Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 11 November 2024

Research Article

Unveiling Genetic Insights of *Klebsiella Pneumoniae* with Whole Genome Sequence Analysis

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DOI: 10.31080/ASMI.2024.07.1449

Received: September 30, 2024

Published: October 30, 2024

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Abstract

The emergence of secondary infections, particularly among post-COVID patients, presents a challenge to healthcare systems. Among post Covid infections, Klebsiella pneumoniae is a significant pathogen, by contributing increased morbidity and mortality rates. In this study, conducted a comprehensive investigation into the genetic characteristics and clinical relevance of K. pneumoniae samples were isolated from urine and sputum of post-COVID patients. Through cultural, biochemical, and genetic analyses, confirmed the identity of K. pneumoniae serotype O2a and elucidated its genomic architecture, antibiotic resistance profiles, and virulence determinants. Whole genome sequencing (WGS) revealed an in-depth of genomic features, including coding sequences, RNA elements, and mobile genetic elements. Analysis of antibiotic resistance genes highlighted the potential for multidrug resistance islands, while the presence of 16 virulence genes, Bio film genes, 114 Pathogenic families, 5 plasmids and 608 transposons, highlighted the pathogenic potential of this isolates. Phylogenetic analysis provided insights into the evolutionary relatedness of the isolates, while the identification of 1 complete virus, 7 prophage regions and 7 viral signal detections indicated genomic plasticity and possible horizontal gene transfer events. The research findings contribute to deeper understanding of genetic basis as well as clinical implications of K. pneumonia infections in post-COVID patients, emphasizing the importance of targeted therapeutic strategies and infection control measures in mitigating the spread of multidrug-resistant pathogens in vulnerable patient populations.

Keywords: Post COVID infections, Klebsiella pneumoniae, Whole genome sequencing, Antibiotic resistance

Introduction

The emergence of *Klebsiella pneumoniae* as a significant pathogen has long been recognized, posing challenges to patient care and infection control measures [1-2]. With the global healthcare issues evolving in response to the COVID-19 pandemic, the incidence of secondary infections, particularly among post-COVID patients have momentum [3]. Among these secondary infections, *Klebsiella pneumoniae* has been identified as a significant cause of illness and fatality, causing problems to already complex clinical scenario [4]. Understanding the molecular mechanisms of the virulence factors and antibiotic susceptibility of K. pneumoniae are crucial for devising effective therapeutic strategies. Whole genome sequencing (WGS) has now become a highly effective tool for describing genetic blueprint of pathogens, offering unparalleled insights into their virulence factors, evolutionary dynamics, and resistance determinants [5]. In the context of post-COVID patients, the application of Whole Genome Sequencing to study *K. pneumoniae* may assist for unraveling the mechanism of its adaptation and pathogenesis in this vulnerable population [6]. This research paper aims to explore the genetic landscape of *K. pneumoniae* isolated from post-COVID patients through comprehensive Whole Genome Sequencing analysis with the help of whole genome data and bioinformatics tools, it is sought to characterize the genomic diversity, virulence ability and antimicrobial susceptibility pattern of this clinical isolate. Furthermore, aim to find any unique genomic signatures or adaptive traits that may distinguish *K. pneumoniae* strains associated with

post-COVID infections. The outcomes of this study are believed to contribute to the understanding of the molecular epidemiology of *K. pneumoniae* in relation to COVID-19 pandemic. Moreover, understanding genomic analyses hold the potential to enlighten clinical management practices, infection control strategies, and the development of targeted therapeutics to mitigate the burden of *K. pneumoniae* infections in post-COVID patients. The current study provides insight for understanding the development of multidrug resistance and high virulent strains.

Materials and Methods

Isolation of bacteria and growth conditions

Samples: Sputum and urine samples of post COVID-19 patients were collected from Krishna Institute of Medical Sciences (KIMS), Hyderabad, India after approval of a written Consent.

Five *Klebsiella pneumoniae* strains were obtained from patients that tested positive for COVID-19 that were collected from KIMS hospital, Hyderabad, India. Among these, two of the strains were isolated from sputum and three were from the urine samples.

These bacterial isolates were first grown on MacConkey agar and further sub-cultured onto Luria-Bertani agar plates at 37°C [7-8].

Characterization

Morphology

Morphological characteristics were performed by Colony morphology on MacConkey agar and microscopic morphology after gram staining.

Biochemical tests

The following are the biochemical tests performed.

Citrate Utilization test, Urease test, Indole test, Methyl Red test, Voges-Proskauer test, Triple Sugar Iron Agar (TSI) test, and Oxidase test.

Antibiotic sensitivity assay

Antibiotic sensitivity assay was performed on Muller Hinton Agar. *K. pneumoniae* was spread and placed 10mg antibiotic discs of Ampicillin, Tetracycline, Cephalosporin, Monobactam, Sulfonamide, Carbapenem, Nitroimidazole, Rifamycin, Macrolide, Fluoroquinolone, Ceftazidime, Norfloxacin, Elfamycin, Cefepime, Chloramphenicol, Levofloxacin, Streptomycin, Kanamycin, Augmentin, Gentamycin and Penicillin-G.

