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Whole Genome Sequence for *Klebsiella pneumoniae* Isolate from Burn Skin

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***Klebsiella pneumoniae* is a type of gram-negative bacterium that was initially discovered and isolated by Carl Friedlander in the year 1882. *Klebsiella pneumoniae* is responsible for a range of nosocomial and community-acquired illnesses, including urinary tract infections (UTIs), pneumonia, surgical site infections, and bloodstream infections. According to a number of studies, gram-negative sepsis ranks as the second most prevalent cause. A comprehensive analysis of the entire genome of *Klebsiella pneumoniae* was conducted using Illumina sequencing by synthesis (SBS) technology, specifically the Illumina HiSeq platform. The genetic material of the organism was comprised of a single circular chromosome of three thousand base pairs in length, exhibiting a GC content of 55.90%. In total, the genome exhibited a collective count of 164 genes associated with transfer RNA, 9 genes associated with ribosomal RNA, and 11,132 protein-coding sequences. Moreover, the analysis of genetic diversity entailed the application of sequence data, which was then compared to the reference genome that shared the highest degree of similarity. The genome annotation service offered by PATRIC and the RAST annotation system have successfully detected many antimicrobial resistance elements, clusters of genes associated with antibiotic resistance, as well as efflux pumps. This study represents the first known case of conducting a comprehensive genome sequencing of *Klebsiella pneumoniae* within the geographic area of Iraq, based on our current knowledge. The current investigation holds potential for advancing our comprehension of the antibiotic resistance traits demonstrated by *Klebsiella pneumoniae*, therefore enabling the healthcare facility to efficiently handle and alleviate outbreaks.**

Keywords: *Klebsiella pneumoniae*; Skin, burn; Whole genome sequencing.

Carl Friedlander isolated *Klebsiella pneumoniae*, a gram-negative bacterium, in 1882. *Klebsiella pneumoniae* causes a variety of nosocomial and community-acquired illnesses, including urinary tract infections (UTIs), pneumonia, surgical site infections, and bloodstream infections. Multiple investigations[1-3] have found that it is the second main cause of gram-negative sepsis.

Klebsiella pneumoniae is gram negative bacteria first isolate in 1882 by Carl Friedlander. It is the second leading cause of gram-negative

sepsis. Capsular polysaccharide (CPS) and a critical virulence component that is a substantial contributor in developing sepsis are both present in *K. pneumoniae* [4]. Two pathotypes, or clusters, of Klebsiella pneumoniae clinical isolates have been identified. Patients with compromised immune systems are the most common sources of classical strains (cKp), and these strains are often carbapenem-resistant (CR). On the other hand, carbapenem is effective against hypervirulent strains (hvKp), which are linked to invasive infections in the community[5, 6]. The HMV phenotype may be determined and hypervirulent *K. pneumoniae* can be distinguished from classical *K. pneumoniae* with the help of a simple microbiological test called the string test. Hyper-capsule generated by hypervirulent *K. pneumoniae* (hvKP)[7] confers the hyper-mucoviscous phenotype. Human defense mechanism expression and neutrophil and macrophage phagocytosis are both suppressed by CPS[8]. Additionally, the hyper-capsule enhances defenses against a wide variety of humoral defense mechanisms. This means that hvKP is protected against complement killing to a greater extent than cKP[9]. Siderophores are low-molecular-weight secondary metabolites that can chelate iron. To transport ferric ions across the cell membrane, these compounds use small peptide molecules with side chains and functional groups that have high affinity ligands[10]. Microbial siderophores are able to chelate iron and increase its uptake even at low concentrations thanks to the formation of a ferric-siderophore complex[11]. For *K. pneumoniae* to cause disease, siderophores are also required[5]. To thrive during an infection, *K. pneumoniae* need a small amount of iron from the surrounding environment. As part of its nonspecific immune response, the host sequesters this metal during infection in an effort to curb the spread of multiple possible pathogens. Iron in the host plasma is usually bound to transferrin and other iron transport molecules, so there isn't much free iron floating around[14]. During a bacterial infection, mammals switch iron binding to lactoferrin, a naturally occurring defense protein found in bodily fluids[12, 13]. The creation of siderophores, molecules with a higher affinity for iron than host transport proteins, is the primary mechanism by which many infections, including *K. pneumoniae*, acquire iron. Siderophores are able to scavenge iron from the environment or the host's iron-chelating proteins[14]. Enterobactin, yersiniabactin, salmochelin, and aerobactin are just few of the siderophores that *Klebsiella pneumoniae* expresses[15]. Only about 2–4% of nosocomial *K. pneumoniae* strains contain the salmochelin siderophore, which is expressed by the iroB gene. Since there are very few evidence explain the mechanism for *Klebsiella pneumoniae*, in current project try to study the mastery for multi antibiotics resistance by employ the NEXT GENERATION SEQUENCE NGS and bioinformatics tools to annotated the *Klebsiella pneumoniae* genome.

