Detection of Polymorphism in the Gene *bla*$_{KPC2}$ of Local *Klebsiella pneumoniae* Isolated from Iraqi Patients

تحديد التغايرات الوراثية لجين bla_{KPC2} لعزلات محلية لبكتيريا الكليبسيلا الرئوية والمعزولة من المرضى العراقيين

Saadi Abd-Alkareem Jasim Mohammed Nadhir Maaroof Najwa Shihab Ahmed*

College of Education Pure Sciences/ Tikrit University

* Biotechnology Research Center/ Al-Nahrain University

سعدي عبد الكريم جاسم محمد نظير معروف نجوى شهاب أحمد*

كلية التربية للعلوم الصرفة/ جامعة تكريت/ صلاح الدين

* مركز البحوث للتقنيات الاحيائية/ جامعة النهرين

E- mail: Saadee1988@yahoo.com

Abstract

Carbapenemases are clinically important because they destroy and may confer a resistance to carbapenems, severe infections caused by carbapenemase producers is associated with increased mortality. To achieve this goal, 180 samples were collected from different clinical sources included 92 urine, 33 smears of wounds, 13 smears of burns and 42 sputum. The samples were taken from patients attended Al-Yarmouk Teaching Hospital and Ibin Baladi Hospital in Baghdad Governorate. Diagnosis of bacterial isolates was done depending upon the microscopic examination, the cultured characteristics and biochemical tests. DNA extracted from 84 samples. Accordingly, detection of *bla*$_{KPC2}$ gene was conducted by using specific primers for amplification of *bla*$_{KPC2}$ gene. Moreover, the sequencing of 910 bp for *bla*$_{KPC2}$ gene was performed by the biotechnology lab. at the National Instrumentation Center for Environmental Management (NICEM). Such test has been implanted by using 3730XL as a DNA sequences. The obtained results were analyzed by blast at the National Center Biotechnology Information (NCBI) and detect polymorphism in *bla*$_{KPC2}$ based on the Bio Edit. Consequently, 94 variations between 47 transversions, 43 transitions and 4 deletions nucleotide were noticed. In a sense the test showed 79% under sequence ID gb|CP009872.1| from 3037673 -3038166 number of nucleotide from *K. pneumoniae* subsp. pneumoniae strain KPNIH30 of Gene Bank, score (329) and expect 5e-86 with the wild type of *bla*$_{KPC2}$ gene from Gene Bank. Finally, the results illustrated polymorphism between local strains of *K. pneumoniae* isolated from Iraqi patients and strain of *K. pneumoniae* obtained from Gene Bank.

Key words: *bla*$_{KPC,}$ Carbapenem, polymorphism, PCR

الملخص

Carbapenemases تعد مهمة سريريا وذلك لأنها تدمر وتمنح المقاومة لمجموعة الكاربينيم، والاصابات الشديدة التي تسببها البكتريا المنتجة لأنزيم carbapenemase المرتبطة مع زيادة معدل الوفيات. وبغية الوصول الى الهدف، جمع مائة وثمانين عينة من مصادر سريرية مختلفة (92 ادرار، 33 مسحة الجروح، 13 مسحة الحروق و 42 قشع) من مرضى راقدين في مستشفى ابن بلدي وكذلك مستشفى اليرموك التعليمي في محافظة بغداد. وشخصت العينات بالاعتماد على المجهر الضوئي، الصفات المزرعية والاختبارات الكيموحيوية. وعزل الحامض النووي الدي اوكسي رايبوزي DNA من 84 عينة. ووفقا لذلك، تم الكشف عن طريق تضخيم الجين bla_{KPC2} باستخدام بادئات متخصصه للجين. علاوة على ذلك، اجريت عملية تسلسل الاحماض النووية للجين bla_{KPC} والذي هو بحجم 910 زوج قاعدي باستخدام جهاز AB13730XL في شركة مايكروجين في الولايات المتحدة الامريكية. أظهرت نتائج تتابع الحامض النووي لجين bla_{KPC2} مطابقتها مع تسلسل الاحماض النووية انفس الجين والمسجل لدى الموقع العالمي لبنك الجينات. بناءاً على ذلك، لوحظت تغايرات وعددها 94 تغاير ما بين 47 انقلابي، 43 انتقالي و4 حذف قاعدة نيتروجينية. اظهر الاختبار نسبة التطابق 79% وتحت الرقم ID gb|CP009872.1| من الرقم النيوكلوتيدة 3037673 -3038166 من عزلة بنك الجينات Klebsiella pneumoniae subsp. pneumoniae strain KPNIH30 وسكور 329 والقيمة المتوقعة 5e-86 مع جين bla_{KPC2} في بنك الجينات العالمي. اخيراً، استنتج من الدراسة الحالية وجود تغايرات بين العزلة البكتيرية المحلية المعزولة من المرضى العراقيين مقارنة مع العزلة البكتيرية المسجلة في بنك الجينات.

