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Research article

Sequencing characterization of housekeeping genes among *Klebsiella pneumoniae* isolated from burn patients

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Abstract

Burn wound infections are one of the most important impairments that occur in the acute period following injury and colonization by the pathogenic agents, including gram-positive, gram-negative bacteria and yeasts. The study included 210 clinical swab samples have been collected from burn- wound patients and cultured on blood agar, MacConkey agar and Eosin methylene blue agar, the period from 1/3/2016 to 30/8/2016 at different hospitals in Baghdad city. By microscopic characterizations, morphological and biochemical reactions, the results showed that 42 (37.5 %) isolates belong to Klebsiella pneumoniae. The analysis of (10) clinical origin of Klebsiella pneumoniae isolates by multilocus sequence typing show the relationship between the local and global isolates which belonged to 7 housekeeping genes (rpoB;beta-subunit of RNA polymerase, gapA; glyceraldehyde 3-phosphate dehydrogenase, mdh; malate dehydrogenase, pgi; phosphoglucose isomerase, phoE; phosphorine E, infB; translation initiation factor 2, tonB; periplasmic energy transducer). The present study, the results showed the 10 isolates of K. pneumoniae were identified into different sequence type (ST): ST 14 and 15 for (IQK1, IQK2, IQK3, IQK4, IQK5, IQK6 and IQK7), ST 266, 54, 709, 728 and 1177 for (IQK8 and IQK9) and ST 665, 975 and 2149 for (IQK10). In addition, the result showed 100% identities with previously reported genes. There was no information on the sequence type (ST) (an allelic profile) of K. pneumoniae in Iraq. According to the results of present study the most occurrence clones found in Baghdad hospitals were endemic ST14 and 15, which accounted for 70% of the isolates (n=10). The presence of the ST14 and 15 clones in Iraq which came closer to global (14 and 15 STs) clones might be indicating intercontinental transmission because these clones were added to the list of the strains that isolated from different countries.

Key words: Burn wound, *Klebsiella pneumonia*, Housekeeping genes, PCR.

Introduction

The *Klebsiella pneumoniae* is Gram-negative bacterium with a prominent polysaccharide capsule of considerable thickness, which give the colonies their glistening and mucoid appearance on agar plates. It is rod shape 0.3 to 1 µm in diameter and 0.6-6 µm in length arranged singly, in pairs or in short chains (1). *Klebsiella pneumoniae* is a facultative anaerobic bacterium and the colonies appear large, mucoid on MacConkey agar indicating fermentation of lactose acid production (2). *K. pneumoniae* is the most significant pathogen within genus *Klebsiella* being responsible for 75% to 86% of *Klebsiella* spp. infections (3). *K. pneumoniae* nosocomial infections had considered significant opportunistic pathogenic agents, being responsible for infections mainly located in the urinary and respiratory tracts,

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but which might also affect soft tissues, wounds and cause septicemia (4). In the hospital situation, *K. pneumoniae* colonization rates increase in a direct proportion to the duration of the hospitalization, which had found to be four times higher in patients who carry the bacteria in their intestine than in non-carriers (5). Multilocus sequence typing (MLST) is a nucleotide sequence-based method used for characterizing the genetic relationships among bacterial isolates. It carried computerized data that allow multi-user international databases available. MLST is more appropriate for strain phylogeny and large-scale epidemiology (6). Therefore, analysis of nosocomial isolates showed that MLST could discriminate among epidemiologically unrelated isolates (7). In addition, MLST method was previously developed for *K. pneumoniae*. MLST scheme customs internal fragments of the following seven housekeeping genes: *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorine E), *infB* (translation initiation factor 2), *tonB* (periplasmic energy transducer)

Materials and Method

Samples Collection:

A total of 210 swab samples have been collected from burn-wound patients (Samples were collected after patient approval) for the period 1/3/2016 to 30/8/ 2016 from hospitals of Baghdad city: Al-Karama Teaching Hospital, Special Burn Hospital, Central Teaching Laboratories, Child protection Teaching Hospital, Imam Ali Hospital. All specimens were labeled and transported by transport media (Al-Hanoof factor, Jordan) with aseptic technique to the laboratory within 1-2 hrs. Then streaked on blood agar, MacConkey agar and EMB agar.

