *KlcA*$_{HS}$ in pHS092839 with the λ-RED-mediated recombination method. First, a linear DNA fragment, consisting of a Kan cassette with flanking homologous regions of *KlcA*$_{HS}$ in pHS092839, was synthesized using a series of polymerase chain reactions. Second, electro-competent *E. coli* BW25113 cells harboring pHS092839 and pIJ790 were prepared. Third, the electro-competent cells were transformed with linear DNA fragments, and the *KlcA*$_{HS}$ sequence was then replaced with the Kan cassette using the λ-RED-mediated recombination method.

Antimicrobial susceptibility tests. The plasmids pHS10842, mutant Δ*KlcA*$_{HS}$pHS10842, pET24a and recombinant pET24a-KlcA$_{HS}$ were transformed into BL21 (DE3) competent cells. All the transformants were selected to detect the imipenem or Kan MICs using microdilution in cation-adjusted MH broth with inoculation of 5x10^5 CFU/ml according to the CLSI M07-A9 guidelines (19). Serial dilutions from this stock solution were added to freshly prepared MH (BD Biosciences) to achieve a final concentration in the plates of 1-128 μg/ml for imipenem and 32-2,048 μg/ml for Kan. The MIC values were assessed following incubation at 37˚C for 18 h. The experiments were performed in triplicate for each antibiotic and each strain. The lowest concentrations of imipenem or Kan showing no bacterial growth were considered as the MIC values. The assay was repeated in triplicate.

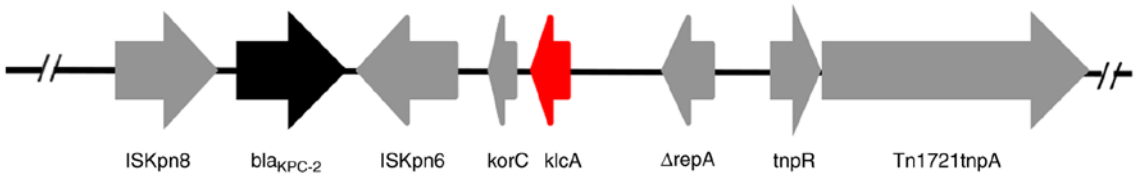

Figure 3. Schematic structure of the backbone region of *bla*$_{KPC-2}$-harboring plasmids. The *bla*$_{KPC-2}$ gene (black) and the *KlcA*$_{HS}$ gene (red) coexist in the same plasmid. The genes and their corresponding transcriptional directions are indicated by horizontal arrows.

In addition, another *bla*$_{KPC-2}$-harboring plasmid, pHS092839, and Δ*KlcA*$_{HS}$pHS092839 were transformed into the NM1049 *E. coli* strain. The imipenem and meropenem MICs of the two transformants were detected using the fully automated microbial identification system VITEK® 2 Compact according to the manufacturer's protocol. The assay was repeated in triplicate.

Determination of the expression of bla$_{KPC-2}$ via reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNAs were extracted from the NM1049 *E. coli* strains harboring the pHS092839 or Δ*KlcA*$_{HS}$pHS092839 plasmids using the E.Z.N.A.® Total RNA Kit I (Omega Bio-Tek, Inc.) and treated with RNase-free DNase (Omega Bio-Tek, Inc.). A total of 0.5 μg of each total RNA was reverse transcribed into complementary DNA with the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. RT-qPCR analysis was then performed on an ABI 7500 Fast Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with SYBR Green RT-PCR kit (Shanghai Yeasen Biotech Co., Ltd.) in a total volume of 20 μl, containing 10 ng RNA and 10 pmol each primer [P15/P16 for *bla*$_{KPC-2}$, P17/P18 for 16S ribosomal RNA (rRNA); Table I]. The following cycling conditions were used for all amplifications: 2 min at 50˚C, 10 min at 95˚C, and 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec, followed by a dissociation step at 95˚C for 15 sec, 60˚C for 15 sec and 95˚C for 15 sec. The baselines were adjusted manually to the same level for the two groups with SDS software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The messenger RNA (mRNA) of the housekeeping 16S rRNA gene was selected as an endogenous reference RNA to adjust the variation of RNA content and amplification efficiency for relative quantification. Relative quantities of *bla*$_{KPC-2}$ mRNA were determined with the comparative quantification cycle (Cq) method. The Cq value indicates the number of PCR cycles at which the fluorescence intensity begins to increase exponentially. The equation $2^{-\Delta\Delta Cq}$ (20) was used to calculate the differences in *bla*$_{KPC-2}$ mRNA expression levels between *E. coli* NM1049 strains harboring pHS092839 and Δ*KlcA*$_{HS}$pHS092839, where $\Delta\Delta Cq=\Delta Cq_{-blaKPC-2}-\Delta Cq_{-16S\ rRNA}$. All experiments were performed in triplicate from three RNA preparations. The expression levels are presented as the mean of three independent experiments. Negative controls were performed using the purified RNA without reverse transcription as the templates. The assay was repeated in triplicate. Data are presented as the mean ± standard deviation.