A solution of Augmentin antibiotic was prepared by mixing 20 mg of Amoxicillin with 10 mg of Potassium Clavulanate. The solution was added to wells, and plates were incubated at 37°C for 24 hrs to observe the zone of inhibition by the antibiotics.

Bacterial DNA isolation

Bacteria was been inoculated in Luria- Bertani broth and was incubated in the orbital shaking incubator for 18hrs at 37°C and 100 RPM. This was followed by centrifugation of the bacterial culture at 5000 RPM for ten minutes for pelleting of cells. DNA was extracted using QIAamp BiOstic Bacteremia DNA kit (QIAGEN Germany) and manufacturer's protocol was followed. The extracted DNA was then placed at -20°C till the time it was required for library preparation.

Preparation of library and next generation sequencing

Preparation of library was carried out by using the Nextera XT DNA Library Preparation kit (Illumina, USA). DNA was extracted, amplified and disrupted by using transposons present into the Nextera XT Kit [9]. Individual adapters were provided for every sample to label appropriately. PCR reaction was performed with the program of 12 cycles of amplification of DNA fragments that were tagged with primers and indices for the generation of dual-indexed sequencing of pooled libraries. After sample normalization, pooling was made and next 300-base paired-end reads sequencing was carried on Illumina (Novaseq 6000), 150PE instrument. Starting from preparation, followed by the sequencing, all the procedures has been done according to the manufactures' guidelines.

Whole genome sequences assembly

The reads that passed through the quality check were used to reconstruct the genomes into contigs using Megahit v1. 2. 9 with k-mer sizes of 21, 49, 77, 105, 133 and 141 [10]. Contigs with length less than 200bp was excluded from the assembly. The assembled genome statistics were examined by of QUAST v5. 0. 2 [11].

Genes annotation

PROKKA tool was used to generate genes annotation of the prokaryotic genomes and predict the coding regions within these genomes (Prokka Software 1.14.6, Version (Proksee)1.1.1 [12].

Genome completeness

Assessment of the genome completeness was done using BUSCO v5. 3. 2 [13] with bacteria_odb10 as the reference. BUSCO is a programme that that gives a qualification of the expected gene content of a certain genome assembly. Circular genome was constructed by Proksee [14].

GC content and GC skew, which is the relative excess of G nucleotides over C nucleotides in the leading strand of the eubacteria replications was evaluated by the toolkit Proksee 1. 0. 2.

MLST Species identification

Species identification was undertaken through Ribosomal Multi locus Sequence Typing (rMLST) [15].

GC content

GC Content and GC skew i. e the relative amount of G nucleotides over C nucleotides on the leading and lagging replication strands eubacteria was analysed with the help of toolkit Proksee 1.0.2[14].

Phylogenetic analysis

Phylogenetic analysis was analysed by the FASTA sequences that were aligned using Muscle and the Neighbour Joining phylogenetic tree was bulit with bootstrap values of 1000 using MEGA7.0 [16-17].

Virus identification

PHASTER tool was used to detect the prophage points in genomic sequence [18].

Virus entry points detection

Phigaro tool was used to identify prophage regions by processing raw genome as input [19].

Viral signal detection

Virsorter 1.1.1 tool was used to detect viral signal in this microbial sequence data [20].

ORF (Open Reading Frames)

Proksee 1.0.0 bioinformatics tool was used for identification of open reading frames in microbial genomic sequence.

Comprehensive Antibiotic Resistance Database (Card) - Resistance Gene Identifier

CARD RGI 6.0.2 Bioinformatical tool of Proksee 1.2.0 was used to identify the antibiotic resistance genes [21].

Biofilm formation

Biofilm producing genes were predicted by using the KEGG Annotation [22].

Virulence genes

The virulence genes were identified by VFDB 2022 Toolkit [23].

Mobile elements or transposons

Mobile elements were analysed by Software mobileOG-db (beatrix-1.6) of Proksee 1.1.3 [24].

Capsular typing

The capsule and lipopolysaccharide serotype prediction were performed using Kaptive v2.0.4 database [25].

Pathogen detection

The pathogenicity was predicted using PathogenFinder v1.1 [22].

Toxin detection

The presence of toxin synthesis genes in the genome were predicted using ToxFinder v 1.0 server hosted by DTU, UK [26].

Plasmid finder

The presence of Plasmids in the genome were predicted using PlasmidFinder-2.0 Server [27].

SNP

Single nucleotide polymorphism is predicted by GATK (Genome Analysis Toolkit) [28].

Statistical analysis

The Experiments were repeated thrice in triplicate (n = 9) and values with standard deviation are presented.