Identification of *K. pneumoniae* had done by: Morphological Characteristics (Colony shape): looks creamy or mucoide light-purple/pink, lactose fermentation) and biochemical tests (Catalase production test, Oxidase production test, Indole production test, Methyl red test, Voges-Proskauer test, Simmons Citrate test, Kligler Iron agar test, Urease production test and Esculin hydrolysis test) according to (8**)**.

DNA extraction

The genomic DNA of the *K. pneumoniae* was extraction according to manufacturer instructions (Geneaid, Korea). DNA preparations were then analyzed by electrophoresis in 1.5% agarose gel.

Oligonucleotide primers and PCR amplification for seven housekeeping genes:

The seven Oligonucleotide primer pairs (table 1) used to amplify the genes *rpoB*, gapA, *Mdh*, *Pgi*, *phoE*, *infB*,*tonB* . The expected amplicon sizes listed in table (1). The specific primers designed according to (6). PCR assays performed in a DNA AMP thermocycler system (TECHNE, USA) as a final volume of 25 µl total containing AccuPower PCR premix (Bioneer, Korea), 0.2 µM of each primer and 5 of DNA template. PCR buffer added to obtain 25µl final volume in the PCR tube. The conditions of the PCR program as follows: An initial activation step at 94 C^{o} for 2 min. followed by 35 cycles of denaturation 94 C^{o} for 20 sec., annealing 50 C^{o} for 30 sec. and extension 72 C^{o} for 30 sec. followed by one cycle consisting of 5 min. at 72 C^{o}. After amplification, The PCR products analyzed by 1.5 agarose gel electrophoresis.

DNA sequencing method:

DNA sequencing method was performed to study the sequence variation in a number of housekeeping genes to define sequence types or clones which led to the definition of major sequence types (STs) (6) and submission in NCBI-GenBank data base of 7 housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB, tonB*) in 10 local *K. pneumoniae* isolates. The PCR products of 7 housekeeping genes in 10 local *K. pneumoniae* isolates were purified from

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agarose gel by using EZ EZ-10 Spin Column DNA Gel Extraction Kit, (Biobasic. Canada). As the following steps:

1. The specific PCR products excised from the gel by clean, sharp blade. Then, transferred into a 1.5 mL microcentrifuge tube.
2. Four hundreds µl. Binding Buffer II was added to gel fragment. Then, incubated at 60ºC for 10 min. and shake until the agarose gel was completely dissolved.
3. Added the above mixture to the EZ-10 column and let stand for two min. then centrifuged at 10,000 rpm for two min. and discarded the flow-through in the tube. Seven hundreds µl. Wash Solution was added to each tube and centrifuged at 10000 rpm for one min. Then, solution discarded.
4. After that, the step 4 repeated. Then, centrifuged at 10000 rpm for an additional minute to remove any residual wash Buffer. The column placed in a clean 1.5pml microcentrifuge tube, added 30 µl of Elution Buffer to the center of the column, and incubated at room temperature for 2 min. Then, the tube centrifuged at 10000 rpm for 2 min. to elute PCR product and store at -20ºC. After that, the purified PCR products samples sent to Macrogen Company in Korea for performed the DNA sequencing by AB DNA sequencing system.

Table (1): Primers used in the study

Primer Name		DNA sequence (5′-3′)	Size (bp)	Reference
rpoB	**F**	GGCGAAATGGCWGAGAACCA	501	(6)
	R	GAGTCTTCGAAGTTGTAACC		
gapA	**F**	TGAAATATGACTCCACTCACGG	450	
	R	CTTCAGAAGCGGCTTTGATGGCTT		
Mdh	**F**	CCCAACTCGCTTCAGGTTCAG	477	
	R	CCGTTTTTCCCCAGCAGCAG		
Pgi	**F**	GAGAAAAACCTGCCTGTACTGCTGGC	432	
	R	CGCGCCACGCTTTATAGCGGTTAAT		
phoE	**F**	ACCTACCGCAACACCGACTTCTTCGG	420	
	R	TGATCAGAACTGGTAGGTGAT		
infB	**F**	CTCGCTGCTGGACTATATTCG	318	
	R	CGCTTTCAGCTCAAGAACTTC		
tonB	**F**	CTTTATACCTCGGTACATCAGGTT	414	
	R	ATTCGCCGGCTGRGCRGAGAG		

Results

Isolation and Identification:

All the isolated pathogens were identified depending on the microscopic examination, morphological and biochemical tests. According to these results, 42 isolates (37.5%) were *K. pneumoniae*.

