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Whole Genome Sequence and Comparative Genomics Analysis of *Citrobacter Freundii* Isolate from Burn Skin

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Abstract:

Background: The emergence of resistance to numerous novel antibiotics, which serve as the ultimate resort for treating infections caused by Gram-negative bacteria that are resistant to multiple drugs, poses a significant risk to public health. The *Citrobacter freundii* strain obtained from the burned skin of an adult exhibited resistance to a broad range of antibiotics, including colistin. The genome features that underlie the antibiotic-resistance phenotype of this isolate was characterized using whole genome sequencing (WGS). **Methods:** Genome sequencing on the Illumina platform was performed on the provided DNA samples (reference order number HN00194138; Psomagen/USA). The assembled genome was assembled and annotated by PATRICS. **Results:** The average G+C content was 55.75 percent throughout the 113 contigs in the genome. There are 9,825 protein-coding regions (CDS) in this genome, along with 149 transfer RNA (tRNA) genes and 8 rRNA genes. There were 1,584 putative proteins annotated and 8,241 proteins with known functions. PATRICS can identify 1592 hypothetical proteins. Additionally, 2132 proteins were displayed together with 8233 protein assignments under functional proteins.

Conclusion: The Iraqi multidrug-resistant C. *freundii* strain has not been reported before. These genetic findings help explain *C. freundii's* pathogenicity. Genomic analysis has helped us comprehend the probable resistance mechanism and the isolates' phylogenetic link with public-domain draft genomes.

Keywords: *Citrobacter freundii*; Multidrug resistance; Burn; NGS.

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Introduction:

Citrobacter is a gram-negative, facultatively anaerobic, coccobacillus or rod-shaped Enterobacteriaceae bacterium [1]. Citrobacter spp. may move using their covered flagella, ferment mannitol to produce H2S and utilize turn as their only carbon source [2]. Citrobacter spp. is a rare opportunist medical-building bacteria that causes urinary tract infections, medicine, or baby infections (e.g., meningitis, sepsis, general bacteremia; intra-abdominal sepsis; brain abscesses; or pneumonia). Citrobacter spp. infections were fatal, with 33-48% overall and 30% for children [3]. Infection is risky for children, elderly, and immunocompromised people [4]. Citrobacter spp. may be part of the human microbiome, making it widespread [5]. Some strains of Citrobacter spp. (notably *C. freundii*), enteric bacteria, Providencia, Enterobacter, and Proteus can cause opportunist intestinal flu.

Biofilms are microbic-derived sessile communities with cells that irreversibly stack on a substrate or each other. They're embedded in an EPS matrix they built and have a unique transcription rate and factor makeup. Microorganisms have a strong ability to attach to surfaces and interact in a multi-step process to build biofilms. Thus, microbial biofilms in wounds contribute to the chronicity and pathology of wound infections [6]. Biofilm development is important in several sectors, including oil drilling, paper manufacturing, food processing, and medicine[7]. The basic cellular processes of microbic biofilm production and behavior are postulated as targets for unique intervention techniques to address biofilm difficulties in these many sectors. In today's globalized market of raw, fresh, and minimally processed foods, food spoilage and degradation may cause huge economic losses and threaten food safety. Antibiotics are widely used to treat infectious disorders; however, they may increase bacterial resistance and cause nonheritable antimicrobial resistance in humans [8, 9]. Bacteria may govern biological phenomena by cell density via quorum sensing (QS). The bacteria produce hormone-like autoinducers that act with restriction proteins at a certain concentration. Many quorum-sensing systems modulate microbial pathogen virulence determinants, making them crucial to human health [10]. However, only a few AHL-based quorum-sensing systems, like those in Yersinia [11] and Serratia [11], are reported in the family of Enterobacteriaceae, while several other members, including enteric and enteric are thought to supply and use non-AHL signal molecules [12]. All quorum-sensing systems in eubacteria are managed and have various physiological roles related to their pathology. Quorum sensing may also be critical in the life cycle of commensal eubacteria, whether they are asymptomatic in the gut or infect others. Unlike Serratia, some eubacteria and ATCC strains do not produce AHLs under laboratory conditions. The clinical isolates expressing AHL synthase should have experienced horizontal transmission during viscus establishment [13]. Assemblage sensing helps *Citrobacter Freundii* cells control division, nutrition accessibility, microbial competition, and population density. Many microorganisms have been diagnosed with different diseases in Iraq [15,16].