الكلمات الدالة: bla_{KPC}، مضادات الكاربينيم، تعدد الاشكال، تفاعل البلمرة المتسلسل

Introduction

Klebsiella pneumoniae is considered the second cause of pathogenesis in Enterobactriaceae family after *E.coli*. These bacteria are gram negative bacilli and considered from opportunistic pathogen bacteria as endemic to the intestinal tract and nasopharynx and infect the person, especially the infant and elderly. The infections occur in any position of the body when transits to it, as it cause 17% from UTI and cause respiratory tract infections [1, 2]. Also *K. pneumoniae* is considered one of important cause pathogen from nosocomial infection, especially for

patients who suffer from Immunocompromised or who are taking immunosuppressed drugs and who suffer from increases iron concentration in blood because they possess Siderophore [3]. *K. pneumoniae* possesses number of virulence factors which share a pathogen and such as capsule antigens, adhesion factors, enterotoxin produce like lipopolysaccharide as well as resistance killer effect for serum and system the obtain on iron (Siderophore) and multi resistance for antibiotics which are considered the main reason in spreading acquired infections in hospitals, as the percentage infections 80% which led to go for find alternative treatments [4]. *Klebsiella pneumoniae* Carbapenemases (KPCs) constitute a new variant of class A β-lactamase enzymes capable of hydrolyzing all known β-lactam antibiotics and displaying resistance to β-lactamase inhibitors [5]. As with other class A enzymes, they are carried on a variety of plasmids, thereby facilitating horizontal transmission of bla_{KPC} genes [6]. Since their initial description in 2001, KPC-producing strains of *K. pneumoniae* and other Enterobacteriaceae have spread rapidly in the New York metropolitan region, with increasing numbers of cases reported across the United States [7]. *K. pneumoniae* resistance to Carbapenem group produces a wide range from β- lactamase (ESBLS), it was differentiate isolates which produce β-lactamase by bla_{KPC} genes, which are these genes diagnosis using specific primers via PCR technique. A screening investigation during 2004 of *E.coli* and *K. pneumoniae* collected from four hospitals in Brooklyn, New York, have shown that none of the *E.coli* isolates carried a bla_{kpc} gene, whereas 24% of the *K. pneumoniae* isolates possess a $bla_{\text{KPC-2}}$ or $bla_{\text{KPC-3}}$ gene. The majority (88%) of the bla_{KPC} –carrying isolates belonged to the same ribotype [8]. Aim of the study is to detect of polymorphism in produce β-lactamase by bla_{KPC} gene of local *K. pneumoniae* isolated from Iraqi patients.

Materials and Methods

Sample Collection and Identification

One hundred eighty samples from two clinical sources were collected including 92 urine, 33 smears of wounds, 13 smears of burns and 42 sputum samples and information of the patients like (sex and age). The samples were taken from Al-Yarmouk Teaching, and Ibin Baladi hospitals from external labs for the period between February 2015 to May 2015. Diagnosed bacterial isolates depended on the microscopic, culture characteristics, biochemical tests, also used the API 20E for diagnosis the Enterobacteraceae family and used the modern methods such as VITEK2 System according to previous reports [9,10].

Antibiotics susceptibility

The bacterial inoculum was prepared through transferring a single colony to test tube containing nutrient broth then incubated at 37°C for 24 hour. After preparing incubation and inoculum, sterile swab is taken and swabbed evenly across the surface of a Muller-Hinton agar plate, after inoculation three antibiotic discs put by forceps with hard pressure, in each dish then plate inverted and incubated at 37°C for 18 hour [11].