Result of Conventional PCR

The results of the PCR amplification of the seven housekeeping genes: *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorine E), *infB* (translation initiation factor 2), *tonB* (periplasmic energy transducer) tested are reported in Figure (1). All genes were always detected in *K.pneumoniae* isolates.

Multilocus sequence typing (MLST) of *K. pneumoniae*:

A different allele number was given to each distinct sequence within a locus, and distinct sequence type (ST) number was credited to each distinct combination of alleles. The allele profile and collection of MLST were assigned by using the MLST database of *K. pneumoniae* which available online:http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

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In the present study, The analysis of 10 clinical origin of *K. pneumoniae* isolates were carried out by multilocus sequence typing using 7 housekeeping genes to show the relationship between the local and global isolates. By Multiple sequence alignment analysis of the seven genes sequences from10 isolates showed the similarity (*) and differences in 7 housekeeping genes nucleotide sequences Figure (2). The 7 housekeeping genes was registered in NCBI (National center for Biotechnology Information) Table (2**)**. The isolates (Iraq Klebsiella) IQK1, IQK2, IQK3, IQK4, IQK5, IQK6, IQK7 IQK8, IQK9 and IQK10 submitted to MLST program to determine the genotypes according to the protocol, which described on the *K. pneumoniae* MLST website (www.pasteur.fr/mlst). Ten isolates of *K. pneumoniae* were randomly representative to analyze by MLST. The results of the present study showed the 10 isolates of *K. pneumoniae* identified into different sequence type (ST): ST 14 and 15 for IQK1, IQK2, IQK3, IQK4, IQK5, IQK6 and IQK7, ST 266, 54, 709, 728 and 1177 for IQK8, IQK9 and ST 665, 975 and 2149 for IQK10. In addition, the result showed 100% identities with previously reported genes. There was no information on the sequence type (ST) (an allelic profile) of *K. pneumoniae* in Iraq. The ten isolates of *K. pneumoniae* were classified into three different MLST: First; seven isolates IQK1, IQK2, IQK3, IQK4, IQK5, IQK6 and IQK7 shared an allelic profile of MLST 146-1-114-166-1-115-1 and identity 100% which designated as ST 14 and 15 (Table 3) and formed 70 % of all the current isolates. The allelic profiles of MLST, ST 14 and 15 means that 146 alleles of *gapA*, 1 allele of *infB*, 114 alleles *mdh*, 166 alleles of *pgi*, 1 allele of *phoE*, 115 alleles of *rpoB* and 1 allele of *tonB*.Second; two isolates IQK8 and IQK9 shared un allelic profile of MLST 146-1-114 -166 -16 -107 -4 and identity 100% which designated as ST 266, 54, 709, 728 and 1177(Table 4). The allelic profile of MLST, ST 266, 54, 709, 728 and 1177 means that 146 alleles of *gapA*, 1 allele of *infB*, 114 alleles *mdh*, 166 alleles of *pgi*, 16 alleles of *phoE*, 107 alleles of *rpoB* and 4 alleles of *tonB*. Third; one isolate IQK10 shared un allelic profile of MLST 146 -1-114 -166 -13 -115 -279 and identity 100%, which designated as ST 665, 975 and 2149 Table (4). The allelic profile of MLST, ST 665, 975 and 2149 means that 146 alleles of *gapA*, 1 allele of *infB*, 114 alleles of *mdh*, 166 alleles of *pgi*, 13 alleles of *phoE*, 115 alleles of *rpoB* and 279 alleles of *tonB* Table (5).