MATERIALS AND METHODS

Bacterial isolate

Standard microbiological techniques were used for isolation and identification. The bacteria grow on nutritive and selective medium after being taken from swab samples taken from individuals who suffer from burned skin. After that, the colony that displayed multidrug resistance was isolated, and DNA was extracted from this solitary colony by utilizing a GENEAID, bacterial DNA extraction kit/Korea, extracted genomic DNA the included instructions, which were followed very strictly, albeit with minor adjustments made to cut down on the amount of contamination and boost the amount of pure DNA obtained. This research aimed to get insight into the whole genome of multidrug-resistant microorganisms because so little attention has been paid to doing so in the past.

Genome sequence

Illumina genome sequencing was performed on the DNA samples (Psomagen/USA, reference order number HN00194138). After quality control (QC), samples' DNA was randomly fragmented before 5' and 3' adapter ligation for library creation. Create and sequence the library with NGS. After sequencing, raw results were evaluated for GC (percentage), total bases, and total reads. Quality filtering and Fast QC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) reduced analytical biases. We assessed data quality at the end of each cycle using the phred quality score (Q20(%) and Q30(%)). De novo contigs were built from raw readings using SPAdes v.3.5[8].

Genome analysis

The assembled genome of *K. pneumoniae* was obtained by the PATRS comprehensive

genome analysis facility[9]. The sequencing data were mapped using the reference genome of *Klebsiella pneumoniae*. The genome was fully annotated to identify functional genes within subsystem categories. Comparative mapping was conducted to analyze the findings, highlighting conserved and distinctive sequencing properties. Additionally, high-quality maps were constructed to validate and visually represent the annotated features. The removal of duplicates, identification of variations, and mapping of filtered data reads to the reference genome were accomplished by the utilization of several bioinformatics tools[10,11,12,13,14].

RESULTS

Genome quality

The genome quality was good and the coarse consistency was 96.5 and fine consistency was 55.3. as it displays in figure (1).

Antibiotics resistance profile

Antibiotic resistance was observed in the isolate. Furthermore, as shown in table (1), the isolate showed sensitivity to just nine antibiotics: ertapenem, ciprofloxacin, meropenem, imipenem, aztreonam, levofloxacin, cefoxitin, and piperacillin-tazobactam.

Table 1. Showing the resistance and sensitivity for *Klebsiella pneumoniae* isolate

Taxon ID	Genome ID	Genome Name	Antibiotic	Resistant Phenotype
573	573.37830	Klebsiella pneumoniae	tetracycline	Resistant
573	573.37830	Klebsiella pneumoniae	ertapenem	Susceptible
573	573.37830	Klebsiella pneumoniae	tobramycin	Resistant
573	573.37830	Klebsiella pneumoniae	amikacin	Resistant
573	573.37830	Klebsiella pneumoniae	ciprofloxacin	Susceptible
573	573.37830	Klebsiella pneumoniae	meropenem	Susceptible
573	573.37830	Klebsiella pneumoniae	imipenem	Susceptible
573	573.37830	Klebsiella pneumoniae	aztreonam	Susceptible
573	573.37830	Klebsiella pneumoniae	cefepime	Susceptible
573	573.37830	Klebsiella pneumoniae	levofloxacin	Susceptible
573	573.37830	Klebsiella pneumoniae	trimethoprim sulfamethoxazole	Resistant
573	573.37830	Klebsiella pneumoniae	gentamicin	Resistant
573	573.37830	Klebsiella pneumoniae	cefoxitin	Susceptible
573	573.37830	Klebsiella pneumoniae	piperacillin tazobactam	Susceptible

