RESEARCH ARTICLE

Gene Expression of kfu gene of *Klebsiella Pneumonia* Isolated from Clinical and Environmental Samples

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ABSTRACT

The rise of multidrug-resistant *Klebsiella* species is a serious worldwide problem. *Klebsiella pneumoniae* has been identified by the World Health Organization as a worldwide priority pathogen in desperate need of next-generation medicines. In comparison to other gram-negative infections, *K. pneumoniae* accumulates a larger variety and frequency of antimicrobial-resistant genes. for molecular detection the *kfu* resistance gene. The genomic deoxyribonucleic acid (DNA) of *K. pneumoniae* isolates was extracted using a wizard genomic DNA purification kit, The genomic DNA collected was examined. using 1% agarose gel electrophoresis, and the concentration and quality of the isolated genomic DNA were determined using a Nanodrop spectrophotometer device. To detect *K. pneumoniae* isolates using molecular methods, genomic DNA extraction of these isolates analyzed (2.5%) *kfu.*

Susceptibility testing was performed on all *K. pneumoniae* (28) isolates against a panel of eighteen different antibiotics using the VITEK 2 Compact system. The findings indicated that the isolates were resistant to tetracycline (100%).

To study the gene expression of *kfu* in *K. pneumoniae*, total RNA was isolated from *K. pneumoniae* using the TRIzol purification kit and converted to cDNA before being submitted for further amplification to investigate the gene expression of *kfu* gene using the RT-qPCR methodology before and after treatment with Chalcone.

The findings described the gene expression of *kfu* in connection to housekeeping genes; the gene demonstrated changes in Ct and Folding values after being treated with various concentrations of Chalcone (0.125 g/mL). This result implies that the bacteria's growth rate was increased to boost the gene expression of *kfu* after treatment with Chalcone.

Keywords: Gene Expression, *Klebsiella Pneumonia,* Next-generation medicines.

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INTRODUCTION

Because of the emergence of antibiotic-resistant strains, Bacteria such *as Staphylococcus aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species are examples of pathogens that exist (ESKAPE) share the primary risk presented by *K. pneumoniae.*[1]

Antimicrobial resistance *K. pneumoniae* especially to antibiotic carbapenems has made treating pneumonia infections more complicated. Carbapenem tolerance may be achieved by several mechanisms, including carbapenemase production, alterations in outer membrane permeability caused by porin deficiency or absence, and overexpression of the efflux system.[2]

Bacteria that are resistant to drugs produce antibiotic-resistant illnesses.

Many disease-fighting drugs lose their effectiveness as bacteria and microbes evolve resistance. Antibiotic-resistant diseases kill over 700,000 people per year across the world. By 2050 up to 10000000 people a year could die of anti-microbial resistance.[3]

It was determined that molecular antibiotic resistance genes might aid in the prediction of antibiotic resistance in bacteria.[4]

The kfu gene, which codes for an iron absorption mechanism, is thought to be pathogenic. Because the *kfu* gene has been linked to virulent tissue infections, capsule development, and purulent tissue infections hypermucoviscosity, it is assumed to be a key to the iron gene absorption originating in the host cell.[5]

MATERIALS AND METHODS

Extraction of genomic DNA from *K. pneumoniae*

Total DNA was extracted from all 43 isolates detected as *K. pneumoniae*, using a Total DNA Extraction Kit (ABIOpure TM Total DNA) DNA concentration and purity were measured by QuantusTM Fluorometer using QuantiFluor® dsDNA System which is a specific system for DNA quantitation. All the

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isolates had DNA concentrations between (80–120 ng/µL) with high purity (1.82). High quality of DNA without fragmentation was shown with agarose gel electrophoresis (1).