Materials and methods:

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.

Citrobacter freundii was isolated from burn skin and it showed multidrug resistance. Furthermore, the isolate has been confirmed by different traditional and molecular approaches.

Genomic DNA Extraction

Genomic DNA was isolated from *C. freundii* isolates using a genomic DNA purification kit (GENEAID, Korea). The DNA concentration was (100 ng/l) and

purity of (1.7-1.8) was determined by a Nano Drop spectrophotometer. Extraction of genomic DNA is confirmed by using universal primers for 16rs [6]. The Sanger sequence result aligns with a blast to confirm the isolate.

Genome sequencing on the Illumina platform was performed on the provided DNA samples (reference order number HN00194138; Psomagen/USA).

Genome analysis:

PATR's comprehensive genome analysis service received a *C. freundii* assembled genome [9]. Reference genome Serratia marcescens was utilized to map sequencing reads. BWA—Burrows-Wheeler Aligner—mapped filtered data reads to the reference genome [10]. After reading mapping, Picard and SAMTools removed duplicates and found variants [11]. The completed genome was annotated for functional genes in subsystem categories using RAST and RASTtk [12] and SEED [13] to compare mapping findings. The PROKSEE service (https://proksee.ca/) identified conserved and unique sequencing characteristics and generated high-quality maps to verify and show the annotated features [14].

Phylogenetic Analysis

PATRIC's phylogenic analysis tool [9] classified reference and representative genomes. PATRIC's Comprehensive Genome research phylogenetic analysis used both reference and representative genomes. Mash/MinHash determined which genomes were the closest references and representations. PATRIC global protein families (PGFams) from these genomes determined this genome's evolutionary location. After MUSCLE aligned protein sequences from these families, nucleotide sequences were mapped.

Results:

The *Citrobacter freundii* isolated from burn skin showed resistance to many antibiotics.

Genome Assembly:

The bacterium *Citrobacter freundii* was generated with the use of auto [16]. The average G+C content was 55.75 percent throughout the 113 contigs in the genome. N50, the length of the shortest sequence that represents half of the genome, is calculated to be 468,423 bases. There are only seven contigs (L50) whose combined lengths equal fifty (N50) (Table 1).

Genome Annotation:

The RAST tool kit (RASTtk) [17] was used to annotate the *Citrobacter freundii* genome, and a genomic ID of 546.1277 was generated. This genome was annotated using genetic code 11, and it belongs to the bacterial super kingdom. There are 9,825 protein-coding regions (CDS) in this genome, along with 149 transfer RNA (tRNA) genes and 8 rRNA genes. In Table 2 we can see a summary of the annotated characteristics.

Features of proteins detect in the *Citrobacter freundii*:

There were 1,584 putative proteins annotated and 8,241 proteins with known functions (Table 3). There were 2,583 proteins assigned an EC number, 2,132 proteins assigned a GO term, and 1,841 proteins linked to KEGG pathways among the proteins with functional annotations. There are 7,387 proteins in the genus-specific protein families (PLFams) for this genome and 9,413 proteins in the cross-genus protein families (PGFams) that are annotated using PATRIC annotation [7].

Number of proteins assignment to various pathways:

PATRICS can identify 1592 hypothetical proteins. Additionally, 2132 proteins were displayed under GO together with 8233 protein assignments under functional proteins. Finally, there are 7387 and 9413 records under PLfam and PGfam, respectively. The

information about this feature is displayed in Figure (1).

Specialty genes:

259 virulence factors were discovered using Victor's program. Additionally, compared to VFDB, which can only discover 60 virulence factors, the PATRIC_VF bioinformatics tool was able to recognize 200 virulence factors. However, 941 transporters were visible in TCDB. By using DrugBand and TDD, the drug target was shown as 519 and 94, respectively. Three bioinformatic stools were utilized to demonstrate differences in antibiotic resistance. The information is shown in Table (4).

The circular genome for *Citrobacter freundii*:

The details for the isolate are shown in the figure (2) from outside to inside. The GC content, the GC skew, open reading frames, and revers strands.

Phylogenetic Analysis

PATRIC offered reference and representative genomes for phylogenetic research. Figure 3 shows the closest reference and typical genotypes. Neighbor-joining phylogenetic tree based on 16s RNA sequence figure 3.