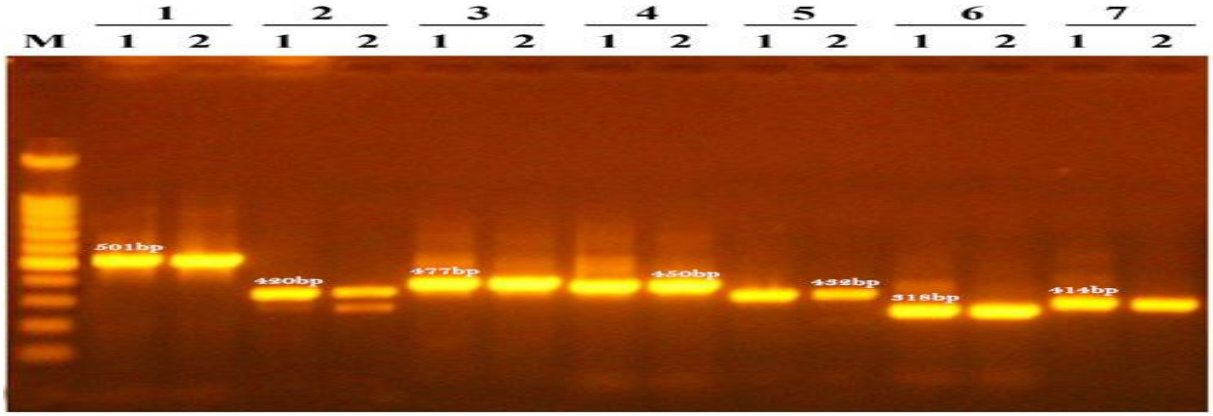

Figure (1): PCR products analyzed by 1.5% agarose gel electrophoresis. The size of amplified DNA fragments were identified by comparison with molecular size marker DNA (M) (100 bp DNA Ladder). the agarose gel electrophoresis of amplified *rpoB*(501bp) ,*gap A(450bp)*, *Mdh* (477bp), *Pgi* (432bp), *phoE* (420bp), *infB* (318bp), *tonB* (414bp). 1 and 2: *K. pneumoniae* 1 and 2; 1-7: numbers of housekeeping genes.

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Discussion

Isolation and Identification

According to the results of the isolation, it has been found that *K. pneumoniae* isolated in high percentages 37.5%. Previous studies indicated that *K. pneumoniae* were preceding all nosocomial gram-negative bacteria, so they accounted in an average 15 - 42 % among different hospitals in Iraq (19, 20). Other study had indicated that *K. pneumonia*e as nosocomial infections were a major cause of morbidity and mortality among several burn patient inhabitants (11).

Result of Conventional PCR

The PCR described is a fast, specific and reliable method, which can be routinely used as an alternative to time consuming traditional tests (16).This method used to conform the presence of study genes in all *K. pneumoniae* isolates.

Multilocus sequence typing (MLST) of *K. pneumoniae*:

It was an excellent method to study the clonal origin and evolution of *K. pneumoniae*. MLST was based on sequence analysis of fragments from seven *K. pneumoniae* housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB and tonB*). According to the results of present study the most occurrence clones found in Baghdad hospitals were endemic ST14 and 15, which accounted 70% of the isolates (n = 10). The presence of the ST14 and 15 clones in Iraq, which came closer to global 14, and 15 STs Clones, might be indicating intercontinental transmission, because these clones added to the list of the strains that isolated from different countries (12). The other minor clones STs 266, 54, 709, 728 and 1177 STs and 665, 975 and 2149 STs that found identically in alleles represented 30% with 3 Iraqi isolates IQK8 , IQK9 and IQK10 . The presence of the minor clones STs indicating that these strains carrying low genotypes and had lower development compared with 14 and 15 STs Strains. By combining the seven gene loci, in the current study 9 distinct sequence types (STs) identified. Most groups of strains sharing the same ST belonged to suspected epidemiological clusters revealed the existence of two clonal complexes, one including ST14 and ST15 in seven Iraqi strains, the other including ST266 ,54,709 and 728 in 2 Iraqi strains and 3 STs 665, 975 and 2140 in one Iraqi isolate. Since the first description in late 2009 in Sweden from *K. pneumoniae* and *E. coli* isolates, *NDM*-1 had established as a major public health threat (13). In addition, NDM-1-producing members of the *Enterobacteriaceae* had isolated in various parts of the world, including Australia, Bangladesh, Belgium, Canada, France, India, Japan, Kenya, the Netherlands, New Zealand, Pakistan, Singapore, Taiwan, and the United States (12). *K. pneumoniae* ST14 had previously to be prevalent in many countries, including India, Sweden, and the United Kingdom (14).The first identified from ST15 *K. pneumoniae* isolates in Morocco (15) and widespread as previously described in Europe, Denmark, Hungary and in Asia; Korea, Malaysia, Singapore and Taiwan (16). The clone ST709 had reported in China as a result from a 3-years period (17).The occurrence of this type in Iraq might be due to the travel frequency for business purposes with China. The widespread distribution is a major concern, both as a source of therapeutic failure and as a potential reservoir of resistance determinants. The main factor in the spread of multidrug-resistant *K. pneumoniae* clones may be human mobility, as explain by the spread of NDM-1-producing strains from India and Pakistan to the other countries. So, the accomplishment of antibiotic-resistant genes (such as *bla* NDM), it is increasingly difficult to cure carbapenem-resistant *K. pneumoniae*. Therefore, the early diagnosis of this pathogen by MLST has become increasingly important and will differentiate most epidemiologically unrelated strains (18).