Results

The five bacteria from sputum and urine samples were isolated on MacConkey Agar were identified as *Klebsiella spp*. It is observed that the colonies were large, mucoid, dark pink, indicating lactose fermentation.

Bacterial isolates were gram negative and rod shape under the microscope. Biochemical analysis results showed Indole negative, Methyl Red test negative and positive for Voges-Proskauer (VP) test. The Triple Sugar Iron Agar (TSI) test showed a yellow-coloured butt, with cracks of the agar, indicating fermentation of glucose, lactose, and sucrose with acid production. The upper part (slant) of the agar remained red, suggesting alkaline conditions due to peptone utilization. Additionally, there was no blackening of the medium was observed, indicating the bacterium does not produce hydrogen sulfide.

Moreover, isolates were urease positive and negative in the oxidase test.

By above biochemical tests it is confirmed that the present study isolates were identified as *K. pneumoniae.*

Antibiotic sensitivity

The bacterial strain *K. pneumoniae* was found to be resistant to majority of drugs tested (Table 1).

It is observed that the strain was sensitive to Streptomycin, Kanamycin and Augmentin.

Antibiotic tested	Zone of inhibition (mm)
Ampicillin	04 ± 0.02
Cephalosporin	04 ± 0.04
Macrolide	05 ± 0.03
Tetracycline	03 ± 0.01
Monobactam	05 ± 0.03
Carbapenem	04 ± 0.03
Sulfonamide	09 ± 0.05
Nitroimidazole	05 ± 0.04
Macrolide	06 ± 0.05
Rifamycin	05 ± 0.03
Fluoroquinolone	04 ± 0.03
Elfamycin	05 ± 0.02
Ceftazidime	03 ± 0.01
Cefepime	05 ± 0.04
Norfloxacin	08 ± 0.03
Levofloxacin	05 ± 0.02
Chloramphenicol	05 ± 0.04
Streptomycin	26 ± 0.01
Augmentin	24 ± 0.04
Kanamycin	22 ± 0.03
Penicillin-G	06 ± 0.01
Gentamycin	08 ± 0.02

Table 1: Antibiotic-sensitivity profile of *K pneumoniae* zone of inhibition (mm) with 30mg concentration.

Whole Genome Sequence submission to NCBI

The resulted Whole Genome sequence was been submitted NCBI and the Accession number of present study *K. pneumoniae* strain is SAMN41088543.

Genome Annotation

Genome assembly was annotated by Prokka. The annotation statistics are provided below.

- Number of contigs: 4
- Number of bases: 5681040
- Number of Coding sequences (CDS): 5474
- Number of misc_RNA: 146
- Number of rRNA: 25
- Number of tRNA: 88
- Number of tmRNA: 1

Genome Completeness

The genome completeness was analysed using BUSCO v5.3.2 with bacteria_odb10 as reference. 99.8% of the genes are present (Figure 1).

MLST Species identification:

The species was identified by Ribosomal Multilocus Sequence Typing (rMLST). rMLST results assessed that sample belongs to *K. pneumoniae* (Table 2).

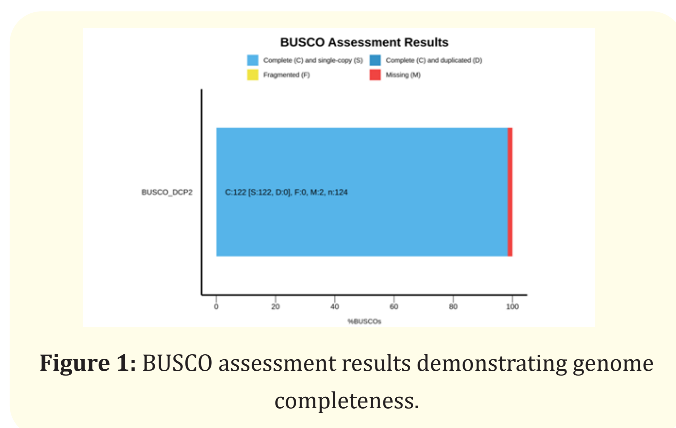

Figure 1: BUSCO assessment results demonstrating genome completeness.

Rank	Taxon	Support	Taxonomy
Species	Klebsiella pneumoniae	100%	*Pseudomonadota>Gammaproteobacteria> Enterobacterales> Enterobacteriaceae> Klebsiella> Klebsiella pneumoniae*

Table 2: Species identification done by rMLST species identification predicted taxa.

GC Content and GC Skew

57.12 % of GC content with rich GC in few regions was observed (Figure 2, Table 3).

