WHOLE GENOME SEQUENCE ANALYSIS OF MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE* STRAIN ISOLATED FROM URINARY TRACT INFECTION

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Abstract

Antimicrobial resistance (AMR) is a significant public health issue, as it can lead to prolonged illnesses, increased healthcare costs, and higher mortality rates. It is also a global health security threat, as resistant infections can spread rapidly across borders, making it challenging to control outbreaks and pandemics. To combat AMR, it is essential to promote responsible use of antimicrobial drugs, such as only prescribing them when necessary, and completing the full course of treatment. Urinary tract infections are the most common infectious disease, it is estimated that more than 150 million UTIs in the world reported per year and it bears as economic and medical burden worldwide and about 35% of healthy individuals suffer from symptoms of UTI at some stages in their lives. Multidrug resistance is a significant public health concern worldwide, as it makes the treatment of infectious diseases more challenging, costly, and sometimes impossible. This study aimed to investigate the antimicrobial resistance mechanism towards MDR *Klebsiella pneumoniae* spp. Whole genome sequencing (WGS) was carried out on most common Gram-negative pathogen *Klebsiella* spp. through Next Generation Sequencing (NGS) platform by using an instrument Ion Torrent S5 (540 Chip). The draft genome was annotated using RAST tool kit (RASTtk) and assigned a unique genome identifier of 573.52981. Antimicrobial resistance genes were identified using ResFinder, AMRFinderPlus (NCBI) and BV-BRC (PATRIC) Pipeline. WGS analysis detected several antimicrobial resistance genes like ESBL genes, including carbapenems (*blaSHV-11, blaTEM-1, ompK36TD, blaOXA-181, blaCTX-M-15, blaSHV-67*). Furthermore, quinolone resistance including *oqxA/oqxB, gyrA_S83I, parC_S80I, qnrB.* In addition to aminoglycoside modifying enzymes genes (*rmtF1, aac(6')-Ib, aph(6)-Id, aph(3'')-Ib*), trimethoprim (*dfrA12*), Sulfonamide (*sul2*), fosfomycin (*fosA*) resistance genes were identified.

Keywords: AMR, MDR, UTIs, Whole genome sequence, *Klebsiella pneumoniae*

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Introduction

Antimicrobial resistance (AMR) is a growing concern in the field of medicine, and it poses a significant threat to public health worldwide. Urinary tract infections (UTIs) are among the most common bacterial infections, and uropathogens are frequently involved in the development of these infections. Antimicrobial resistance genes (ARGs) have been identified in uropathogens and are responsible for the resistance to several commonly used antibiotics. The misuse and overuse of antibiotics are major contributors to the development of AMR. Preventing AMR requires a multifaceted approach that includes reducing the unnecessary use of antibiotics, improving infection, prevention and control, increasing the development of new antibiotics, and promoting responsible use of antibiotics in both humans and animals. It is estimated that by 2050, AMR could cause 10 million deaths annually worldwide, and have a significant economic impact on the global economy. The emergence and spread of AMR in uropathogens have significant implications for the treatment of UTIs. It is essential to identify the presence of ARGs in uropathogens to determine appropriate antibiotic treatment. Furthermore, the development of new antibiotics and the implementation of effective infection control measures are necessary to combat the spread of AMR in uropathogens and to ensure the effective treatment of UTIs.

UTIs are a common health problem, especially in women, and they can range from mild to severe, and even life-threatening in some cases. Uropathogens are a common cause of UTIs and include *E. coli*, *Klebsiella*, *Pseudomonas* and *Enterococcus*. Multidrug-resistant uropathogens has become a significant concern due to the limited options for treating these infections. These bacteria have developed resistance to multiple classes of antibiotics, making it challenging to find effective treatment options. Commonly used antibiotics such as penicillin, cephalosporins, and fluoroquinolones may not work against MDR uropathogens. The emergence of MDR uropathogens is mainly due to the overuse and misuse of antibiotics, which leads to the development of antibiotic resistance. In addition, poor hygiene practices, use of contaminated catheters, and compromised immune systems can increase the risk of UTIs.