DNA Extraction

DNA was extracted from 84 samples by DNA extraction kit (Genomic DNA Mini Kit, USA, Catalog #: GB 100) according to the manufacturer's protocol [12]. Detection of bla_{KPC2} gene was conducted by using primers for amplification of bla_{KPC2} gene. A fragment 910bp of bla_{KPC2} gene was amplified using a forward primer and reverse primer which supplied by IDT (Integrated DNA Technologies) company, Canada (bla_{KPC2F}:5'TGTCACTGTATCGCCGTC-3′) and a reverse primer (bla_{KPC2R}:5-'CTCAGTGCTCTACAGAAAACC-3') [13].

PCR Procedure

The PCR amplification is performed in a total volume of 25µl containing 1.5µl DNA, 12.5 µl Taq Master Mix PCR (Promega, USA), 1µl of each primer 10 pmol then distilled water is added into a tube to a total volume of 25µl. PCR amplification was conducted under the following conditions: 5 minutes at 95°C, followed by 35 cycles at 94 °C for 45second, 56°C for 80second, 72 °C for 90 second and a final extension of 72°C for 7 minutes using a thermal Cycler made by Labnet (Labnet international, Inc, MultiGene OptiMax, Catalog #: TC9610-230, USA). The PCR products were separated on an 1.5% agarose gel electrophoresis and visualized by exposure to ultra violate light 302 nm after ethidium bromide staining [14].

Purification of samples from Gel

After DNA extraction from the samples, purification and concentration were carried out to measure the DNA and to diagnose it by polymerase chain reaction (PCR), results must be sent for the purpose of sequencing processing but before that must be purified from the gel after PCR. After electrophoresis the PCR products then stained and visualized under U.V light. When a band appears, it was taken by cutting it and the Gel extraction

method was followed; after extracting bands from gel, purification and of he product was measured by Nanodrop [12]. After extraction processing 20 samples were sent to sequencing analysis. The sequencing of bla_{KPC2} gene was performed by National Instrumentation Center for Environmental Management (NICEM) biotechnology lab, machine was DNA sequencer 3730XL, Applied Biosystem, and compatibility search was conducted using Basic Local Alignment Search Tool (BLAST), a program which is available at the National Center Biotechnology Information (NCBI) and Bio Edit program.

Results and Discussion

Isolation, Identification and Susceptibility Test

Initial isolation results showed that there were 82 isolate given the qualities of colonies *Klebsiella spp*. from the total collected samples 180. Those samples had the ability to lactose fermentation when it was cultured on MacConkey agar medium and gave the pink glamorous colonies with mucus texture of which it is one of the important qualities of *Klebsiella spp*. Microscopic examination, cultured characteristic and biochemical tests showed that 82 isolate of the total 180 samples gave a positive results to *Klebsiella* spp. which represented 45.6%. This percentage clarifies the importance of this genus between other pathogens [15,16]. Furthermore, 82 isolates of *Klebsiella spp*. were tested by API20E a test used to differentiate between the *Klebsiella spp.* which gave a positive results in biochemical tests. When the diagnosis of all isolates, it observed that there is diversity among species where the highest percentage appeared to *Klebsiella pneumoniae* 61 isolates (74.4%), the appearance of a higher percentage of *K. pneumoniae* were compatible with other studies [17], and 12 isolate of *K. terrigena* (14.6%) and fewer isolates 9 isolates (11%) appeared to be *K.oxytoca* Table (1).

Table (1): Percentage of Diagnosis by API 20E

Source / Type of bacteria	Urine	sputum	Wound smear	Burn smear	Total
K. pneumoniae	**37**	**18**	**6**	**0**	**61 (74.4%)**
K. terrigena	**2**	**1**	**2**	**0**	**5 (6.1%)**
K. oxytoca	**8**	**4**	**4**	**0**	**16(19.5%)**

Some studies indicated that *K. pneumoniae* was predominant species 86% among *Klebsiella spp.* that clinically isolated [17] and also have noted diversity in these bacteria in clinical isolates [16,18], 61 isolates from *K. pneumoniae*, it explains that high percentage obtained from urine (37) isolates 60.5%, sputum (18) isolates 30% and Wound smear (6) isolates 9.5% while the burn smear didn't show any isolate from *K. pneumoniae.* The blood samples are diagnosed, (60) samples in Ibin Al-Baladi Hospital, and gave the diagnosis results by VITEK2 System. It showed high percentage to *K. pneumoniae* 23 isolates, while 17 isolates appeared to be from *Staphylococcus aureus* and other isolates showed very low percent as *Escherichia coli* (4) isolates, *Enterococcus faecium* (3) isolates, *Salmonella typhi* (1) isolate and *Pseudomonas aeruginosa* (1) isolate Table (2).