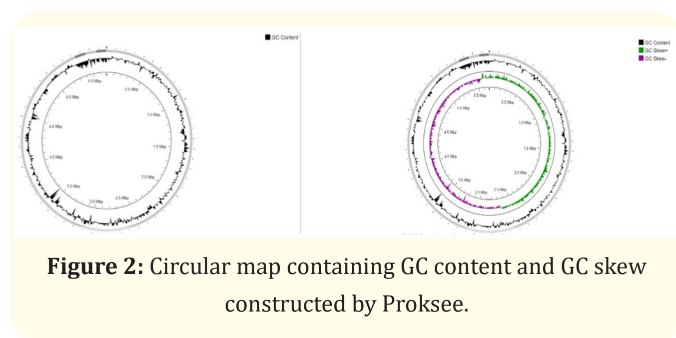

Figure 2: Circular map containing GC content and GC skew constructed by Proksee.

Base	Count	Percentage
A	1219348	21.46
C	1623057	28.57
G	1622127	28.55
T	1216507	21.41

Table 3: Nucleotide base count and percentage.

Phylogenetic analysis

Phylogenetic analysis for the reference genome (Figure 3) showed that isolated strain DCP2 is similar to *Klebsiella pneumoniae.*

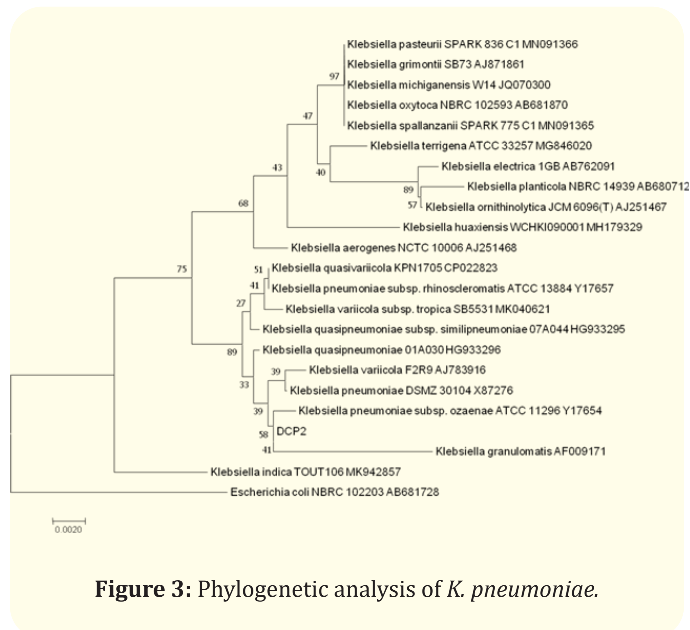

Figure 3: Phylogenetic analysis of *K. pneumoniae.*

Virus identification (PHASTER)

Totally 1 intact prophage region has been identified at region 1 of length 122.5Kb having completeness score of intact (150).

Virus entry points

Seven (7) Prophage regions were identified in the given nucleotide sequence by using the tool Phigaro 2.3.0 of Proksee 1.0.1 [19].

Viral signal detection

Seven (7) viral signal detections were identified in the given nucleotide sequence by using the tool Software VirSorter 2.2.4 [20] of Proksee 1.1.1.

Open reading frames

The total ORF's identified in the nucleotide sequence of *K. pneumoniae* of this study is 18678 (Figure 4).

Antibiotic resistance genes

The genes for antibiotic resistance were predicted from genome analysis by CARD database, major antibiotics found to be resistant were rifamycin, carbapenem, aminocoumarin, cephalosporin, tetracycline, macrolide, monobactam, cephamycin, sulfonamide and various other drugs (Figure 5).