Table 2. Summary for the genome assembly and annotated features details

Genome Annotation Pipeline (PGAP)	Results
Total length:	11,068,038 bp
GC Content %	55.91
Number of Contigs:	167
Number of Subsystems	278
Genes (total)	2,530
CDSs (total)	5,074
Genes (coding) proteins	11,132
rRNA	9
tRNAs	134
Contig L50	13
Contig N50	264,921
Plasmids	0

Based on annotation statistics and PATRIC genomes of the same species, this genome looks to be high-quality. After readings filtering, phred quality scores of bases above Q20 and Q30 were 97.8% and 94.0 %. The Comprehensive Genome Analysis revealed 167 contigs, 11,068,038 bp, and 2,454 coding proteins in this assembled genome (Table-3). GC averages 55.90%. Figure-1 shows GC content and GC skew analysis schematically. A protein subsystem implements a biological activity or structural complex. 278 genome-specific subsystems were identified during annotation. Figure-2 provides a genome subsystem overview.

Subsystem Analysis

A subsystem is a set of proteins that together implement a specific biological process

or structural complex and PATRIC annotation includes an analysis of the subsystems unique to each genome. An overview of the subsystems for this genome is provided in Figure 2.

Phylogenetic Analysis

PATRIC offered reference and representative genomes for phylogenetic research. Figure-3 shows the closest reference and typical genotypes.

Table 3. Summary proteins features details

Proteins features	Patrics
Hypothetical proteins	3053
Proteins with functional assignments	8079
Proteins with EC number assignments	0
Proteins with GO assignments	1928
Proteins with pathway assignments	0
Proteins with Subsystem assignments	0
Proteins with PATRIC genus-specific family (PLfam) assignments	5374
Proteins with PATRIC cross-genus family (PGfam) assignments	10435