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Table (2): Gene Bank accession numbers of *K. pneumoniae* and housekeeping genes

Genes	Seq. NO.	No. of Isolates
rpoB	Seq.1-Seq9	IQ-KP2-IQ-KP10
gapA	Seq.10-Seq19	IQ-KP1-IQ-KP10
Mdh	Seq.20-Seq.28	IQ-KP1-IQ-KP10
Pgi	Seq.29-Seq38	IQ-KP1-IQ-KP10
phoE	Seq.39-Seq48	IQ-KP1-IQ-KP10
infB	Seq49-Seq58	IQ-KP1-IQ-KP10
tonB	Seq59-Seq68	IQ-KP1-IQ-KP10

Table (3): Allele numbers assigned in sequencing type database (ST) 14 and 15 of *K. pneumoniae* for IQK1, IQK2, IQK3, IQK4, IQK5, IQK6 and IQK7 isolates at the seven loci.

Locus	Identity (%)	HSP Length	Allele Length (bp)	Gaps	Allele
rpoB	100.00	501	501	0	rpoB-146
gapA	100.00	450	450	0	gapA-1
Mdh	100.00	477	477	0	mdh-114
Pgi	100.00	432	432	0	pgi-166
phoE	100.00	420	420	0	phoE-1
infB	100.00	318	318	0	infB-115
tonB	100.00	414	414	0	tonB-1

HSP, High scoring segment pairs; bp, base pair.

Table (4): Allele numbers assigned in sequencing type (ST) 266, 54,709,728 and 1177 database of K. *pneumoniae* for IQK8 and IQK9 isolates at the seven loci.

Locus	Identity (%)	HSP Length	Allele Length (bp)	Gaps	Allele
rpoB	100.00	501	501	0	rpoB-146
gapA	100.00	450	450	0	gapA-1
Mdh	100.00	477	477	0	mdh-114
Pgi	100.00	432	432	0	pgi-166
phoE	100.00	420	420	0	phoE-16
infB	100.00	318	318	0	infB-107
tonB	100.00	414	414	0	tonB-4

HSP, High scoring segment pairs; bp, base pair.

Table (5): Allele numbers assigned in sequencing type (ST) 665, 975 and 2149 of K. *pneumoniae* for IQK10 isolate at the seven loci.

Locus	Identity (%)	HSP Length	Allele Length (bp)	Gaps	Allele
rpoB	100.00	501	501	0	rpoB-146
gapA	100.00	450	450	0	gapA-1
Mdh	100.00	477	477	0	mdh-114
Pgi	100.00	432	432	0	pgi-166
phoE	100.00	420	420	0	phoE-13
infB	100.00	318	318	0	infB-115
tonB	100.00	414	414	0	tonB-297

HSP, High scoring segment pairs; bp, base pair.