Phylogenetic Analysis

Reference and representative genomes that the experts at the National Center for Biotechnology Information (NCBI) deem to be of high quality and value to the scientific community are selected and categorized by hand. In the phylogenetic analysis that is a part of the Comprehensive Genome Analysis report, PATRIC offers reference and representative genomes. Mash/MinHash [16] located the nearest reference and representative genomes to use as a point of comparison. The phylogenetic position of this genome was determined by selecting PATRIC global protein families (PGFams) from among these genomes. Nucleotide sequences were aligned with MUSCLE [18] to protein sequences from these families and then mapped to the alignment. Amino acid and nucleotide alignments were combined into a data matrix and analyzed with RaxML [19], and the tree's support values were calculated by rapid bootstrapping [20] (Figure 3).

Subsystem distribution of *Citrobacter freundii*

Subsystems (Subsystems, Genes) for metabolism was (133,2018), for protein processing (46,535), for stress responses, Defense, and Virulence (45,392), and for Energy (40,688) as shown in Figure 4.

Table (1): assembly details for Citrobacter freundii genome

Table 1. Assembly Details	
Contigs	113
GC Content	55.75
Plasmids	0
Contig L50	7
Genome Length	9,934,761 bp
Contig N50	468,423
Chromosomes	0
Job ID	assembly_8678
Job Started	October 23rd 2021, 9:18:14pm
Job Completed	October 24th 2021, 12:53:16am
Total Time	3h35m2s
Selected Recipe	auto

Table (2): Annotated details for Citrobacter freundii genome

Table 2. Annotated Genome Features	
CDS	9,825
tRNA	149
rRNA	8
Partial CDS	0
Miscellaneous RNA	0
Repeat Regions	0
Job ID	annotation_8678
Job Started	October 24th 2021, 12:53:16am
Job Completed	October 24th 2021, 1:06:04am
Total Time	12 minutes and 48 seconds

Table (3): proteins feature for Citrobacter freundii genome.

Table 3. Protein Features	
Hypothetical proteins	1,584
Proteins with functional assignments	8,241
Proteins with EC number assignments	2,583
Proteins with GO assignments	2,132
Proteins with Pathway assignments	1,841
Proteins with PATRIC genus-specific family (PLfam) assignments	7,387
Proteins with PATRIC cross-genus family (PGfam) assignments	9,413

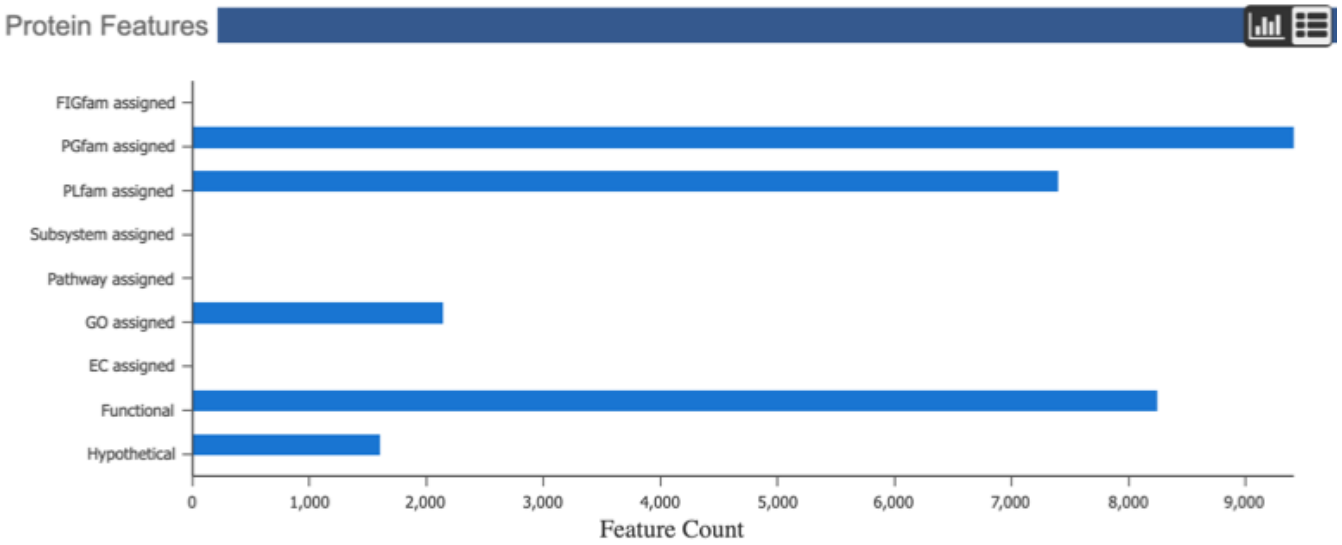

Figure 1 protein features show the protein's assignment to different pathways.

