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Whole Genome Sequencing of the Multidrug-Resistant Proteus mirabilis MORAY37 Recovered from a Urinary Tract Infection case in Mosul, Iraq

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ABSTRACT

INTRODUCTION: *Proteus mirabilis* is a common member of the intestinal microbiota in humans and animals but can cause serious infections, including urinary tract infections (UTIs) and sepsis. The rise of multidrug-resistant (MDR) P. mirabilis strains, particularly those producing extended-spectrum β-lactamases (ESBLs), poses a significant global health challenge. This study aimed to sequence and analyze the genome of P. mirabilis MORAY37, a highly resistant isolate from a UTI case in Mosul, Iraq, to elucidate its genetic determinants of antibiotic resistance and virulence.

METHODS: The genome of P. mirabilis MORAY37 was sequenced using next-generation sequencing technology. Bioinformatics tools, including SPAdes for assembly, QUAST for quality assessment, and RAST for annotation, were employed. Phylogenetic analysis was conducted using the Type Strain Genome Server (TYGS), and antibiotic resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD).

RESULTS: The genome of P. mirabilis MORAY37 comprises 4,131,367 bp with a GC content of 39.1%. It contains 3,844 coding sequences and 75 RNA genes, predominantly involved in amino acid metabolism (294 genes), protein metabolism (203 genes), and carbohydrate metabolism (198 genes). Phylogenetic analysis confirmed its close relationship to P. mirabilis ATCC 29906 (89% isDDH value). Ten antibiotic resistance genes were identified, conferring resistance to aminoglycosides, fluoroquinolones, monobactams, cephalosporins, chloramphenicol, and sulfonamides.

CONCLUSION: The study provides valuable genomic insights into P. mirabilis MORAY37, highlighting its multidrug resistance profile and phylogenetic lineage. These findings underscore the need for further research to explore the mechanisms of resistance gene dissemination and potential therapeutic strategies, such as phage therapy or CRISPR-based interventions, to combat MDR P. mirabilis infections

Keywords: Antibiotic resistance genes, GC content, *Proteus mirabilis*, Whole-genome sequencing

INTRODUCTION

Proteus mirabilis, a type of bacteria that has a rod-like shape and is classified as Gram-negative, is a primary culprit behind urinary tract infections (UTIs). It is especially prevalent in individuals who have severe UTIs or those who are connected with catheter use [1]. Its inherent resistance

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to polymyxin, nitrofurantoin, and tigecycline, together with the acquisition of antimicrobial resistance genes, poses a significant danger in terms of both epidemiology and treatment. The prevalence of multi-drug resistance (MDR) in P. mirabilis is consistently globally rising, primarily attributed to the synthesis of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and/or carbapenemases [4-5]. Since the 1990s, a number of metallo-β-lactamases (MβLs) encoded by mobile DNA have appeared in significant Gram-negative pathogens such as Enterobacteriales, Pseudomonas aeruginosa, and Acinetobacter baumannii [6]. There have been only a small number of reported instances of P. mirabilis New Delhi metallo-beta-lactamase (NDM)-producers in China [7], Tunisia [8], India [9], and New Zealand [10].

P. mirabilis, a bacterial pathogen, is recognized as a secondary cause of urinary tract inflammation, following Escherichia coli. It is commonly found in patients admitted to hospitals, individuals using urinary catheters for extended periods, and those with structural abnormalities in the urinary tract [11]. The pathogenicity of these bacteria is linked to the presence of numerous virulence factors, such as pili (fimbriae), flagella, urease, protease, hemolysin, and lipopolysaccharide, also known as endotoxin [12]. Both the protease and urease enzymes are regarded as virulence factors that are synthesized by every strain of Proteus spp.

P. mirabilis naturally exhibits resistance to specific antibacterial agents, including tigecycline, polymyxin, and nitrofurantoin [2-3,13]. Additionally, the prevalence of drug resistance in P. mirabilis has escalated due to the utilization of broad-spectrum antibiotics, specifically AmpC β-lactamases and Carbapenemases [4,14]. Furthermore, the production of biofilms in certain bacteria aids in their ability to withstand environmental stress and evade the effects of antibiotics. An observation has been made that the resistance to antibiotics is increasing significantly, ranging from 10 to 1000 times, in microorganisms that can produce biofilm [15-16].