Table (2): Percentage of Diagnosis by VITEK2 System

No. of specimens	*K. pneumoniae*	*Staphylococcus aureus*	*E.coli*	*Enterococcus faceium*	*Salmonella typhyi*	*Pseudomonas aeruginosa*
100	**23**	**17**	**4**	**3**	**1**	**1**

Susceptibility tests are performed for 50 isolates of *K. pneumoniae*, susceptibilities of all isolates to Carbapenem group such as: Meropenem, Imipenem, Ertapenem and Doripenem are determined by disc diffusion method. From gained results, noted that all isolates were resistant to Carbapenem group, but each antibiotic differs from the other in the inhibition zone formed around the colony, where it showed highest resistance to Imipenem then Doripenem then Ertapenem and Meropenem respectively [10,18] and each tetracycline, gentamycin and ampicillin don't have any effect with *K. 28neumonia*.

Optimization of the PCR Conditions

At the beginning, all the 61 isolates from urine, sputum , wound and 23 isolates from blood were grown on Blood agar medium to activate bacteria, reserved on the Maintenance medium and then transferred to the Nutrient broth instead of Luria – Berloni and brain-heart infusion medium, used in other studies. After ending the DNA extraction, the concentration and purity of DNA by Nano drop should be done. Results showed a

concentration between 54- 294 ng/µl and purity 1.69 - 2.02. Through the use of PCR kit according to the Promega Company's, the interaction was done in volume 25µl. Taken 12.5µl from Master Mix which consists of $MgCl_2$, dNTPs and Taq polymerase and this was used as constant volume for 84 samples. The special compounds for diagnosing the *bla*$_{KPC2}$ gene 910bp Green Master Mix 12.5µl, Forward primer 10 Pico mole, Reverse primer 10 Pico mole, DNA 1-1.5 µl and D.W. 9.5-9 µl and changing condition for purpose of reaching to optimum condition for each primer through the manipulation of annealing temperature, time and cycle number.

Diagnosis The *bla*$_{KPC2}$ Gene

This gene identified through determination of the optimal condition of interaction, where used 1 µl from Forward and Reverse primers 10 picomole/ µl concentration and used 1.5 µl from DNA template, placing the initial temperature for beginning interaction 94°C for 2 minute for one cycle and also the work of gradient PCR through using varied temperature in annealing stage 53, 54,55,56,57 and 58°C for 1:20 minute and when the product deported electrically it showed the best temperature to annealing primer 56°C, Figure (1) shows the product and marker DNA for the package 910bp.

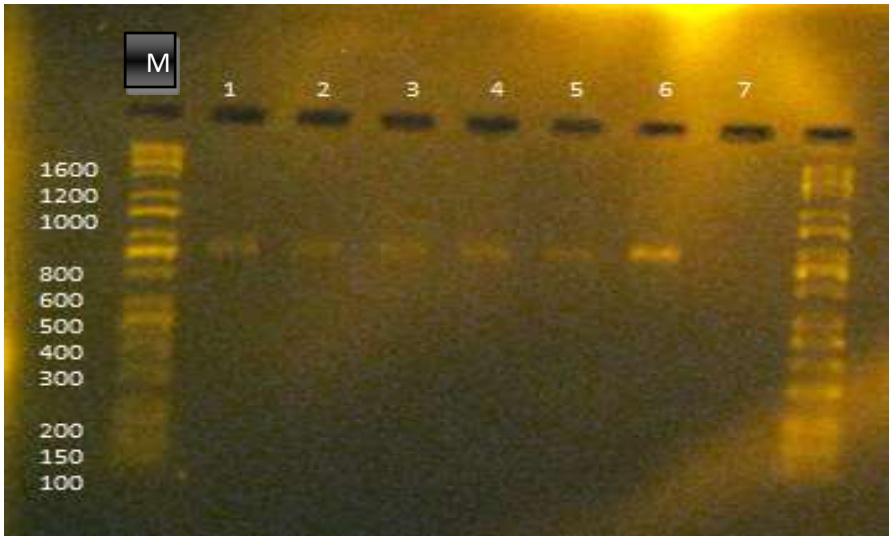

Fig. (1): PCR product the band size 910bp. The product is electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 2:00 hours. M: DNA ladder 100-10000bp, lane 1-7 PCR product of band size 910bp. visualized under U.V light.