The amount and quality DNA recovered are adequate for PCR amplification. Higher volumes about DNA templates enhance the possibility of producing non-specific PCR results. Lower volumes of templates diminish amplification accuracy. A pure DNA molecule is determined by the extinction coefficients of nucleic acids at 260 and 280 nm. preparation has an anticipated of 1.8.[6] The outcomes of the current study showed the important use of commercial kits as a rapid extraction method for genomic DNA compared to conventional DNA extraction methods such as alkaline lysis, boiling and salting methods, phenol-chloroform method, which eliminated the need of these inconvenient and tedious methods.[7,8]

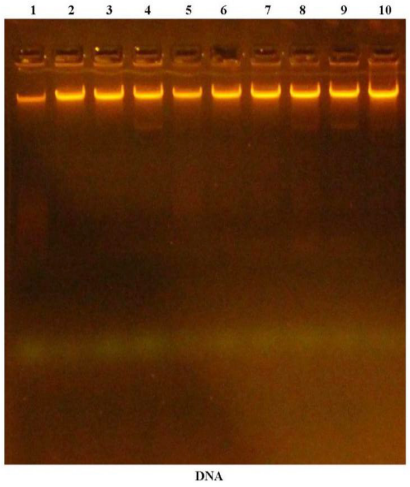

Figure 1: 1 percent electrophoresis on agarose gels of genomic DNA from isolates of K. pneumoniae at 7volt/cm for 1 hour. Extracted genomic DNA (lanes 1–10)

Primers

Table 1: The Sequences of primers used for conventional PCR and Real-time PCR

No.	*Seq.*	*Annealing temp. (°C)*	*Product Size (bp)*
(kfu)F	5`-GAAGTGACGCTGTTTCTGGC-3`	58	797
(kfu0 R	5`-TTTCGTGTGGCCAGTGACTC-3`		

Table 2: PCR reaction conditions for detection of kfu genes based on experimentation: after many attempts, the polymerase chain reaction was optimized.

The steps of the loop	*Temperatures*	*Time/ m:s*	*Cycle number*
Initial Denaturation	(95)ºC	05 : 00	(1)
Denaturation	(95)ºC	00 : 30	
Annealing	(55 OR 58)ºC	00 : 30	(30)
Extension	(72) ºC	01 : 00	
Final extension	(72)ºC	07 : 00	(1)
Storage	(10) ºC	Hold	

Cycling Conditions of the *kfu* Gene

Detection of *kfu* gene used to identify *K. Pneumoniae.* The following table explains the software used for polymerase chain reaction (PCR) amplification after many experiments.

RESULTS AND DISCUSSION

PCR Detection and Prevalence of the Virulence gene (*kfu* gene) in *K. pneumoniae*

Virulence factor *kfu* gene was The ferric iron uptake system gene *kfu* is essential for the metabolism of iron for the host to maintain its development.[9,10]

The current study *kfu* genes were PCR-amplified using a primer pair Iron metabolism is required for the host to sustain its development, and the ferric iron uptake system gene *kfu* is required for this. *kfu* gene. The amplified DNA with the kfu primers produced a PCR product with a molecular size of roughly 797 bp, as indicated in the Figures 2 and 3.

43 *K. pneumoniae* isolates were subjected to amplification using these primers, 7 isolates (16.28%) were positive for *kfu* genes. The presence of these genes may indicate the isolates' pathogenicity potential.[11]

Amplified the product size of 797 bp of the sequence of the *kfu* gene. However, detected the prevalence of virulence-associated gene *kfu* gene at the rate of 35%.

The discovery of these genes at the molecular level opens the door to early identification of infection in vulnerable individuals. Given the results, further research is required to clarify the function of additional host and pathogen variables that may aid in disease development at the physiological and molecular levels.[11]

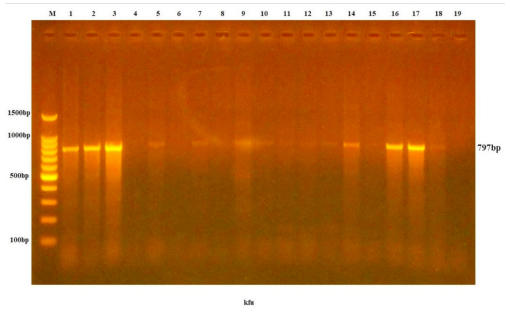

Figure 2: The enhancement of *kfu* gene of *K.pneumoniae* sample were fractionated on 1.5% Electrophoresis of agarose gels dyed with Eth. Br. M: 100bp ladder marker. Lanes 1-19 resemble 797bp PCR products.