Genome sequencing is increasingly utilized in clinical microbiological investigations to analyze the genetic makeup of an organism and enhance our comprehension of microbial pathogenicity and evolution [15,17]. Although the public health risks are evident, the current level of studies and whole-genome sequencing for P. mirabilis is considerably lower compared to that of ESKAPE pathogens. For example, only 3647 whole-genome assemblies of this species are available in GenBank (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=584, accessed on 26 April 2024) as opposed to Escherichia coli (265072 assemblies; https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=562), Klebsiella pneumoniae (65145 assemblies; https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=573) and Acinetobacter baumannii (26779 assemblies; https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=470). This study represents the most important antibiotic resistance and virulence genes in P. mirabilis MORAY37 isolated from a UTI specimen in Mosul, Iraq.

METHODS

Source and Identification of *P. mirabilis MORAY37*: *P. mirabilis* MORAY37 was isolated in a previous study from a UTI case visiting Ibn Sina Teaching Hospital in Mosul, Iraq. The isolate produced smooth, non-lactose fermenting colonies on MacConkey agar and swarmed on blood agar. Identification was further confirmed via 16S rRNA gene sequencing [13].

Genomic DNA extraction and sequencing: Genomic DNA was gently extracted from P. mirabilis MORAY37 using the Presto Mini Genomic DNA Bacterial Kit supplied by Geneaid (Taiwan). Genomic DNA was sent to Psomagene sequencing company (Maryland, USA).

Genome submissions to NCBI GenBank: The genomic sequence of Proteus mirabilis MORAY37 has been submitted to DDBJ/ENA/GenBank and assigned the accession number NZ_JAWUZK000000000.1.

Genome Assembly and Annotation: The raw reads were de novo assembled to contigs using the SPAdes 3.5 bioinformatics tool [18] applying settings of k-mer length of 21,33,55,77. QUAST software [19] was used to generate assembly statistics. The assembled genome was annotated using the RAST server [20]. The SEED tool [21] was used for predicting functional genes in subsystem categories.

Whole Genome Based Phylogenetic Tree: The Type Strain Genome Server (TYGS) [22] was utilized to deduce the phylogenetic tree of Proteus mirabilis MORAY37 and its closest strains based

on whole-genome sequencing. The genome, formatted in FASTA, was uploaded to the server using the default parameters. The tree was constructed using the FastME 2.0 program [23], which is incorporated into the TYGS.

In silico DNA-DNA (isDDH) Hybridization analysis: GGDH bioinformatics tool [24] was used to measure isDDH values between Proteus mirabilis MORAY37 and the most closely related strains based on whole genome sequences data.

16S rRNA gene phylogenetic tree analysis: The BLASTn tool was utilized to search for homology between the Proteus mirabilis MORAY37 sequence and sequences included in the NCBI GenBank database. The phylogenetic tree was constructed by bootstrap analysis (100X) using the MEGA-11 software [25].

Detection of the antibiotic resistance genes in the Genome of Proteus mirabilis MORAY37: Antibiotic resistance determinants in the Proteus mirabilis MORAY37 genome were detected using the Comprehensive Antibiotic Resistance Database (CARD) program version 3.2.6 [26].

RESULTS

General Genome Features of Proteus mirabilis MORAY37

General genome features of P. mirabilis MORAY37 are shown in Table 1. Results of the draft genome sequence showed that the general size of the genome was 4,131,367bp. The whole genome sequence of P. mirabilis MORAY37 yields a GC content of 39.1% distributed within 68 contigs; the largest contig was 406,777 bp, and the smallest was 503 bp with an N50 value of 179,311.

Table 1: General genome features of *Proteus mirabilis MORAY37* generated using QUAST software and RAST server

Feature	Value
Genome total length (bp)	4,131,367
Number of contigs	68
Largest contig (bp)	406,777
Smallest contig (bp)	503
GC content (%)	39.1
Total of protein-coding sequences (CDSs)	3,844
Number of RNA genes	75
N50	179,311

Using the Rapid Annotation System Technology (RAST) server, we were able to detect 3844 coding sequences in addition to 75 RNA genes from different categories.