Sequencing and Alignment of NCBI

Results illustrated in Figure (1) indicate that a yield of single band of the desired product with a molecular weight of 910bp for *bla*$_{KPC2}$ gene of *K. pneumoniae* was obtained from 20 samples. Twenty PCR product samples were sent for sequence analysis; 8 of *K. pneumoniae* isolates from blood and 10 samples isolates isolated as follows: 4 from urine, 4 from sputum and 4 from wound. The samples were treated with AB13730XL Applied Biosystems machine in National Instrumentation Center for Environmental Management NICM/USA Company. Results of the sequence analysis were analyzed by blast in the National Center Biotechnology Information (NCBI) and BioEdit program to detect the polymorphism in *bla*$_{KPC2}$ gene, the results obtained showed that 94 variations between 47 transversions (refers to the substitution of a (two ring) purine for a (one ring) pyrimidine) , 43 transition (a point mutation that changes a purine nucleotide to another purine (A ↔ G) or a pyrimidine nucleotide to another pyrimidine (C ↔ T)) and 4 deletion nucleotide, have shown 79% compatibility as showed in Figure (2), under sequence ID gb|CP009872.1| from 3037673 -3038166 number of nucleotide from *Klebsiella pneumoniae* subsp. pneumoniae strain KPNIH30 of Gene Bank, and score 329 and expect 5e-86 with the wild type of *bla*$_{KPC2}$ gene from Gene Bank as shown in Figure (2) and Table (3) and isolated from perirectal swab. The expectation value was defined to give an estimate of the number of times expected to get the same coincidental similarity and the lower value of E. This indicates that the degree of similarity was high between sequences which gave greater confidence. The value very close to zero means that these sequences were identical and the bit Score: statistical measure of the moral similarity and the higher value

indicates that the high degree of similarity, if dropped from the class of 50 points, the sense that there is no similarity mention.

Klebsiella pneumoniae subsp. pneumoniae strain KPNIH30

Sequence ID: gb|CP009872.1|

Score	Expect	Identities	Gaps	Strand
329 bits(178)	5e-86	392/497(79%)	8/497(1%)	Plus/Plus