Klebsiella pneumoniae is a common uropathogen, that can cause urinary tract infections (UTIs). This type of bacteria is often found in the gastrointestinal tract and can sometimes spread to the urinary tract, causing an infection. UTIs caused by *Klebsiella pneumoniae* are typically more common in individuals with weakened immune systems, such as those with diabetes or those who have recently undergone surgery. Symptoms of UTI caused by *Klebsiella pneumoniae* can include painful urination, frequent urination, cloudy or foul-smelling urine, and lower abdominal pain. If left untreated, the infection can spread to the kidneys and lead to more serious complications. Gram-negative pathogens are becoming resistant to nearly all the antibiotic drug which creating situations reminiscent of the pre-antibiotic era and MDR gram-negative pathogens are also becoming increasingly prevalent in the community [6].

Whole genome sequence analysis (WGSA) is the process of analyzing an organism's complete DNA sequence, including all of its genes and non-coding regions. This technology has revolutionized the field of genomics, enabling scientists to study an individual's complete genetic makeup and providing insights into the underlying genetic basis of diseases. WGSA involves several steps, including DNA extraction, library preparation, sequencing, and bio-informatic analysis. WGSA has numerous applications, including the identification of disease-causing mutations, personalized medicine, and evolutionary studies. It has been used to identify genetic variants associated with various diseases, including cancer, heart disease, and rare genetic disorders. By identifying these variants, researchers can develop new treatments and therapies that are tailored to an individual's genetic makeup.

Materials and Methods

1. Identification of bacteria and Antibiotic susceptibility test

The studies were carried out on uropathogens. The bacteria were isolated from urinary tract infected urine samples and identification of bacteria were carried out by performing biochemical test and then performed the antibiotic susceptibility test by Kirby-buer disc diffusion method and towards the commonly recommended antibiotics and determine the minimum inhibitory concentration (MIC) in µg/mL against 19 antibiotics using the Epsilometer test (*E* test) (bioMerieux). The most resistant spp. of *Klebsiella pneumoniae* was selected for further analysis.

2. Whole genome sequencing

2.1 Genomic DNA Isolation, Qualitative and quantitative analysis

DNA was isolated from sample by Qiagen gDNA kit. Quality of gDNA was checked on 0.8 % agarose gel (loaded 5 µl) for the single intact band. The gel was run at 110 V for30 min. 2 µl of the sample was loaded in BioTeK Epoch for

determining A260/280 ratio. The DNA was quantified using Qubit dsDNA HS Assay kit (Life Tech). 1 µl of each sample was used for determining concentration using Qubit® 2.0 Fluorometer.

2.2 Preparation of libraries for Run Chemistry

Preparation of libraries were conducted via Ion Xpress™ Plus Fragment Library Kit (Thermo Fisher Scientific, USA) according to manufacturing instruction (including fragmentation, purification of fragments, ligation, amplification and quantification steps). For quantitation step, Ion Library Taqman Quantitation kit was used.

2.3 Bioanalyzer profile of final library

The fragment size was checked (QC Step) for purified fragmented DNA. This performed according to the instructions of Agilent™ High Sensitivity DNA Kit with by Agilent™ 2100 Bioanalyzer.

2.4 Template preparation & Sequencing

After Library preparation, the template was prepared (Template Preparation Step) according to manufacturer instruction of Ion 540™ Kit (Thermo scientific, USA) by using Ion OneTouch™ 2 System. The library after that were loaded on chip using Ion 540™ Chip Kit and sequenced by Ion GeneStudio S5 System (Ion Torrent, Thermo scientific, USA).

2.5 Method - Data Analysis

2.5.1 De novo Assembly & Annotation

Raw Sequencing reads are subjected to QC and pre-processing by Torrent Suite Software. Trimming of low-quality 3' ends, removal of adaptor sequences and quality parameter was set to ≥Q20 for filtering out low quality bases by the Ion Torrent Suite. High quality sequence reads de-novo assembled using SPAdes assembler 3.1.0 (Torrent browser) with the default settings. Resulted scaffolds were filtered based on the scaffolds length (>500bp). The quality of the assembled genomes was assessed using CheckM v1.2.1 for completion and contamination. The final assembled genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), Rapid Prokaryotic Genome Annotation (PROKKA) tool and BV-BRC (PATRIC) server.

2.5.2 Species Identification

16S & rMLST*:* Annotated sequences of marker genes 16s rRNA were aligned with the National Centre of Biotechnology Information (NCBI) database, using BLAST algorithm for species identification. Ribosomal Multilocus Sequence Typing (rMLST, https://pubmlst.org) with whole genome sequences input were also used for species identification of the isolates.