Most of the genes predicted in the subsystem categories contribute to the metabolism of amino acids and their derivatives (294), protein

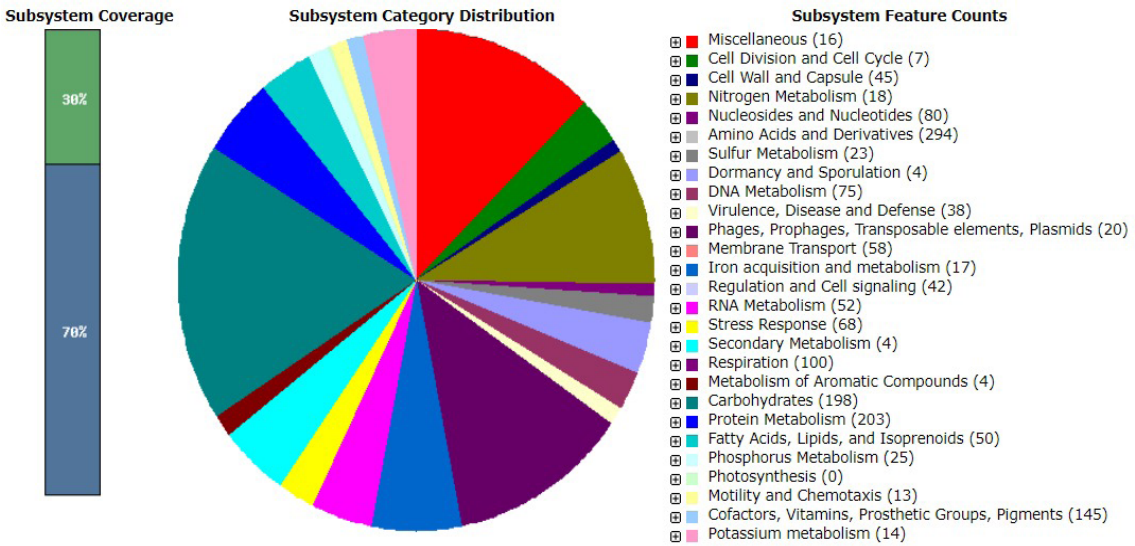

Figure 1: Subsystem category distribution statistics of *P. mirabilis MORAY37*

The genome was annotated using the RAST server. The pie chart depicted the frequency of each subsystem feature, while the SEED viewer showcased the extent of subsystem coverage. The green bar represents the proportion of proteins that are part of the subsystems, whereas the blue bar represents the proportion of proteins that are not part of the subsystems.