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Query  66       GTGAAGATTTTGGGGTGAAAGGCGTCTGAACTGGTGATCCCAATTGACAGGCTGCCGTTC  125
                 ||||||||||  || || || || || |||||||| || || |  ||||||||||||||||
Sbjct  3037673  GTGAAGATTTGCGGATGGAAAGCATCAGAACTGGTAATGCCTAGCGACAGGCTGCCGTTC  3037732
Query  126      -GCCCGCGGGCAATGCCCCGGGCTTTCTCCAGCGCGGCGTCGCTCACCGCCAGGATCTTA  184
                  |||||| |||||||||  ||| ||||||||||| || |||||||  || |||||||
Sbjct  3037733  ATCCCGCGCGCAATGCCCTTGGCCTTCTCCAGCGCCGCATCGCTCATGGCGAGGATCTGG  3037792
Query  185      CACGCGTCCTCATATAAGGCTTCTCCCGCTTCGGTCGACTCCACGCCTCGCGTCTCGCGT  244
                |  || |||||||| || |  |||||||||| |||  |||||| || || ||    ||
Sbjct  3037793  CGGGCATCCTCATAGAAAGACTCTCCCGCTTCCGTCAGCTCCACCCCGCGGGTTAAACGC  3037852
Query  245      CTGAATAACGGCGTGCCCACTTCTTCCTCGAGCCGTTTAATCTGCTGACTCATAGGAGGC  304
                | ||| | ||| || ||||||||   ||| ||||| | ||| |||||||| |  ||||||
Sbjct  3037853  CGGAACAGCGGGGTTCCCACTTCGCGCTCAAGCCGCTGAATTTGCTGACTTAACGGAGGC  3037912
Query  305      TGTGAAATACCCAGCGCTTTGGCCGCTTTGGTGAAGTGTCGC-TCACGTGCAACGGCGAC  363
                |||||||||||||| | ||||| || | ||||||||| ||| || |  || ||||||||
Sbjct  3037913  TGTGAAATACCCAGCTCCTTGGCGGCCTGGGTGAAGTGCCGCGTC-CTGGCGACGGCGAC  3037971
Query  364      AAAATACCGCACATAACGAAGTTCCATATCAAAAACGTCTCAAACCAGCATGGATTCTAT  423
                |||||| || | |||||||||||||||||| |||||||||||||||||||||| ||||||
Sbjct  3037972  AAAATAGCGAAGATAACGAAGTTCCATATCGAAAACGTCTCAAACCAGCATGGTTTCTAT  3038031
Query  424      ATTGGAACTCTATGCTGAATCGGGTCAACATTTATTTAACCTTTCTAA-ATAAAGTTGAA  482
                |||||||||  |  |||||||||||||||||||||||||||||||| | ||   ||||||
Sbjct  3038032  ATTGGAACTGTGAGCTGAATCGGGTCAACATTTATTTAACCTTTCTTATATTT-GTTGAA  3038090
Query  483      -GAGGACGAGC-ATGATGGTGCATTCATCTGCATGCGACTGTGAGGCCAGTTTGTGCGAG  540
                 |||||  | |  || |||   |||||  ||| ||||  ||| || |  ||| | |||||
Sbjct  3038091  CGAGGAAGTGGTAT-ATGAATCATTCTGCTGAATGCACCTGCGAAGAGAGTCTATGCGAA  3038149
Query  541      ACCCTGCGAGGGTTTTC  557
                 |||||||| | ||||||
Sbjct  3038150  ACCCTGCGGGCGTTTTC  3038166
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Fig. (2): Sequencing of sense flanking the partial *bla*$_{KPC2}$ gene compared with standard *bla*$_{KPC2}$ gene, obtained from Gene Bank. Query represents of sample; Subject represent of database of National Center Biotechnology Information (NCBI).

Table (3): Represent type of polymorphism in of sense flanking the partial *bla*$_{KPC2}$ gene of *Klebsiella pneumoniae* subsp. pneumoniae strain.

No.	Location of substitution		Type of substitution	No.	Location of substitution		Type of substitution	No.	Location of substitution		Type of substitution
1	3037683	G>T	Transversion	32	3037829	A>G	Transition	63	3037959	T>G	Transversion
2	3037684	C>G	Transversion	33	3037830	G>A	Transition	64	3037960	G>T	Transversion
3	3037687	A>G	Transition	34	3037837	C>G	Transversion	65	3037963	G>A	Transition
4	3037690	G>A	Transition	35	3037840	G>T	Transversion	66	3037978	G>C	Transversion
5	3037693	A>G	Transition	36	3037843	G>C	Transversion	67	3037981	A>C	Transversion
6	3037696	A>G	Transition	37	3037846	T>C	Transition	68	3037983	G>C	Transversion
7	3037699	A>T	Transversion	38	3037847	A>T	Transversion	69	3038002	G>A	Transition
8	3037708	A>G	Transition	39	3037848	A>C	Transversion	70	3038041	G>C	Transversion
9	3037711	G>C	Transversion	40	3037849	A>G	Transition	71	3038043	G>A	Transition
10	3037714	T>A	Transversion	41	3037854	G>T	Transversion	72	3038044	A>T	Transversion
11	3037716	G>T	Transversion	42	3037858	C>T	Transition	73	3038080	T>-	
12	3037717	C>T	Transition	43	3037860	G>A	Transition	74	3038083	T>A	Transversion
13	3037733	A>-		44	3037864	G>C	Transversion	75	3038084	T>A	Transversion
14	3037734	T>G	Transversion	45	3037867	G>T	Transversion	76	3038097	A>C	Transversion
15	3037741	C>G	Transversion	46	3037876	G>T	Transversion	77	3038099	T>A	Transversion
16	3037751	T>C	Transition	47	3037877	C>T	Transition	78	3038101	G>C	Transversion
17	3037752	T>G	Transversion	48	3037878	G>C	Transversion	79	3038102	T>-	
18	3037756	C>T	Transition	49	3037882	A>G	Transition	80	3038108	A>G	Transition
19	3037768	C>G	Transversion	50	3037888	C>T	Transition	81	3038109	A>T	Transversion
20	3037771	A>G	Transition	51	3037890	G>T	Transversion	82	3038110	T>G	Transversion
21	3037779	T>C	Transition	52	3037894	T>C	Transition	83	3038116	T>A	Transversion
22	3037780	G>C	Transversion	53	3037903	T>C	Transition	84	3038117	G>T	Transversion
23	3037783	G>C	Transversion	54	3037905	A>T	Transversion	85	3038121	A>C	Transversion
24	3037794	G>A	Transition	55	3037906	C>A	Transversion	86	3038126	A>G	Transition
25	3037795	G>C	Transversion	56	3037928	T>G	Transversion	87	3038127	C>A	Transversion
26	3037798	A>G	Transition	57	3037930	T>C	Transition	88	3038131	C>T	Transition
27	3037807	G>T	Transversion	58	3037936	G>C	Transversion	89	3038134	A>G	Transition
28	3037810	A>G	Transition	59	3037939	C>T	Transversion	90	3038136	A>C	Transversion
29	3037812	A>C	Transversion	60	3037941	G>T	Transversion	91	3038137	G>C	Transversion
30	3037813	C>T	Transition	61	3037951	C>T	Transition	92	3038141	C>T	Transition
31	3037825	C>G	Transversion	62	3037955	G>-		93	3038143	A>G	Transition
								94	3038158	G>A	Transversion