Pathogen watch Pipeline*:* MLST, core genome MLST, sublineage, clonal group identification (https://bigsdb.pasteur.fr/klebsiella/) and In Silico K-Type (Capsule polysaccharide) and O-type (lipopolysaccharide) serotypes were determined (https://github.com/katholt/Kaptive).

Similar Genome Finder Service of BV-BRC (PATRIC) server was used to find similar public genomes or compute genome distance estimation using Mash. It returns a set of genomes matching the specified similarity criteria. Results were verified by manually run Mash (v2.3) software.

2.5.3 Phylogeny and SNP analysis Phylogeny

The bacterial Phylogenetic Tree Service of BV-BRC enables construction of customphylogenetic trees built from user-selected genomes. The Codon Tree method selects single-copy BV-BRC PGFams and analyzes aligned proteins and coding DNA from single-copy genes using the program RAxML. The service returns a Scaled Vector Graphics (SVG) image of the final tree, as well as a Newick file which can be rendered in the interactive Phylogenetic Tree Viewer in BV-BRC or downloaded and viewed in FigTree or other software.

General Phylogenetic tree was constructed through comprehensive genome analysis. 54 close reference genomes were selected using BV-BRC similar genome finder services and phylogenetic tree was built using codon tree method from single-copy genes using theprogram RAxML.

2.5.4 Identification of genes related to Antibiotic Resistance

Antimicrobial resistance genes were identified using ResFinder v4.1, AMRFinderPlus v3.11.2 (NCBI) and BV-BRC (PATRIC) Pipeline.

Results:

1. Genome Accession Numbers

Raw genome data were submitted to the National Center for Biotechnology Information (NCBI). The assembled genomic sequences of *Klebsiella pneumoniae* (KP_2703) isolate were deposited under the Bio-Projects (accession number-PRJNA927155) and Bio-sample (accession number-SAMN32903713).

2. Raw reads, Pre-processing and Quality Control

Below is the statistics of raw sequencing reads processed by Torrent Suite Software. Final raw data (fastq) has total 229,064,099 of ≥Q20 bases with mean read length of 154 bp and GC content of 46%.

Table 1: Sequencing Results

Sample	Res-3569 (UP Strain-1)
Total Raw Bases	265,551,834 bp
Total ≥Q20 Bases	229,064,099 bp
Total ≥Q20 Reads (Sequences)	1,718,941
Mean Read Length	154 bp
Mean GC percent	46 %

3. Genome Assembly and Assessment

A total of 1,718,941 raw reads were used for genome assembly using SPAdes assembler, version 3.1.0. The raw reads were assembled into 148 contigs (≥500bp) yielding a genome size of 5,583,705 bp with N50 value of 99,646 with an average G+C content of 46.35%. Genome quality was found to be good using CheckM tool.

Table 2: Final Genome Statistics

Contigs	148
Genome Length	5,583,705 bp (5.58 Mb)
GC Content	46.35%
Contig N50	99,646
Contig L50	18
Check M Completeness	99.8
Check M Contamination	4.8
Genome Quality	Good

4. Species Identification

Result of Blastn using 16S rRNA gene found in draft genome sequence of *Klebsiella pneumoniae* strain showed closest match with other *Klebsiella pneumoniae*. In addition, the web-based PubMLST.org was used for strain prediction, rMLST identify the 55 ribosomal marker genes of species *Klebsiella pneumoniae.* The draft genome was annotated using RAST tool kit (RASTtk) and assigned a unique genome identifier of 573.52981. This genome is in the superkingdom, Bacteria and annotated using genetic code 11.

The taxonomy of this genome is: Cellular organisms > *Bacteria* > *Proteobacteria* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Klebsiella/Raoultella group* > *Klebsiella* > *Klebsiella pneumoniae*

The Sequence Type/Sublineage of *Klebsiella pneumoniae* strain is ST-147 as identified by Pathogen watch server using MLST/cg MLST tools. Results of Similar Genome Finder Service of BV-BRC server finds similar >50 public genomes and compute genome distance estimation using Mash (v2.3) with strain *Klebsiella pneumoniae*, which was manually verified with selected genomes.