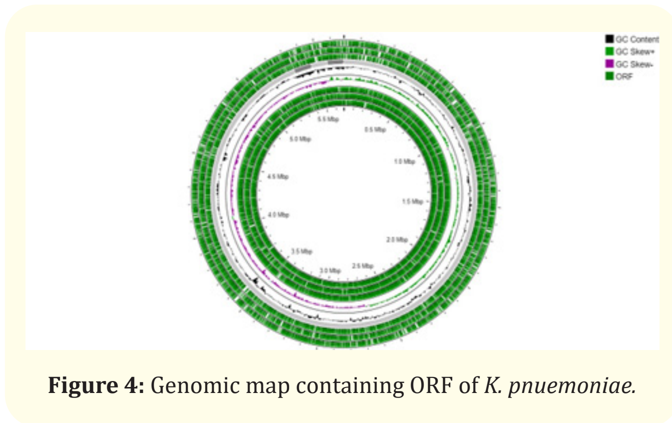

Figure 4: Genomic map containing ORF of *K. pnuemoniae.*

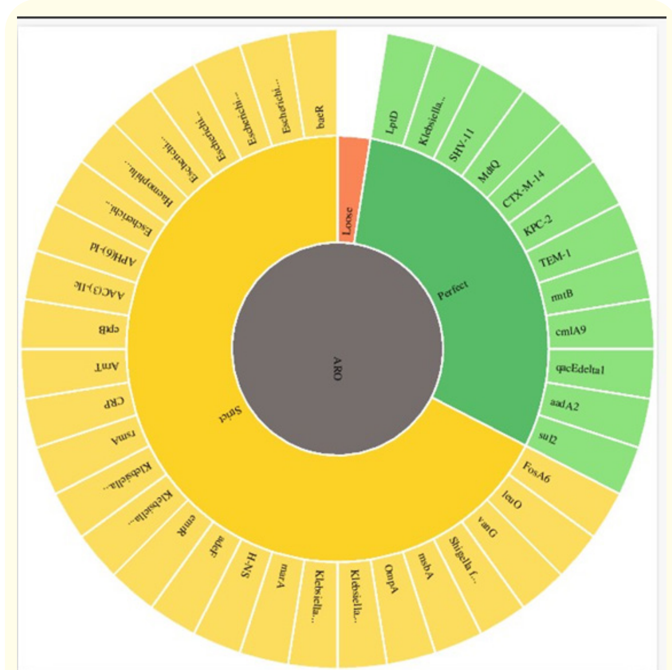

Figure 5: Representation of antibiotic resistance genes by CARD.

Islands of antibiotic resistance gene islands:

The antibiotic resistance genes which are closely associated towards each other which are within less than 1.5kb length, forms antibiotic resistance gene islands TEM-1, rmtB, Cm1A9, aada2, qacEdeltal, AA (3)-11C, Su12, APH 16)-Id. TEM – 13, CTX-M-14, reside closely within 1.5 kbp length forming antibiotic gene island (Figure 6).

The circular genomic map was generated by using IslandViewer4 describing the location of genomic islands in the genome. The circular genomic map was observed (Figure 7).

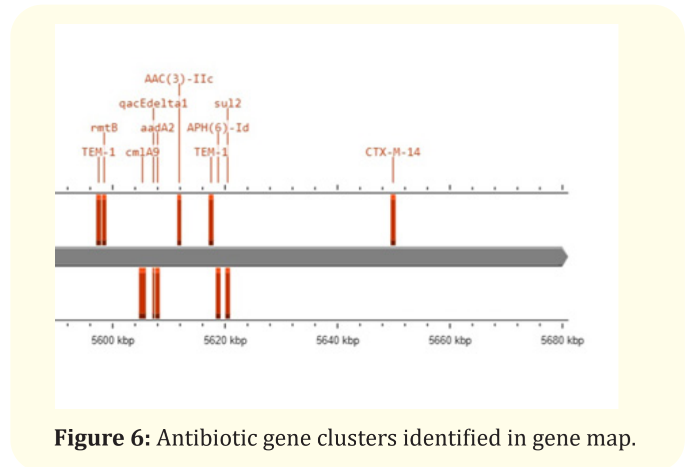

Figure 6: Antibiotic gene clusters identified in gene map.

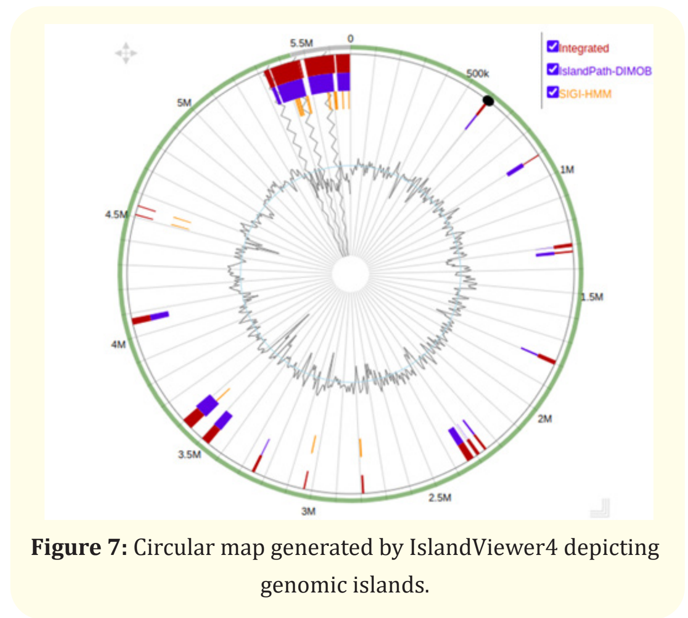

Figure 7: Circular map generated by IslandViewer4 depicting genomic islands.

Biofilm associated genes

The biofilm production genes are predicted by performing the KEGG Annotation [22] and the map is given in Figure 8. The strain is found to make biofilm.

Virulence genes

Sixteen (16) virulence genes were detected from the isolated bacterial nucleotide sequence, the virulence genes were Type 3 fimbriae, Type I fimbriae, Capsule, AcrAB, Aerobactin, Entsiderophore, Salmochelin, Yersiniabactin, Allantoin utilization, RcsAB, RmpA, T6SS-I, T6SS-II, T6SSS-III, LPS rfb locus, Colibactin.