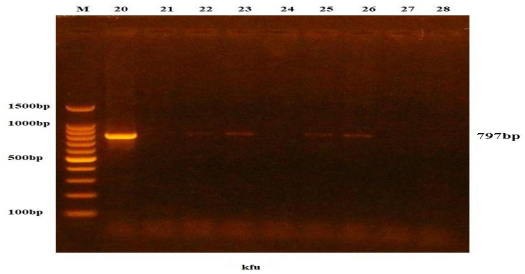

Figure 3: The enhancement of *kfu* gene of *K. pneumoniae* sample were fractionated on 1.5% Electrophoresis of agarose gels dyed with Eth. Br. M: 100bp ladder marker. Lanes 1-19 resemble 797bp PCR products.

The prevalence of *kfu* genes of *K. pneumoniae* isolates. There was a variation in the prevalence of *kfu*. genes between isolates. Results showed the distribution of *kfu* gene was 21 (91%), 8 (72.7%), respectively.

The incidence Thus the distribution of *kfu* as a virulence factor associated with diverse capsule *K* serotypes in *K. pneumoniae* may represent the organisms responsible for the infection. Seroepidemiology.[12]

Antibiotic Susceptibility Test

All the 28 *K. pneumoniae* employing the automated VITEK 2 compact system, were evaluated for antibiotic susceptibility. After growing on MacCkonkey agar plates, each isolate received a McFarland 0.5 standard solution in 0.45% sodium chloride. The VITEK equipment was filled with a liquid suspension of all isolates and left overnight to produce the results. 18 types of antibiotic were depended as the following included within the VITEK 2 Compact system gram negative susceptibility card.

According to the findings, 28 of the 28 (100%) clinical isolates were Ticarcillin resistant, and all of the Ticarcillin-resistant isolates were also piperacillin resistant. The *kfu* gene was included in both Ticarcillin and piperacillin resistant strains, indicating that they both have the AcrAB efflux mechanism.[13]

Treatment-resistant was seen to be important as because many of the strains displayed resistance to at least two or more treatment groups of antibiotics. Any of the isolates are highly resistant to both Ticillin and penicillin. this conclusion is in concordance with a previously reported finding.[14]

Immune to other antibiotics (also called lactic acid hydrolases) such as resistance to (technically known as an anti-lactamases, or suramin, which are produced by microorganisms known as Escherichoiromonadaceae, hydrolyses antibiotics, such as cefzideonylams, and thus renders them ineffective.[15]

A total of 8% of all isolates of *K. pneumoniae* are shown to be immune to meropenem. The findings found that of the eight percent of urine samples that were examined, there was no increase in *K. pneumoniae's* resistance (phagocytosis). It is seen in a research done on Iraqi medical professionals done in Najaf; a distinction in the resistance trends maybe because of its patients, which is caused by the analysis being done in various hospitals, or by different geographical locations; this study only includes hospitals in Najaf.

The present study found an improvement in meropenem, a carbapenem antibiotic, was associated with increased antibiotic tolerance in *K. pneumoniae* treatments. will lead to guess that this recent exposure to broad-spect antibiotics, along with being a contributing factor, may raise the present percentage of antibiotic-resistant bacteria.[16]

Jarallah and Abbas' results confirm (that) 17.6% of *K. pneumonia* isolates are immune to meropenem (antibiotic). The study by other researchers[17] found that 18.7% of the *K. pneumoniae* clinical isolates were immune to meropenem in Egypt. But another study done in Taiwan found that the

Table 3: Antimicrobial susceptibility percentage rates of (28) *K. pneumoniae* isolates against 18 antimicrobial agents