5. Phylogenetic analysis

PATRIC provides the reference and representative genomes, and includes them in the phylogenetic analysis that is part of the Comprehensive Genome Analysis report. PATRIC global protein families (Pfam) were selected from these genomes to determine the phylogenetic placement of this genome. The protein sequences from these families were aligned with MUSCLE, and the nucleotides for each of those sequences were mapped to the protein alignment. The joint set of amino acid and nucleotide alignments were concatenated into a data matrix, and RaxML was used to analyze this matrix, with fast bootstrapping was used to generate the support values in the tree (Figure 1).

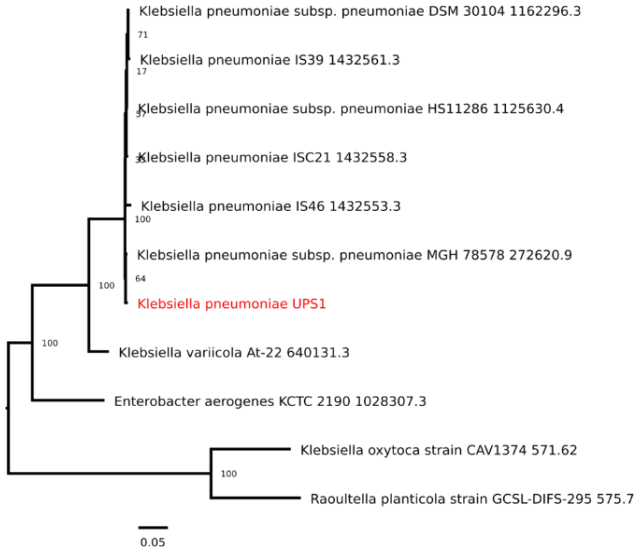

Figure 1: Phylogenetic tree

54 close reference genomes were selected from BV-BRC similar genome finder services and single copy genes analyzed by BV-BRC server, from which 100 genes were used for building phylogenetic tree by RAxML (Figure 2).

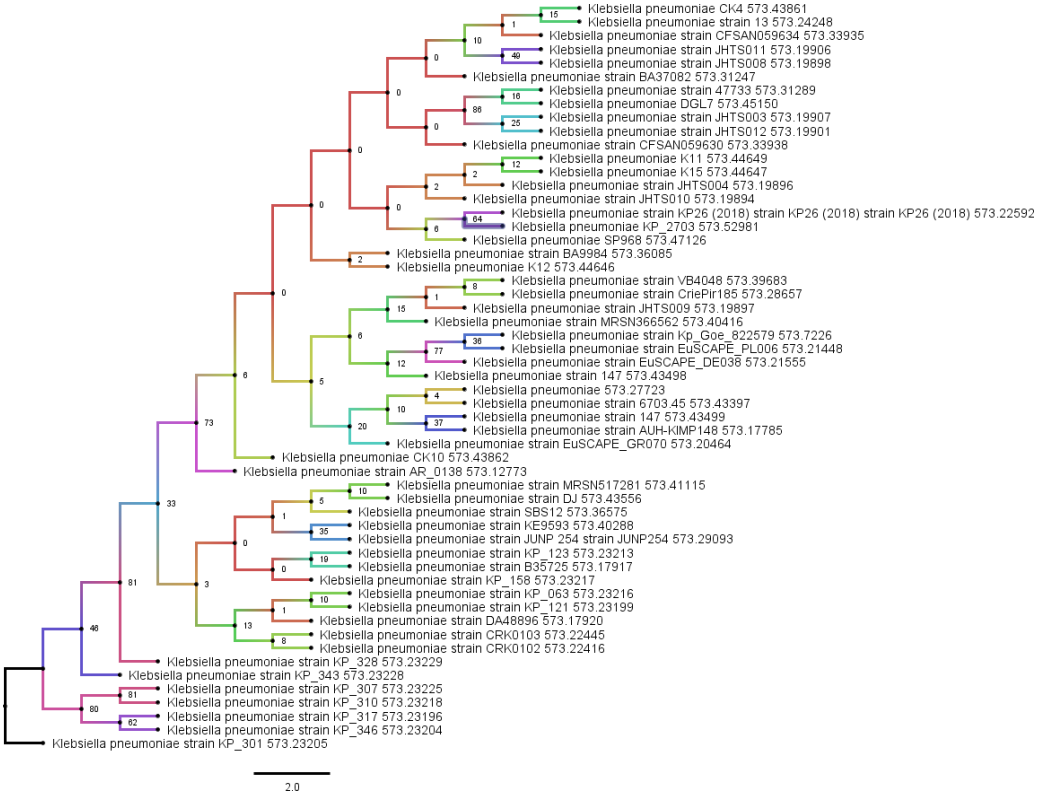

Figure 2: Phylogenetic tree based on single copy genes

6. Genome Annotation

A circular graphical display of the distribution of the genome annotations is provided. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reversestrand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to know virulence factors, GC content and GC skew. The colors of the CDS on the forward and reverse strand indicate the subsystem that these genes belong to (see Subsystems below).