Mobile elements

608 Mobile elements were identified.

Capsular typing

The results shows that the given strain belongs to O-locus ie., O2a (Figure 8,9)

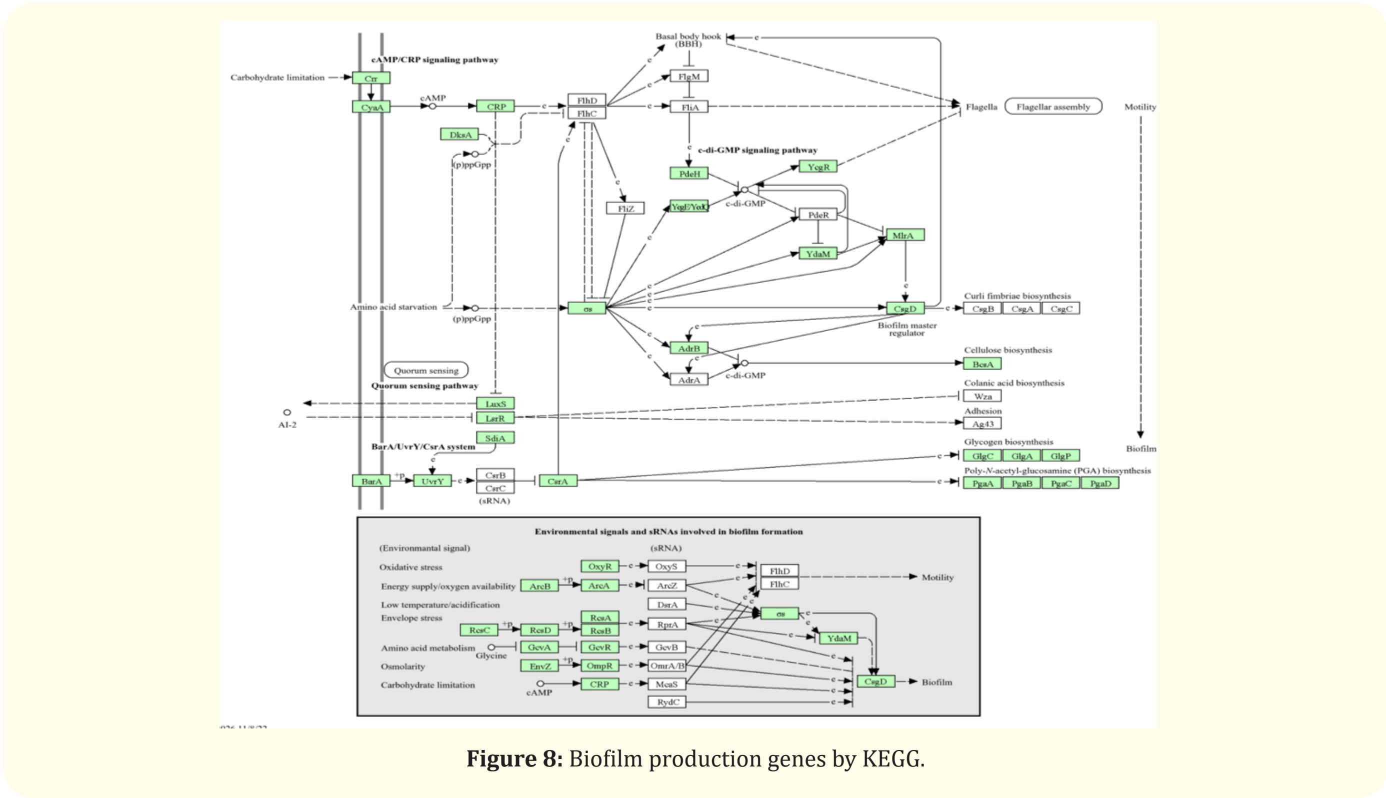

Figure 8: Biofilm production genes by KEGG.

Sample | Results | Download raw results table (TXT) | Download raw results (JSON)

DCP2 | Best locus: O1/O2v1 | Best type: O2a | Match confidence: Very high | Cov: 100.00% | ID: 97.72% | Genes: 7 / 7

O1/O2v1 reference:

wzm Cov: 100.00% ID: 100.00%
wzt Cov: 100.00% ID: 99.19%
wbbM Cov: 100.00% ID: 99.21%
glf Cov: 100.00% ID: 98.44%
wbbN Cov: 100.00% ID: 97.65%
wbbO Cov: 100.00% ID: 98.68%
kfoC Cov: 100.00% ID: 96.58%

Other genes found in locus: 0 | Other genes found outside locus: 4

Assembly pieces: Download as FASTA | O1/O2v1 reference size: 8064 | Length discrepancy: -2 bp

Contig name	Start position	End position	Length
DCP1	3543739	3551800	8062

Figure 9: Capsular typing results done by Kaptive v2.0.4.