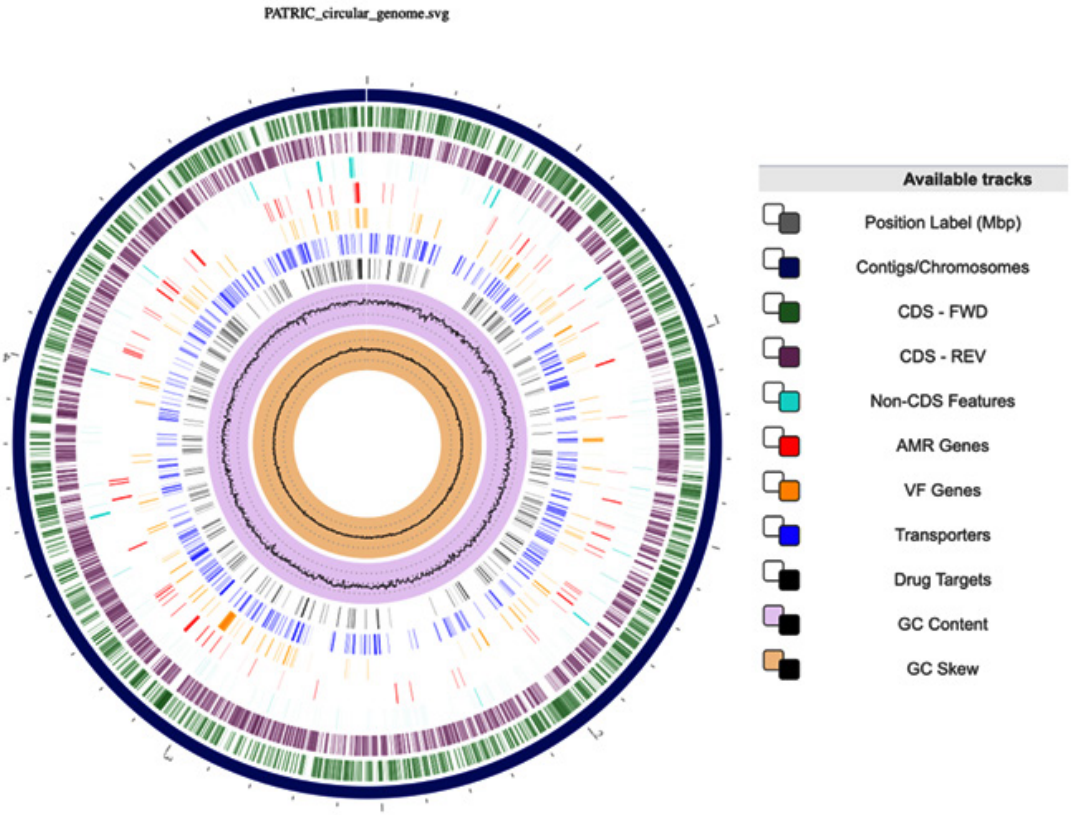

Fig. 1. *K. pneumoniae* circular genome. The red ring indicates genomic backbone (contigs) while the innermost ring reflects chromosomal position. A- GC content (black) and GC skew (green) are larger than the genome average, whereas purple is less. B- The forward and reverse strand open reading frames. The colors of the CDS on the forward and reverse strand indicate the subsystem that these genes belong to (see Subsystems below). This genome has too many contigs to be rendered clearly. The circular display has been limited to the 90 longest contigs of the 352 contigs in the genome.

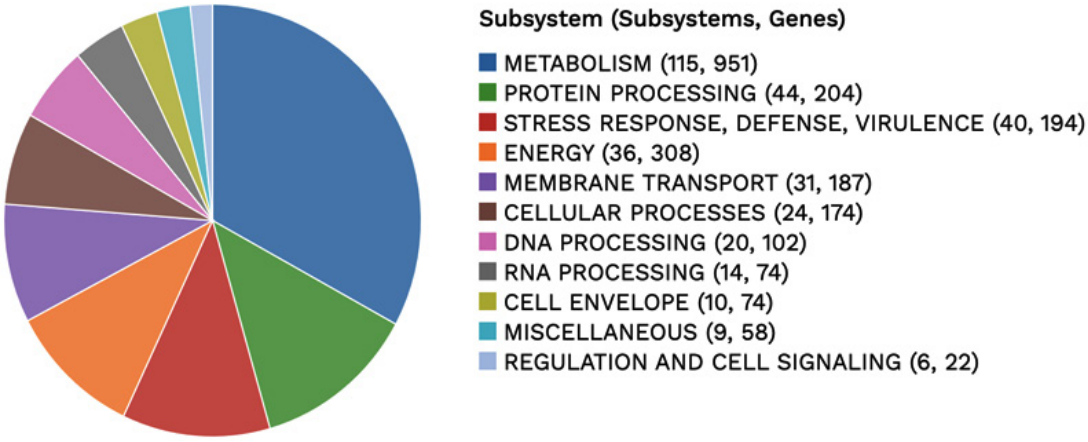

Fig. 2. Subsystem distribution of *Serratia marcescens*. RAST annotated the genome. The pie chart and SEED viewer showed subsystem feature counts and coverage. The green bar of the subsystem coverage shows the fraction of proteins in the subsystems, while the blue bar shows those omitted