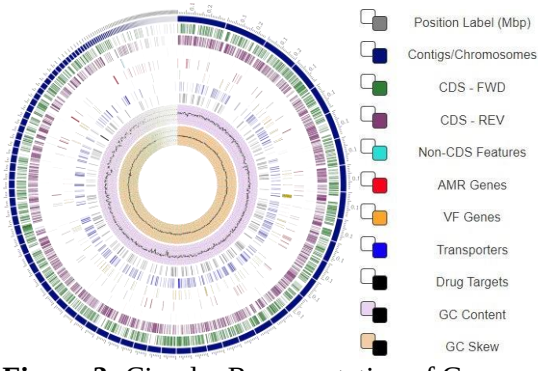

Figure 3: Circular Representation of Genome

A subsystem is a set of proteins that together implement a specific biological process or structural complex and analysis of the genome on BV-BRC using the annotated genes provided a general overview of the coded biological features with a subsystem coverage of 49%. An overview of the subsystems for this genome is provided in below figure.

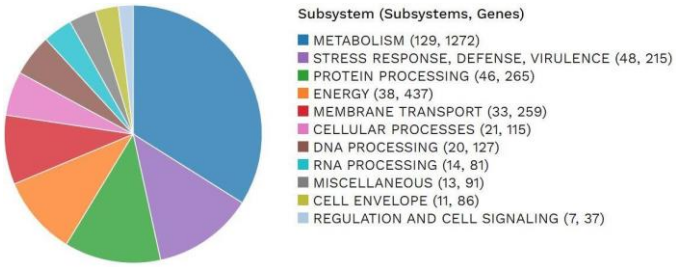

Figure 4: Biological features identified by RAST toolkit

7. NCBI PGAP Annotation

NCBI Genome annotation is a multi-level process that includes prediction of protein-coding genes, as well as other functional genome units such as structural RNAs, tRNAs, small RNAs, and pseudogenes. Recent pipeline includes utilization of curated protein profile hidden Markov models (HMMs), and curated complex domain architectures for functional annotation of proteins and annotation of Enzyme Commission numbers and Gene Ontology terms. Annotation of the draft genome using the NCBI PGAP predicted a total of 5597 genes, including 4934 protein-coding genes, 10 rRNAs, 73 tRNAs, 10 ncRNAs, 570 pseudogenes and 1 CRISPR Array.

8. Antibiotic Resistance

Antibiotic resistance genes were investigated and found to be present in following methods (1) AMRFinderPlus, (2) ResFinder (3) BV-BRC Annotation (DB: CARD, NDARO, PATRIC). NCBI AMRFinderPlus has identified total 22 AMR genes which have identity > 90% and query coverage > 80%. List covers 11 Class and 15 subclasses of Antibiotics. The gene identified was *rmtF1, aac(6')-Ib, aph(6)-Id, aph(3'')-Ib, blaSHV-11, blaTEM-1, ompK36_T136TDT, blaOXA-181, blaCTX-M-15, pmrB_R256G, fosA, mph(A), oqxA, oqxB, gyrA_S83I, parC_S80I, qnrB, arr-2, sul2, dfrA12*. ResFinder has predicted total 20 genes related to Antibiotic resistance, which covers 13 antibiotic/drug classes: *fosA, dfrA12, aac(6')-Ib-cr, qnrB1, rmtF, aph(6)-Id, aph(3'')-Ib, blaCTX-M-15, blaOXA-181, blaSHV-11, blaSHV-67, blaTEM-1B, catA2, mph(A), ARR-2, OqxA, OqxB* and *sul2* gene.