Pathogen detection

- Min Identity Threshold: 96.927
- Z-THRESHOLD: 3.0
- Prediction Score: 351.922
- Probability of being human pathogen: 0.798
- Matches: 127
- Genome Coverage (%): 2.32
- Pathogenic Families Matched: 114
- Non-Pathogenic Families Matched: 13
- The organism is predicted as human pathogenic: Yes

Toxin detection

The genome showed no toxin genes. However, the genome showed part of genes encoding for the following toxins

- mRNA interferase toxin RelE
- Serine/threonine-protein kinase toxin HipA
- Orphan toxin OrtT
- Ribosome association toxin RatA
- Putative toxin HigB2

Plasmid finder

Five (5) plasmids were identified by PlasmidFinder-2.0. and presented in Figure 10.

PlasmidFinder-2.0 Server - Results

Organism(s): *Enterobacteriales*

Enterobacteriales						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
Col(KPHS6)	100	178 / 178	NC_016839.1 Klebsiella pneumoniae subsp. pneumoniae HS11286 plasmid pKPHS3, complete sequence	112796..112973		NC016841
IncC	100	417 / 417	NC_016839.1 Klebsiella pneumoniae subsp. pneumoniae HS11286 plasmid pKPHS3, complete sequence	103602..104018		JN157804
IncFIB(pKPHS1)	100	560 / 560	NC_016838.1 Klebsiella pneumoniae subsp. pneumoniae HS11286 plasmid pKPHS1, complete sequence	201..760		CP003223
IncFII(K)	98.65	148 / 148	NC_016846.1 Klebsiella pneumoniae subsp. pneumoniae HS11286 plasmid pKPHS2, complete sequence	39090..39237		CP000648
IncR	99.2	251 / 251	NC_016846.1 Klebsiella pneumoniae subsp. pneumoniae HS11286 plasmid pKPHS2, complete sequence	110833..111083		DQ449578

Figure 10: Number of Plasmids identified by PlasmidFinder-2.0.

SNP

93 SNPs are found.

Discussion

The detailed characterization of *Klebsiella pneumoniae* isolated from samples of post-COVID patients provides valuable information into the genetic makeup, antibiotic islands and virulence, pathogenesis of this clinically significant pathogen. The combined microbiological, biochemical, and genomic analyses explain the pathogenicity, antibiotic resistance, and potential virulence factors associated with these isolates. In this study *K. pneumoniae* was isolated from post COVID patients, collected from KIMS hospital. Two of them were isolated from sputum and three from the urine samples. Sheng., *et al*. (2023) [29] isolated eight *K. pneumoniae* isolates from various clinical specimens (urine, sputum, throat, blood, and stool) from three hospitals in Armenia between January 2019 and August 2019. Similarly, Runcharoen., *et al*. (2017) [30] isolated and sequenced *K. pneumoniae* from patients from hospital located in Thailand, hospital sewage, and surrounding canals. Whereas, Wylie., *et al*. (2019) [31] isolated *K. pneumoniae* from a 26-year-old female with a history of recurring urinary tract infections, as part of a larger prospective study of women with recurrent urinary tract infections.

The confirmation of isolated *K. pneumoniae* through both cultural and biochemical methods explain the accuracy and reliability of the identification process. The distinct morphological characteristics observed on MacConkey Agar, along with positive results in biochemical tests, confirms the identity of the isolates as *K. pneumoniae*. Furthermore, molecular techniques such as rMLST further confirm the species identification. Multilocus sequence typing (MLST) was performed using Ribosomal Multilocus Sequence typing (rMLST)and it results that the given nucleotide sequence belongs to the organism *Klebsiella pneumoniae* of having taxonomy i.e., Psuedomonadota>Gammaproteobacteria>Enterobacterales> Enterobacteriaceae>klebsiella >klebsiella pneumonia. However, Sheng., *et al*. (2023) [29] reported that *K. pneumoniae* ARM01 belonged to sequence type 967 (ST967), capsule type K18, and the O

antigen type O1 genotype. Whole genome sequencing (WGS) and subsequent bioinformatic analyses disclose the genetic structure of *K. pneumoniae* isolates. The genome assembly and annotation reveal a depth of genomic features, including coding sequences (CDS), RNA elements, and mobile genetic elements. Several antibiotic resistance genes and making clustered islands highlight the potential for these isolates to transfer of multidrug resistance, posing challenges to treatment regimens.