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13          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
14          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
20          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
19          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
16          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
17          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
18          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
12          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
11          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
15          AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
gapA_gene   AAAGACGGTCATCTGGTCGTTAACGGTAAAAAAATCCGTGTTACCGCTGAACGTGACCCG
            ************************************************************

sequence5   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence10  GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence8   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence9   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence7   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence3   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
1           GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence6   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
sequence4   GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
mdh_gene    GCGTAAACCGGGTATGGATCGTTCCGACCTGTTTAACGTTAACGCCGGCATCGTGAAAAA
            ************************************************************

6           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
10          ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
2           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
8           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
5           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
4           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
7           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
9           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
3           ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
rpoB_gene   ATGGACCAGAACAACCCGCTGTCTGAGATTACGCACAAACGTCGTATCTCCGCACTCGGC
            ************************************************************

43          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
50          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
41          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
sequence9   CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
44          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
49          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
46          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
47          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
45          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
42          CTTCGCCGTCAGCGCAGCCTACACCAGCTCCGACCGTACCAACGATCAGAACCTGCTGGC
phoE_gene   CTTCGCCGTCAGCGCGGCCTACACCAGCTCCGATCGCACGAACGATCAGAACCTGCTGGC
            ********** ********** ** ** **********

66          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
67          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
62          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
65          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
tonB_gene   ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
61          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
63          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
64          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGCCTACGCTACTGTCTGTG
68          ATGAGCGCAATGACCTTTGATTTACCTCGCCGCTTTCCGTGGAATACGCTACTGTCTGTG
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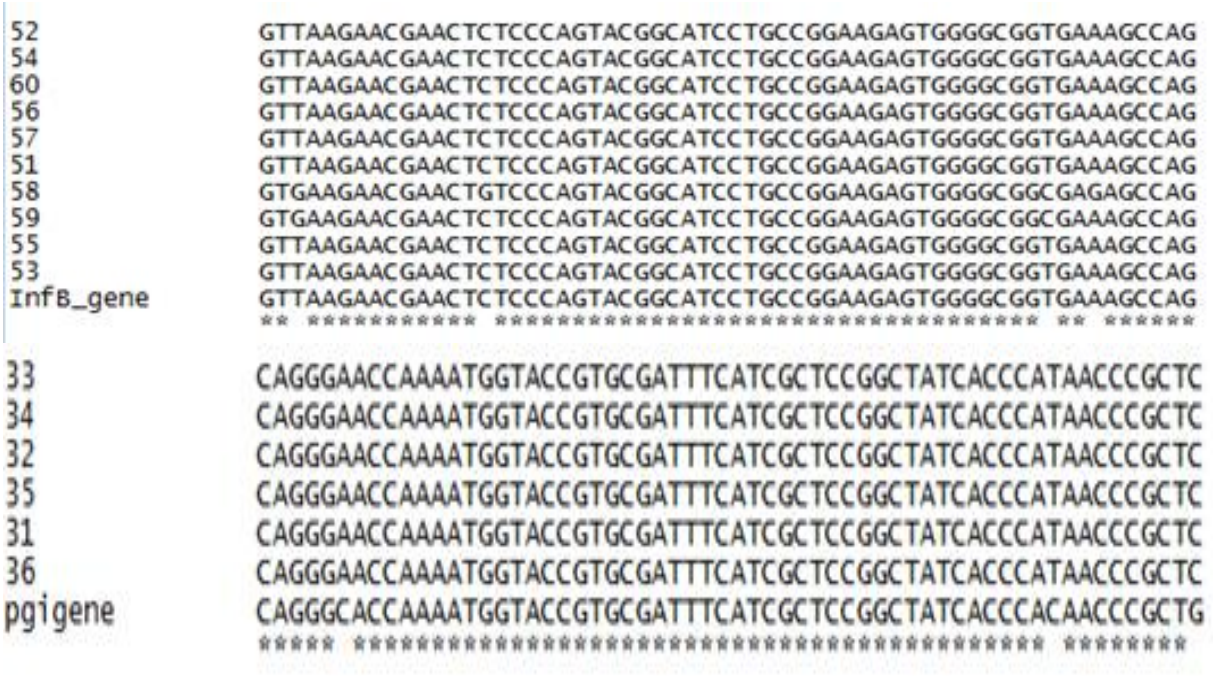

Figure (2): Multiple sequence alignment analysis from (10) isolates of *K. pneumoniae* showed the similarity (*) and differences in 7housekeeping genes nucleotide sequences (Multiple sequence alignment program version 6) (online).

Conclusion

Multilocus sequence typing (MLST) was an excellent method to study the clonal origin and evolution of *K. pneumoniae*.

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