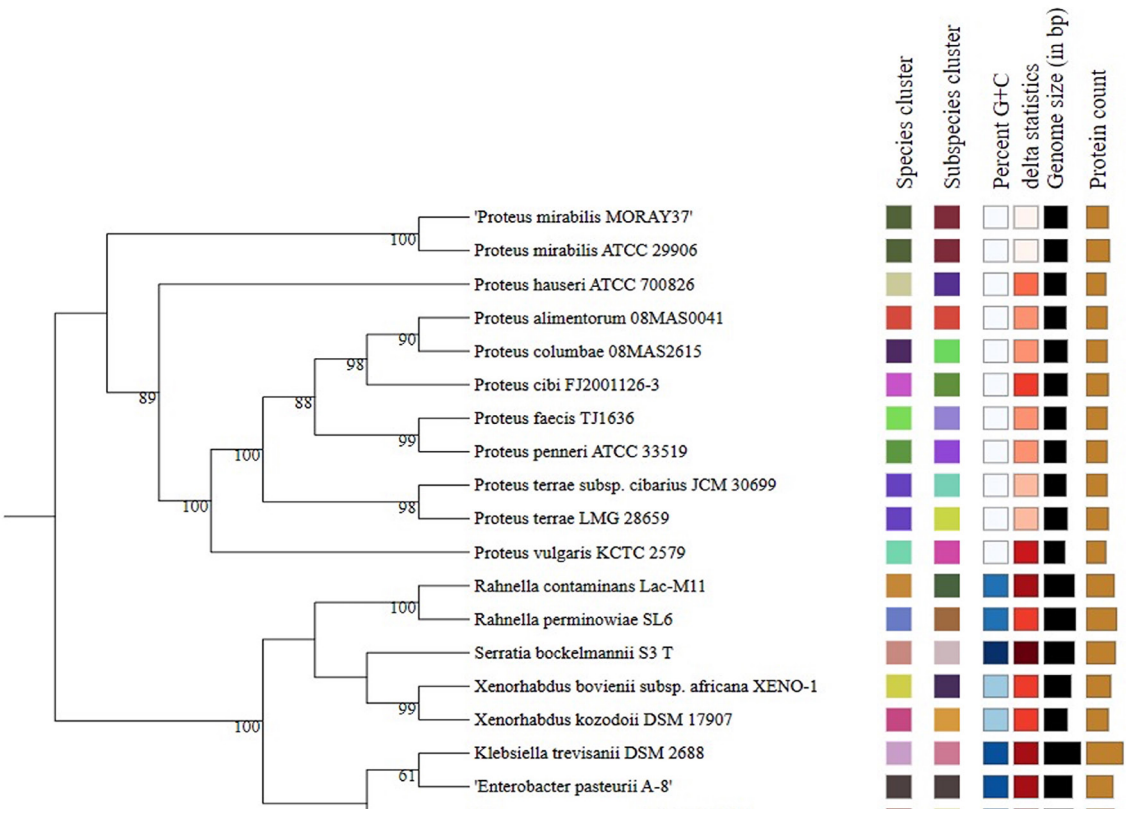

Figure 2: The TYGS server used to construct a phylogenetic taxonomy tree of Proteus mirabilis MORAY37.
The ultimate tree was built using the Fast ME 2.0 technique, which relies on the balanced minimum evolution method and includes 100X pseudo-bootstrap support values. Leaf labels are determined based on their connection to clusters of species and subspecies, as well as their genomic GC percent, δ-values, overall genome size, and total number of proteins..

metabolism (203), carbohydrates metabolism (198), cofactors, vitamins, prosthetic groups, and pigments production (145) (Figure 1).

In silicon DNA-DNA (*is*DDH) Hybridization analysis

The phylogenetic taxonomy tree of Proteus mirabilis MORAY37 using TYGS server identified the closest type strains were Proteus mirabilis ATCC 29906 (accession number: CAWLFQ000000000.1) Proteus faecis TJ1636 (accession number: NZ_PENZ00000000.1), Proteus penneri ATCC 33519 (accession number: NZ_PHFJ00000000.1), Proteus alimentorum 08MAS0041 (accession number: NR_163665.1), Proteus terrae subsp. Cibarius JCM 30699 (accession number: NZ_PGWT00000000.1), Proteus terrae LMG 28659 (accession number: NZ_PENS00000000.1), and Proteus vulgaris KCTC 2579 (accession number: NZ_PHNN01000004.1) with isDDH values of 89.0, 43.1,43.1,42.5,42.4, 42.1 and 41.0%, respectively (Figure 2 and Table 2).

The complete 16S rRNA gene of P. mirabilis MORAY37 was isolated from the sequenced genome and used to draw a phylogenetic tree (indicated in a black circle). The phylogenetic tree was drawn using MEGA-11 software with a scale length of 0.01. The phylogenetic tree shows that P. mirabilis MORAY37 strain is the closest to Proteus mirabilis ATCC 29906, which confirms the results we obtained from isDDH. However, other species from Proteus were also located in different branches of the dendrogram, as shown in Figure 3.

Acquired antibiotic resistance genes in the genome of *Proteus mirabilis MORAY37*

Acquired antibiotic-resistance genes from P. mirabilis MORAY37 have been identified in the genome using CARD. The results showed that there are about 10 genes in *P. mirabilis* MORAY37 genome conferring resistance to aminoglycosides, fluoroquinolones, monobactams, cephalosporins, chloramphenicol, and sulfonamides as shown in Table 3.