The prediction of antibiotic resistance genes using the CARD database disclose the molecular basis of resistance mechanisms within the *K. pneumoniae* genome. The identification of closely associated antibiotic resistance gene islands indicates the potential for horizontal gene transfer and the dissemination of resistance. Additionally, the presence of mobile elements further facilitates the adaptability and evolutionary potential of these pathogens in response to antibiotic selective pressures. In the whole genome analysis, many resistant genes which are resistant to various Antibiotics such as fluoroquinolone, cephalosporin, glycylcycline, penam, tetracycline, rifamycin and phenicol were observed. Sheng., *et al*. (2023) [29] revealed that isolates belonged to sequence type 307 (ST307), with ST37, ST147, ST807, and ST967 were resistant to ampicillin, amoxicillin- clavulanic acid, ceftazidime and cefepime and sensitive to meropenem. Enany., *et al*. (2022) [32] identified resistant genes aph(3'')-Ib, aph(6)-Id, blaTEM -234, fosA, fosA6, oqxA, oqxB, sul2 and tet(D) in *Klebsiella pneumoniae*, these genes are responsible for antibiotic inactivation, antibiotic efflux, and antibiotic target replacement resistance mechanisms. The most predominant resistance mechanism identified is the antibiotic inactivation by aph(3'')-Ib and aph(6)-Id responsible for aminoglycoside phosphotransferase that has a prominent role for aminoglycoside resistance. Present study had similar resistance observed as the isolate contained aph(6)-Id gene responsible for aminoglycoside resistance due to antibiotic inactivation mechanism. In this Genome analysis reported 608 Mobile elements were identified on other hand Enany., *et al*. (2022) [32] reported thirteen insertion sequences with the analysis using ISfinder IS1R, IS1X2, IS26, IS903B, ISEc15, ISEcl1, ISKpn1, ISKpn14, ISKpn26, ISKpn28, ISKpn49, ISKpn54, and ISSen9.

In another study by Wang., *et al*. (2023) [33] 29 class-1 integrons with 12 different groups of gene cassette arrays among 17 complete genomes. Antibiotic genes were clustered together to make antibiotic resistance islands, were also reported by Wang., *et al*. (2023) [33]. These antibiotic resistance islands are responsible for joint transfer of antibiotic resistance genes in to sensitive strains and making then multi drug resistance strains. In this genomic analysis genomic islands were identified containing antibiotic resistance genes TEm-1, rmtB, qacEdeltal, aadA2, cm1A9, AAC (3)-IIc, sul2, APH (6)-Id residing closer.

The detection of numerous virulence genes indicates the pathogenic potential of these *K. pneumoniae* isolate. The absence of complete toxin genes, while notable, does not evidenced by the presence of other virulence factors and incomplete toxin genes. Moreover, the prediction of pathogenicity based on genomic analysis confirms the clinical significance of this isolate in causing human infections. In this study 16 virulence genes were detected from the given nucleotide sequence which include Type 3 fimbriae,Type I fimbriae ,Capsule ,AcrAB, Aerobactin, Ent siderophore, Salmochelin, Yersiniabactin, Allantoin utilization, RcsAB, RmpA, T6SS-I, T6SS-II, T6SS-III, LPS rfb locus, Colibactin. Sheng., *et al*. (2023) [29] identified virulence factor, yagZ/ecpA, in all *K. pneumoniae* ST967 isolates. ERR7672084 (recovered from Switzerland) and ERR4782243 (recovered from Philippines) contained additional aerobactin-associated genes: iucC and iucB. On other hand Enany., *et al*. (2022) [32] identified 65 virulence factors from the four samples belonged to ST 627. In this study the Capsular typing results showed that the given strain belongs to O-locus ie., O2a. Enany., *et al*. (2022) [32] recorded more than 130 capsular types based on their genomic data of *K. pneumoniae*.

Phylogenetic analysis showed that the given sequence was similar between *K. pneumoniae subsp*. Ozaenae ATCC 11296Y17654 and *Klebsiella granulomatis* AF009171. Sheng., *et al*. (2023) [29] showed ArM01 was phylogenetically related to two of the human isolates, forming a single monophyletic cluster. The identification of intact prophage regions and viral signal detections indicates the genomic plasticity and potential for phage-mediated horizontal gene transfer, which may contribute to the evolution and adaptation of these pathogens in diverse ecological niches. In this study one intact prophage regions have been identified at region 1 of length 122.5Kb having completeness score of intact (150) whereas Enany., *et al*. (2022) reported intact 3 bacteriophages in Entero p88, in sample K75 2 bacteriophages were identified. Five plasmids are reported in present study. Wang., *et al*. (2023) [34] reported Eight

plasmids consisiting relatively high nucleotide sequence similarity (coverage ≥80% and identity ≥90%) with pKP167-261 obtained from the NCBI nucleotide database. The findings presented in this study have significant clinical inferences for the management and treatment of infections caused by *K. pneumoniae* in post-COVID patients. Understanding the genomic determinants of virulence, pathogenicity and antibiotic resistance islands can inform targeted therapeutic strategies and infection control actions to regulate the spread of multidrug resistant pathogens.

Conclusion

In conclusion, the multidisciplinary approach employed in this study, combining microbiological, genomic, and bioinformatic analyses, provides a comprehensive understanding of genetic basis and clinical relevance of *Klebsiella pneumoniae* isolates in post-COVID patients. By understanding of interplay between virulence, pathogenicity, antibiotic resistance islands, and genomic plasticity, this research contributes to the broader efforts aimed at combating infectious diseases and improving patient outcomes in the post-pandemic era.

Data Availability

The data has been submitted to NCBI with the Accession number of SAMN41088543.

Conflict of Interest

Authors declare no conflict of interest.

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