Table (4) Specialty genes for different functions

	Source	Genes
	Victors	9
Antibiotic Resistance	CARD	98
Antibiotic Resistance	NDARO	7
Antibiotic Resistance	PATRIC	124
Drug Target	DrugBank	519
Drug Target	TTD	94
Transporter	TCDB	941
Virulence Factor	PATRIC_VF	200
Virulence Factor	VFDB	60
Virulence Factor	Victors	259

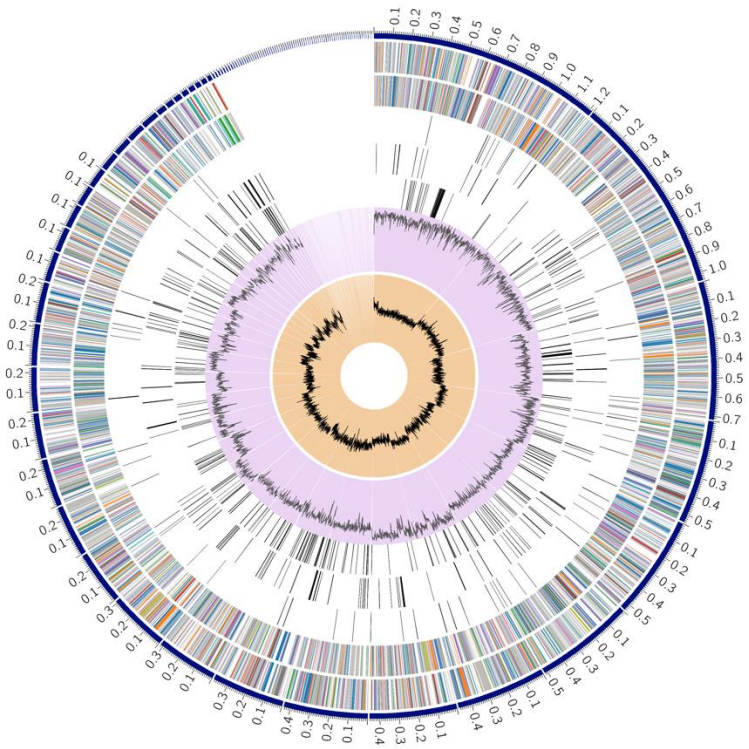

Figure (2) *Citrobacter freundii* circular genome

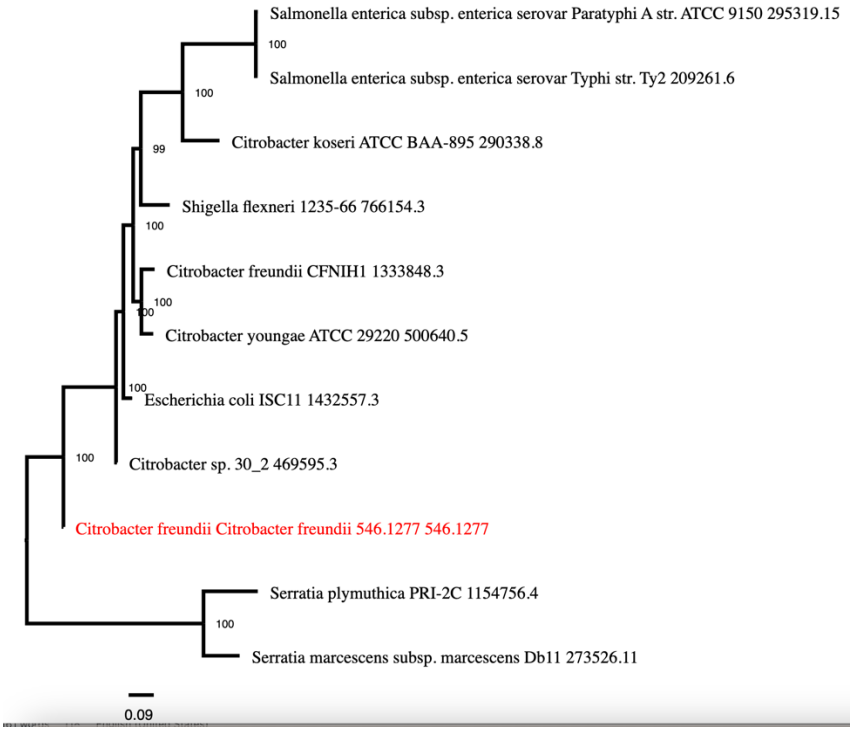

Figure (3) Phylogenic tree for *Citrobacter freundii*

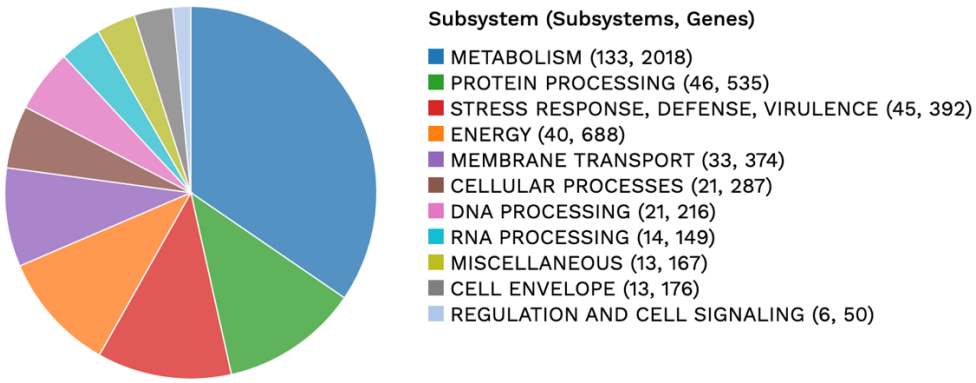

Figure (4) Subsystem distribution of *Citrobacter freundii*

Discussion:

The present investigation provides a comprehensive depiction of the entire genome of *C. freundii*, which was obtained from the cutaneous tissue of an adult burn patient. The genome of our strain appears to contain a range of genes for multi-drug resistant efflux systems with overlapping specificities, as indicated by the annotation of ARGs through CARD. Numerous resistances and transporters have been identified, as an illustration. The lack of comprehensive understanding regarding the seemingly repetitive nature of cellular defense mechanisms against the harmful impacts of toxic substances is a notable issue. The proposition is that the overlapping specificities of MDR efflux systems enable a cell to optimize the expulsion of specific compounds, thereby improving bacterial fitness [14].

Increasing evidence suggests that the colonization of bacteria in the gut commences during the intrauterine phase. Various factors, such as maternal transfer, antibiotic treatment, cesarean delivery, and invasive medical procedures, can influence the composition of the microbiome. This alteration can affect all types of microbiomes [15]. A study examining the microbiome of healthy preterm infants has revealed the presence of antibiotic-resistant genes, indicating a high prevalence of potentially