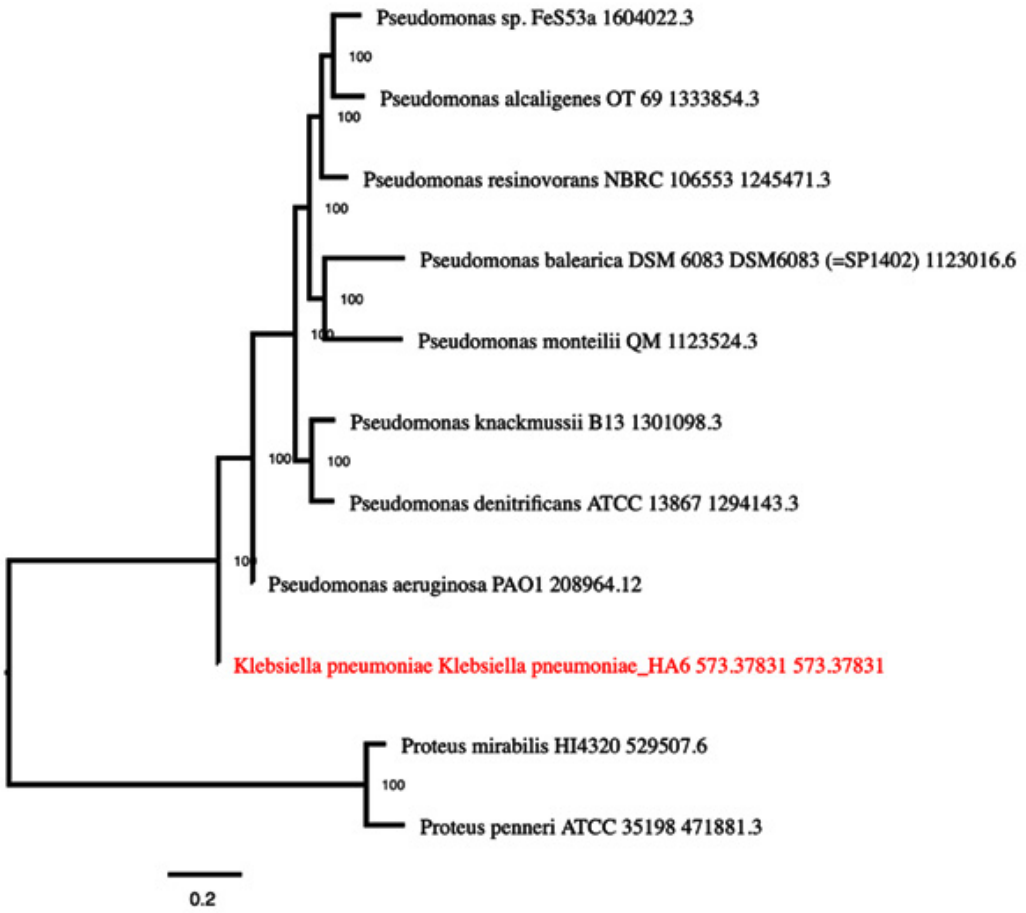

Fig. 3. phylogenic relationship representation of the *K. pneumoniae*

DISCUSSION

Gram-negative bacilli infections are the most dangerous and potentially fatal infectious illness causes in hospitalized patients[1, 2]. *K. pneumoniae* is an opportunistic pathogen that causes pneumonia, abscess, bacteremia, and urinary tract infections in both community-acquired and nosocomial infections[3]. *K. pneumoniae* has become a global problem due to its propensity to rapidly acquire antibiotic resistance, requiring action to avoid the spread of multidrug-resistant bacteria[4]. Whole-genome sequencing (WGS) has grown more accessible and economical in recent years, leading in increased application in a variety of domains, including clinical microbiology[5-7]. One of the primary advantages of employing WGS is the ability to characterize the genetic content of clinically relevant bacteria and tie it to virulence-associated phenotypes, allowing for a better understanding of their transmission within the hospital and the use of timely medicines. The current investigation intended to describe *K. pneumoniae* isolates from burns skin patients hospitalized in Baghdad City. This study consider as is the first study in Iraq to use WGS to characterize an opportunistic pathogen in depth.

CONCLUSION

In conclusion, our study is the first in Iraq to analyze the genome of *K. pneumoniae*. Furthermore, the *K. pneumoniae* genome was compared to those of clinical reference strains, and its display of antibiotic resistance and virulence genes was identified. More whole genome sequencing and comparative genomics research is needed to better understand the background and assessment of multidrug-resistant isolates from different hospital rooms with special attention on delivery room.

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Author Contributions

H. A, N.A and S.J designed the study and performed the experiments. In addition, both authors analyzed the data and write the manuscript.

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Institutional Review Board Statement

Not applicable since the study not involving humans or animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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