The Genome Annotation Service in PATRIC uses k-mer-based AMR genes detection method, which utilizes PATRIC's curated collection of representative AMR gene sequence variants and assigns to each AMR gene functional annotation, broad mechanism of antibiotic resistance, drug class and, in some cases, specific antibiotic it confers resistance to. The presence of AMR-related genes in a given genome does not directly imply antibiotic resistant phenotype. It is important to consider specific AMR mechanisms and especially the absence/presence of SNP mutations conveying resistance. A summary of the AMR genes annotated in this genome and corresponding AMR mechanism is provided in following table.

Table 3: Summary of AMR genes and corresponding AMR mechanism

AMR Mechanism	Genes
Antibiotic activation enzyme	KatG
Antibiotic inactivation enzyme	AAC(6')-Ib/AAC(6')-II, APH(3")-I, APH(6)-Ic/APH(6)-Id, CatA2 family, CatB family, CTX-M family, Mph(A) family, OXA-48 family, SHV family, TEM family
Antibiotic resistance gene cluster, cassette, or operon	MarA, MarB, MarR
Antibiotic target in susceptible species	Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho, rpoB, rpoC, S10p, S12p
Antibiotic target protection protein	BcrC, QnrB10
Efflux pump conferring antibiotic resistance	AcrAB-TolC, AcrAD-TolC, AcrEF-TolC , AcrZ, EmrAB- TolC, EmrD, MacA, MacB, MdfA/Cmr, MdtABC-TolC, MdtL, MdtM, SugE, TolC/OpmH
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA
Protein modulating permeability to antibiotic	OccD6/OprQ, OprB
Regulator modulating expression of antibiotic resistance genes	AcrAB-TolC, EmrAB-TolC, H-NS, OxyR

Discussion

The presence of Antibiotic Resistance Genes (ARGs) in uropathogens is a significant concern as it reduces the effectiveness of antibiotics used to treat UTIs. The ARGs can be transferred between bacteria through horizontal gene transfer, leading to the spread of resistance. The most common types of ARGs found in uropathogens include those that confer resistance to β-lactams, aminoglycosides, fluoroquinolones, and sulfonamides.

β-lactamase genes are among the most common ARGs found in uropathogens. These genes produce enzymes that hydrolyze β-lactam antibiotics, such as penicillin and cephalosporin, rendering them ineffective. Aminoglycoside-modifying enzyme genes confer resistance to aminoglycosides, a class of antibiotics used to treat UTIs. Fluoroquinolone resistance genes are responsible for resistance to fluoroquinolone antibiotics, which are commonly used to treat complicated UTIs. Sulfonamide resistance genes are also common in uropathogens and are responsible for resistance to sulfonamide antibiotics. The prevalence of Gram-negative pathogens has practical importance, especially for treatment options [16]. Our findings showed that the most prevalent ESBLs belong to *blaSHV-11, blaTEM-1, blaCTX-M-15, blaOXA-181* genes. Beta-lactamases are enzymes that can hydrolyze the beta-lactam ring in antibiotics, rendering them ineffective against bacteria.

The prevalence of β-lactamase genes is variable across countries, cities and regions. The prevalence of *blaCTX-M, blaTEM, blaSHV* was 44%, 47.4% and 69.5%, respectively [2, 21]. At the first cases of ESBLs, the prevalence of the *TEM* and *SHV* genes was the most predominant but now more CTX-M type is reported from several countries including Africa, India, Iran and industrialized countries such as France, Canada and the UK [28]. In several similar studies [20, 12]. The coexistence of different beta lactamase genes within the same isolates have been analyzed as well. Data analyses revealed that CTX-M is the most prevalent ESBL; this finding is consistent with previous studies. Production of CTX-M was significantly associated with resistance to most of the antibiotics. Determination of the frequency of β-lactamase genes (*blaSHV*, *blaTEM*, *blaCTX-M*) and phylogenetic groups among ESBL-producing uropathogenic *Escherichia coli* isolated from outpatients.

K. pneumoniae and *E. coli* have been associated with epidemic and endemic nosocomial infections caused by multidrug infections, mainly ESBL-producing bacteria in Sudan and worldwide [24, 5, 14]. The β-lactamases-producing *K. pneumoniae* and *E. coli*, especially TEM, CTX-M, and SHV type, are the most prevalent species that have spread globally within the hospital and environment [1, 3, 30, 17]. The gene *blaCTX-M-15*-positive isolates were phenotypically resistant to cephalosporin; this finding is expected because this gene was documented with a high affinity to hydrolyze cephalosporin [15].