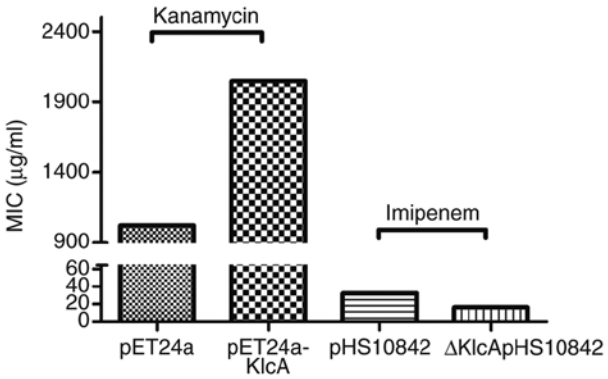

Figure 4. Kanamycin or imipenem MICs increased/decreased by 2-fold in the presence/absence of the *KlcA*$_{HS}$ gene in the pET24a-KlcA$_{HS}$ or pHS10842 plasmids harbored by BL21 (DE3) host strains. MIC, minimal inhibition concentration.

Statistical analysis. Data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The assay was repeated in triplicate. The variables are described as the mean ± standard deviation. The comparison tests were performed using non-parametric methods with the Mann-Whitney U test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Analysis of the genetic structure of bla$_{KPC-2}$-harboring plasmids. The backbone region of the extracted pHS10842 and pHS092839 plasmids extracted was confirmed, in which the *KlcA*$_{HS}$ and *bla*$_{KPC-2}$ genes coexisted (Fig. 3).

Examination of the association between KlcA$_{HS}$ and antibiotic MICs. The imipenem MIC of the transformants harboring Δ*KlcA*$_{HS}$pHS10842 was lower (16 μg/ml) than that of the wild-type pHS10842 (32 μg/ml), and the Kan MIC of transformants harboring pET24a was lower (1,024 μg/ml) than that of pET24a-KlcA$_{HS}$ (2,048 μg/ml) (Fig. 4). In addition, the imipenem MICs of the two NM1049 *E. coli* strains carrying plasmids pHS092839 or Δ*KlcA*$_{HS}$pHS092839 exceeded 16 μg/ml, and the ertapenem MIC of host strains harboring Δ*KlcA*$_{HS}$pHS092839 was 4 μg/ml, compared with ≥8 μg/ml for the host strains carrying pHS092839 (Fig. 5A). Taken together, it was confirmed that *KlcA*$_{HS}$ contributed to the decreased susceptibility to the corresponding antibiotics. However, the MIC alteration was not large, with only a 2-fold decrease in susceptibility to carbapenems in the presence of the *KlcA*$_{HS}$ gene.

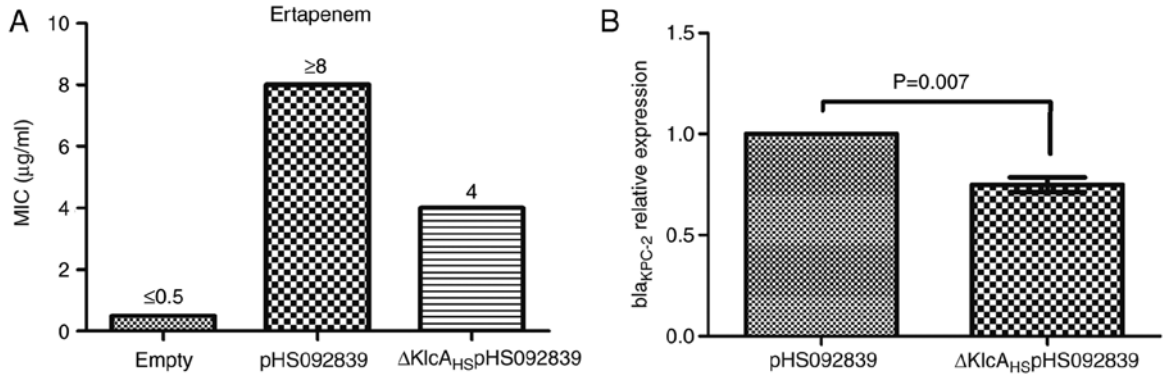

Figure 5. Ertapenem MICs and bla_{KPC-2} mRNA expression levels in the host strain *E. coli* NM1049 harboring different plasmids. (A) The ertapenem MIC of host strains harboring Δ*KlcA*$_{HS}$pHS092839 was 4 μg/ml, whereas the ertapenem MIC of the host strains carrying pHS092839 was ≥8 μg/ml, and that of the host strains alone was ≤0.5 μg/ml. (B) mRNA expression level of bla_{KPC-2} in the mutant pHS092839 was significantly downregulated compared with that in the wild-type pHS092839. MIC, minimal inhibition concentration.

Expression of bla_{KPC-2} is affected by the $KlcA_{HS}$ gene. The results of RT-qPCR analysis revealed that the mRNA expression levels of bla_{KPC-2} in the transformants carrying Δ*KlcA*$_{HS}$pHS092839 were significantly downregulated (P=0.007) compared with those observed in the transformants carrying pHS092839 (Fig. 5B).