Table 2: Comparisons between the genome of Proteus mirabilis MORAY37 and the genomes of type strains using various criteria, including isDDH, GC content, δ-value, genome size, and number of proteins.

Proteus mirabilis MORAY37 vs. type strain genomes	Digital isDDH value (%)	Percent G+C (%)	δ- value	Genome Size (bp)	Number of proteins
Proteus mirabilis ATCC 29906	89.0	38.6	0.158	3,970,390	3812
Proteus hauseri ATCC 700826	38.4	37.4	0.206	2,783,512	3343
Proteus alimentorum 08MAS0041	42.5	38.01	0.193	3,830,912	3426
Proteus columbae 08MAS2615	41.5	37.93	0.191	3,953,210	3530
Proteus cibi FJ2001126-3	40.9	38.13	0.223	4,004,202	3611
Proteus faecis TJ1636	43.1	37.78	0.194	3,880,280	3446
Proteus penneri ATCC 33519	43.1	37.81	0.197	3,771,888	3410
Proteus terrae subsp. cibarius JCM 30699	42.4	37.81	0.18	3,922,849	3512
Proteus terrae LMG 28659	42.1	37.85	0.18	4,098,142	3729
Proteus vulgaris KCTC 2579	41.0	37.79	0.227	3,639,158	3251
Rahnella contaminans Lac-M11	13.3	53.08	0.237	5,225,880	4611
Rahnella perminowiae SL6	13.3	51.84	0.217	5,592,375	5095
Serratia bockelmannii S3T	13.3	59.0	0.256	5,284,737	4907
Xenorhabdus bovienii subsp. africana XENO-1	13.7	44.73	0.213	4,669,241	4036
Xenorhabdus kozodoii DSM 17907	13.8	44.7	0.213	4,124,875	3678
Klebsiella trevisanii DSM 2688	13.2	55.15	0.243	6,215,359	5947
Enterobacter pasteurii A-8	13.2	56.41	0.242	4,810,455	4376
Kluyvera cryocrescens NBRC 102467	13.3	53.85	0.254	5,044,663	4702
Photorhabdus luminescens subsp. mexicana MEX47-22	13.9	42.51	0.248	5,814,352	4774

DISCUSSION

Next-generation sequencing, when integrated with bioinformatics, enables the generation of comprehensive information about the entire bacterial genome, including regions and genes of interest, single nucleotide polymorphisms (SNPs), and pathways on bacterial operons. This technique bypasses the complicated steps of bacterial cloning that were previously used to obtain sequences of bacterial DNA fragments, in addition to having a cost-effective way to maintain diversity in bacterial cultures [27].

The draft genome sequence showed that the general size of the genome was 4,131,367bp, which is acceptable compared to the genome sizes of other sequenced Proteus strains. For example, P. mirabilis MAD23; 3.729 Mb [28], P. mirabilis HI4320; 4.063 Mb [29], while other genome sequences were larger than our sequence such as P. mirabilis CriePir 89; 4,292,030 bp [30]. P. mirabilis WF3225; 4,246,169 and P. mirabilis WF4035; 4,320,254 bp [31]. This indicates that the sequence coverage was sufficient to determine almost the entire genome.

The TYGS addresses the gold standard methods and state-of-the-art estimates in the genomic era to determine the closest type bacterial genome sequences with validly published names [32]. Results from isDDH show that the isDDH value