clinically significant antibiotic-resistant bacteria from birth. This study represents one of the limited investigations on this topic. The current study posits that the initial colonization of the neonatal gut is significantly impacted by the transmission of microorganisms during birth from the mother and the surrounding environment. This is due to the absence of any invasive or antibiotic interventions administered to the subjects. The existence of antibiotic resistance genes (ARGs) in both the intrauterine and extrauterine milieu may ultimately result in the establishment of potentially pathogenic bacterial populations in the gastrointestinal tract of neonates. It is necessary to employ scientific methodologies to determine the likelihood of intestinal carriage of multi-drug resistant (MDR) strains and to develop preventive measures to mitigate unfavorable consequences.

More whole genome sequencing and comparative genomics research are needed to better understand the background and assessment of multidrug-resistant isolates from different hospital rooms with special attention on delivery room.

Conclusion:

To the best of our knowledge, this is the first report on the multidrug-resistant *C. freundii* strain that has been found in Iraq. These discoveries offer novel genomic data for a deeper comprehension of *C. freundii* pathogenicity. To provide more thorough information about the genome diversity of colistin resistant *C. freundii* in Iraq, future research that includes a larger sample size of the local strains should be taken into consideration.

Author Contributions

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

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Institutional Review Board Statement:

Not applicable since the study does not involve humans or animals.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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