The global emergence and dissemination of acquired carbapenemase among Gram-negative bacteria are considered a major public health problem. The carbapenemase-encoding genes are often located on plasmids along with other resistance genes, resulting in multidrug-resistant, extremely drug-resistant and pandrug-resistant bacteria [26]. The *blaOXA-181* gene is a carbapenemase gene that confers resistance to carbapenems, which are broad-spectrum antibiotics often used as a last resort to treat multidrug-resistant infections. This gene has been increasingly reported in *Klebsiella spp*. This study demonstrate that the *blaOXA-181* gene is a significant concern in *Klebsiella* uropathogens, wich is correlate with the reports from various regions worldwide, including India, Lebanon, and North East India. It emphasizes the need for prompt detection and management of antibiotic-resistant infections and the importance of implementing effective infection control measures. Therefore, the co-evolution of carbapenem-resistant strains is potentially the most worrying possibility due to the emergence of invasive *K. pneumoniae* infections, leading to treatment challenges [16].

Aminoglycoside resistance rates in *K. pneumoniae* have been described in many countries, leading to global concerns [4, 18]. The mechanism of resistance to aminoglycosides mainly occurs due to the presence of aminoglycoside modifying enzymes (AMEs) and their different ability to modify aminoglycosides. These enzymes include acetyltransferases (AACs), nucleotidyltransferases (ANTs), and phosphotransferases (APHs) were described in Enterobacterales [32]. In our study, expressing various aminoglycosides gene *as rmtF1, aac(6')-Ib, aph(6)-Id, strA, strB, aph(3'')-Ib. aac(6')-Ib*: This gene encodes an aminoglycoside acetyltransferase that modifies aminoglycosides such as gentamicin and tobramycin. It is commonly found in Gram-negative bacteria and has been reported in a variety

of species, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, *strA and strB*: These genes encode aminoglycoside phosphotransferases that confer resistance to streptomycin, *rrs and rrl*: These genes encode the 16S and 23S rRNA, respectively, and mutations in these genes have been associated with aminoglycoside resistance in bacteria. These mutations can result in reduced binding of aminoglycosides to the ribosome, leading to decreased efficacy of these antibiotics.

The *parC-S80I* gene is type of mutation that confers resistance to the quinolone group of antibiotics, including ciprofloxacin. This gene was found in present study. This mutation is commonly found in multidrug-resistant (MDR) *Klebsiella* uropathogens and can make treating these infections more challenging. Several studies have identified the presence of the *parC-S80I* mutation in MDR *Klebsiella* uropathogens. The prevalence of the *parC-S80I* mutation in MDR *Klebsiella* uropathogens, which can limit the effectiveness of quinolone antibiotics. Clinicians should be aware of the potential for quinolone resistance when treating patients with MDR *Klebsiella* infections.

Insusceptibility to quinolones and fluoro-quinolones happens essentially because of amino acid substitutions in *gyrA* and *parC* genes [9]. Recently, *qepA* and *oqxAB*, multidrug efflux pumps genes, were discovered and reported as the third PMQR mechanism involved in quinolone resistance [31, 13]. Fluoroquinolone treatment is no longer effective in more than half of patients in many countries in the world [29]. Ciprofloxacin resistance observed was higher than that described in prior studies from Egypt. This growing in ciprofloxacin insusceptibility rate may be due to the extensive usage of quinolones, the extended use of small doses of the more effective fluoroquinolones such as ciprofloxacin, also the inappropriate use of fluoroquinolones [22, 8].

In this study also finding other variety of resistance genes, such as *sul* gene and *fosA* gene. Similar study reported the increased prevalence rates of *sul* variants, primarily in Gram-negative bacteria, including *E. coli* strains isolated worldwide [11]. However, *fosA* gene is frequently found in Gram-negative species, including *K. pneumoniae* and *P. aeruginosa* [16]. This may be due to the presence of *fosA* gene on the chromosome in many Gram-negative species, whereas it rarely exists on the *E. coli* chromosome [10]. This data showed that WGS determine accurately the exact mechanisms of antibiotic resistance in *Klebsiella pneumonia* spp. WGS platforms are rapidly spreading in clinical diagnostic laboratories that make genomic data more accessible and easily used in routine clinical settings, due to the lowering of turn around time [16].

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