Table (4) represents comparison between local strain of *Klebsiella pneumoniae* isolated from blood, urine, sputum, and wound with the strain *Klebsiella pneumoniae* subsp. pneumoniae recorded in the National Center Biotechnology Information (NCBI) and isolated from different source swab, urine, sputum, groin, and throat/groin but the same host (Homo sapiens) have under sequence ID (gb|CP009872.1, gb|CP009863.1|, gb|CP009771.1| 17, gb|CP008831.1| 18, gb|CP008827.1| 19, gb|CP007731.1| 17, and gb|CP007727.1| 19, gb|CP008797.1| 17, respectively with source of isolation and showed compatibility 79% and_score 329 and expect 6e-86 with gene bank. However previous study [22], recorded *Klebsiella pneumoniae* subsp. pneumonia, under same sequence ID (gb|CP009771.1) but different sources of isolation, first strain KPNIH32, isolated from swab and second strain KPNIH33 from urine.

Table (4): Sequencing ID in gene bank, score, expects and compatibility of DNA sequences obtained from National Center Biotechnology Information (NCBI).

	ACCESSION	Bacteria	Strain	country	Host	Reference	Source	Compatibility	expect	Score	Range
1	gb|CP009872.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH30	USA	Homo sapiens	[19]	perirectal swab	79%	6e-86	329	3037673 to 3038166
2	gb|CP009863.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH29	USA	Homo sapiens	[19]	perirectal swab	79%	6e-86	329	3043358 to 3043851
3	gb|CP009771.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH32,	USA	Homo sapiens	[19]	perirectal swab	79%	6e-86	329	3023794 to 3024287
4	gb|CP009771.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH33	USA	Homo sapiens	[19]	urine	79%	6e-86	329	3069099 to 3069592
5	gb|CP008831.1|	Klebsiella pneumoniae subsp. Pneumonia	KPR0928	USA	Homo sapiens	[20]	sputum	79%	6e-86	329	3039068 to 3039561
6	gb|CP008827.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH1	USA	Homo sapiens	[21]	groin	79%	6e-86	329	3047535 to 3048028
7	gb|CP007731.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH27	USA	Homo sapiens	[19]	groin	79%	6e-86	329	2985742 to 2986235
8	gb|CP007727.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH10	USA	Homo sapiens	[21]	groin	79%	6e-86	329	3047538 to 3048031
9	gb|CP008797.1|	Klebsiella pneumoniae subsp. Pneumonia	KPNIH24	USA	Homo sapiens	[19]	throat/groin	79%	6e-86	329	2774431 to 2774924

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