Antibiotic	*Resistant*	*Intermediate*	*Sensitive*
Ticarcillin	28 (100.00%)	0 (0.00%)	0 (0.00%)
Ticarcillin/Clavulanic acid	18 (64.28%)	2 (7.14%)	8 (28.57%)
Piperacillin	28 (100.00%)	0 (0.00%)	0 (0.00%)
Piperacillin/Tazobactam	4 (14.28%)	0 (0.00%)	24 (85.71%)
Ceftazidime	27 (96.42%)	0 (0.00%)	1 (3.57%)
Cefepime	27 (96.42%)	0 (0.00%)	1 (3.57%)
Aztreonam	27 (96.42%)	0 (0.00%)	1 (3.57%)
Imipenem	4 (14.28%)	0 (0.00%)	24 (85.71%)
Meropenem	4 (14.28%)	0 (0.00%)	24 (85.71%)
Amikacin	3 (10.71%)	2 (7.14%)	23 (82.14%)
Gentamicin	13 (46.42%)	0 (0.00%)	15 (53.57%)
Tobramycin	14 (50%)	0 (0.00%)	14 (50%)
Ciprofloxacin	2 (7.14%)	0 (0.00%)	26 (92.85%)
Pefloxacin	-	-	-
Minocycline	5 (17.85%)	4 (14.28%)	19 (67.85%)
Colistin	-	-	-
Rifampicin	-	-	-
Trimethoprim/Sulfamethoxaz ole	17 (60.71%)	0 (0.00%)	11 (39.28%)
Chi-Square (χ^2)	17.449 **	4.815 *	15.603

* ($p \leq 0.05$)-Significant, ** ($p \leq 0.01$)-Highly significant.

percentage of drug-resistant *K. pneumoniae* in patients was approximately 16 percent.[18]

Gene Expression

Genes Expression of kfu Gene of K. pneumoniae by Quantitative Real-time PCR

The present study used quantitative reverse transcription-polymerase chain reaction (RT-PCR) to assess the mRNA expression of *kfu* and compare it to that of Chalcone after bacteria were incubated with inhibitors at a concentration of 100 g/mL for 24 hours. The fold change in gene expression was calculated using relative quantification. This is dependent on the normalization of Ct values when calculating the Ct, which is the difference between the mean Ct values of each replicate of *kfu* cDNA amplification and the housekeeping *infB*.

Comparative Ct method was also referred to as the fold change = $2^{-\Delta\Delta\ Ct}$ approach of other researches.[19] which was a golden equation technique for comparing gene expression in various samples based on relative gene expression data. Each sample was compared to an internal control gene in both treated and untreated individuals to establish that the observed differences were due to changes in target gene expression rather than mRNA quality or quantity.

In the control, the fold of gene expression for *kfu* cDNA amplification was (1.00). The expression values (6.11, 47.93, 0.005, 195.41, 7.29, 455.59 and 0.85) had the highest values compared to the control, indicating that they function as an activator. The results are provided in Table 4, Figures 4 and 5.

The melting curve displayed in figure created after the PCR experiment revealed all amplicons of the *kfu* gene. This finding suggested that during the processes shown in Figures 4 and 5, no primer–dimmers were created (Figures 6 and 7).

Antibiotic resistance has developed into a serious issue in public health. In the medical and agricultural fields, antibiotic resistance is caused by horizontal mobile genetic transfer factors that carry multiple resistance genes.[20]

Table 4: Fold of gene expression of *kfu* gene depending on ΔΔCt method

Sample	*infB*	*KFU*	*dct*	*ddct*	*Folding*	
1	26.71	24.02	-2.69	-2.61	6.11	
2	29.20	24.15	-5.05	-5.58	47.93	
3	21.67	23.46	1.79	7.75	0.005	
14	31.40	24.16	-7.24	-7.61	195.41	101.88 ± 17.35 a
16	29.73	24.40	-5.33	-2.87	7.29	
17	33.18	23.87	-9.31	-8.83	455.59	
20	27.12	23.51	-3.61	0.23	0.85	
C1	23.94	23.86	-0.08	0.00	1.0	
C2	23.35	23.88	0.54	0.00	1.0	
C3	23.57	17.61	-5.96	0.00	1.0	
C14	23.59	23.96	0.37	0.00	1.0	1.00 ± 0.00
C16	26.31	23.85	-2.47	0.00	1.0	
C17	24.56	24.08	-0.48	0.00	1.0	
C20	27.86	24.03	-3.84	0.00	1.0	
T-test (p-value)	-	-	-	-	-	2.071 ** (0.0001)