Discussion

It was reported that 27 KPC-producing *K. pneumoniae* isolates exhibit a range of carbapenem MICs, with 11 exhibiting low-level carbapenem resistance to imipenem or meropenem (MIC <4 μg/ml), two exhibiting an intermediate level (MIC=8 μg/ml) and 14 a high level (MIC >16 μg/ml) (7). Other studies have reported that the susceptibility rates to ertapenem, imipenem and meropenem for KPC-producing *K. pneumoniae* were 0-6, 26-29 and 16-52%, respectively (4,5,21). The growth of KPC-producing isolates may be inhibited at carbapenem MIC values lower than the recommended breakpoint concentrations. In our previous study, there was a fraction of isolates showing a lower MIC than the breakpoint concentration in KPC-producing *K. pneumoniae*, namely 4.5% for imipenem in contrast to 3.6% for meropenem (unpublished data), which indicated that plasmid encoding carbapenemases were not the unique factor influencing MICs. Therefore, it is more difficult to detect KPC-producing *K. pneumoniae* clinical isolates with low carbapenem MICs in clinical laboratories. Proper identification of KPC-producing isolates is critical to the therapeutic schedule, as carbapenems are prohibited from being used to control the infections due to KPC-producing pathogens. Although the carbapenem-resistant phenotype is caused by combined mechanisms associated with extended spectrum β-lactamase (ESBL) and outer-membrane permeability defects, the clinical isolates are not KPC-producing pathogens, the use of carbapenems remains controversial (3). Therefore, the CLSI carbapenem breakpoint concentrations were revised in 2010. At the same time, EUCAST (http://www.eucast.org/) recommended a lower cut-off point to identify potential carbapenemase-producing Enterobacteriaceae (CPE). Numerous factors are able to alter carbapenems MIC values. Carbapenems MICs were increased when carbapenems are degraded in clinical strains producing additional ESBLs or AmpC β-lactamases (22). In addition, alterations or losses of porins in *K. pneumoniae* (22,23) or *E. coli* (24) and the enhanced activity of efflux pumps (25) have been shown to reduce susceptibility to carbapenems. In the present study, KlcA$_{HS}$ was shown to elevate the MIC of antibiotics not only for imipenem, but also for any other antibiotics. The results of this study showed that KlcA$_{HS}$ upregulated the expression of mRNA of bla_{KPC-2}. The variation in carbapenems MIC values in CPE may involve diverse combinations of resistance mechanisms in each clinical isolate (7,12). An understanding of the underlying mechanisms associated with the alteration of carbapenem MICs will facilitate anti-infective therapy, particularly for patients infected with KPC-producing pathogens.

In the present study, it was demonstrated that the $KlcA_{HS}$ gene, in addition to the bla_{KPC-2} gene, was located at the backbone region of several plasmids in carbapenem-resistant *K. pneumonia* clinical isolates. The $KlcA_{HS}$ gene upregulated the expression of bla_{KPC-2} and reduced the susceptibility to antibiotics such as carbapenems. The present study also had limitations; for example, the *E. coli* BL21 (DE3) strain was selected as a host strain for ΔKlcA$_{HS}$pHS10842 and pHS10842, whereas the host *E. coli* strain NM1049 was selected as ΔKlcA$_{HS}$pHS092839 and pHS092839; although host background is identical, the MIC of each is comparable and it is better to transform the two plasmids and their derivants into the same strains. In addition, when studying the function of a gene, knockout and complementation experiments are classical experimental methods, when the mutation point is located on the bacterial chromosome. The complementation experiment can be completed by introducing an expression plasmid carrying the target gene into the host cell, but the gene in the present study was located on the plasmid carried by the host cell, which limited the use of the complementation experiment. Additionally, there remains limited understanding of the exact function of the anti-restriction KlcA$_{HS}$; in the present study, it was found that KlcA$_{HS}$ upregulated the transcription of bla_{KPC-2} gene and improving this knowledge is aimed in future studies.

The present findings showed that $KlcA_{HS}$ conferred increased resistance to carbapenems in the host strains. The survival probability of clinical pathogens may

be enhanced by the presence of the $KlcA_{HS}$ gene in antibiotics used on a large scale.

Acknowledgements

Not applicable.

Funding

This study was supported by grants from the National Natural Science Foundation of China (grant nos. NSFC 81571365 and 81372141), the Natural Science Foundation of Jiangsu Province (grant no. BK20191210), the Jiangsu Commission of Health (grant no. H2018073), the Lianyungang Science and Technology Bureau Project (grant no. SH1526) and the Bengbu Medical College Research Project (grant no. BYKY17182).

Availability of data and materials

The datasets used in the present study are available from the corresponding author on reasonable request.

Authors' contributions

HS and GHH conceived and designed the experiments. WL, CZ, YW, WJZ, WCZ, YPS and YZ performed the experiments. WL, CZ, LY, JH, YPS, LY and XL analyzed the data. WL and CZ interpreted the data and WL wrote the first draft of the manuscript. CZ, YW and WJZ developed the structure and arguments for the paper. WCZ and YPS made critical revisions. All authors have reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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