** ($p \leq 0.01$)-Highly significant.

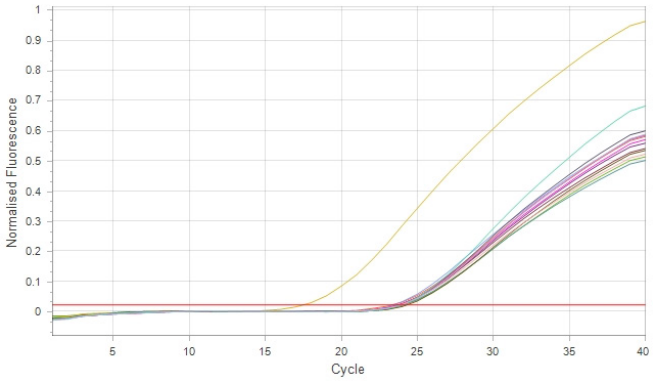

Figure 4: The amplification plots of *kfu* gene after and before the treatment

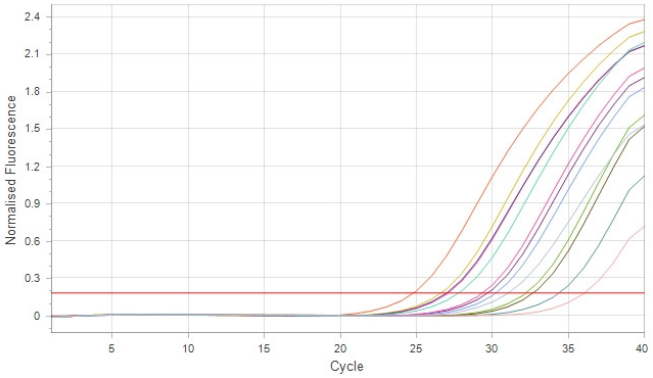

Figure 5: The amplification plots of *infb* gene after and before the treatment.

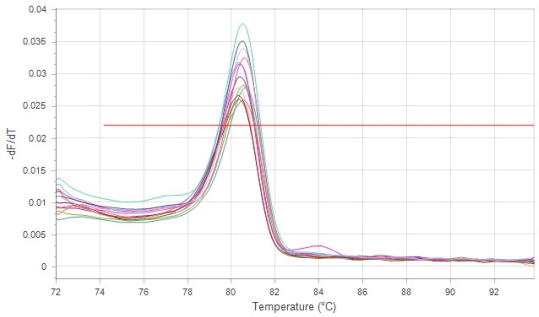

Figure 6: Melting temperature curve, the temperature ranged from 78 to 82°C, after and before the treatment

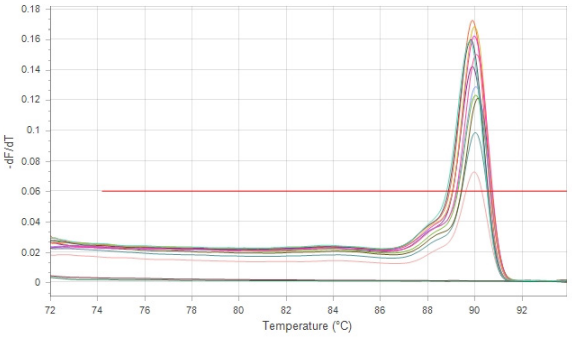

Figure 7: Melting temperature curve, the temperature ranged from 78 to 82°C, after and before the treatment

Plasmids, transposons, and integrons are all types of genetic elements. all play critical Antibiotic resistance is transmitted via a variety of mechanisms. genes across bacteria.[21] Aminoglycosides, chloramphenicol, lactams, trimethoprim, erythromycin, and rifampin are examples of antibiotics. are resistant to antibiotics owing to integron gene transfer.[22]

K. pneumoniae is susceptible to a wide variety of antibiotics, including penicillin, amoxicillin, cefotaxime, and carbenicillin, but resistant to cephalosporins, aminoglycosides, rifamycin, and fluoroquinolones.[23] Because it lacks a chromosomally encoded AmpC cephalosporinase, *K. pneumoniae* is sensitive to the majority of -lactam antibiotics.[24] However, when *K. pneumoniae* gained -lactamases genes, it developed resistance to a wide spectrum-lactamases and AmpC enzymes.[25]

As a result, it is difficult to exactly characterize how the expression of efflux pumps results in the development of antibiotic resistance. Indeed, it seems a link exists between multidrug resistance and efflux pump complexes; efflux pumps may impart and/or acquire resistance to a variety of antibiotic classes.[26]

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