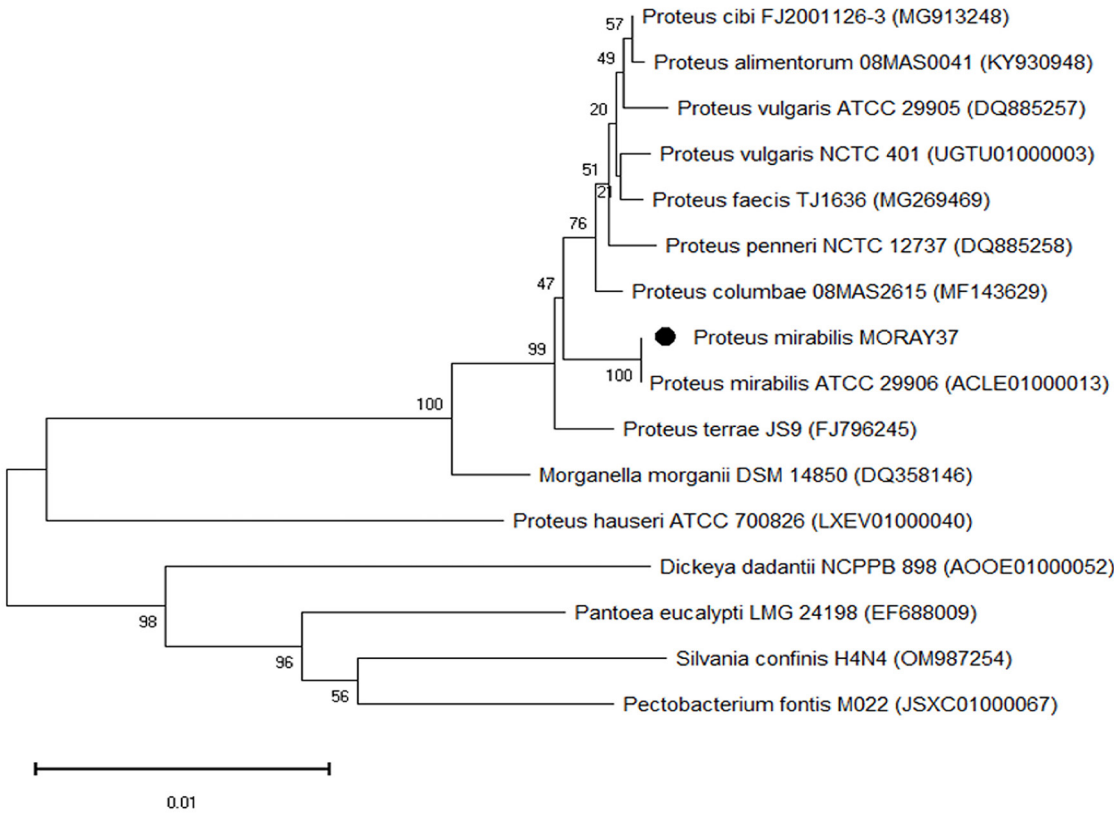

Figure 3: The Neighbor-Joining phylogenetic trees

Tress depict the correlation between Proteus mirabilis MORAY37 (shown by a black circle) and the closely related strains, as determined by analyzing their 16S rRNA sequences using MEGA-11 software. The scale length of the trees is set at 0.01. The bootstrap test (100 repetitions) displays the percentage of replicate trees where the related strains clustered together, indicated next to the branches.

of Proteus mirabilis MORAY37 is ≥70%, above threshold-based, compared with the type strain Proteus mirabilis ATCC 29906; this indicates that the two genome sequences are strongly related and might belong to the same species [33].

The Comprehensive Antibiotic Resistance Database (CARD) is a biological database that gathers and organizes references to antimicrobial resistance genes, proteins, and phenotypes. The database contains all drug classes and resistance mechanisms, and its data is structured using an ontology. Previous studies have detected antibiotic-resistant genes in P. mirabilis, including those required for ESBL resistance [13,30]. We hypothesized that antibiotic-resistance genes in P. mirabilis genome might be disseminated by horizontal transfer or plasmids as a number of resistant genes in P. mirabilis were found to be carried on class II integrons [33]. However, studies have mentioned that antibiotic-resistance genes in P. mirabilis are not carried on plasmids [34]. More studies are required to study the location of antibiotic-resistance genes in this strain, whether on P. mirabilis chromosome or plasmid. Nowadays, plasmid sequencing technologies have evolved, which might also be useful in determining the location of genes in bacteria. This high frequency of antibiotic resistance is most likely due to numerous types of inappropriate antibiotic use in Iraq. Antibiotics, for example, have traditionally been offered without a medical prescription in Iraqi drugstores [35-37].

CONCLUSION

Phylogenetic analysis shows that P. mirabilis MORAY37 strain is the closest to Proteus mirabilis

Table 3: Antibiotic resistance genes detected in the genome sequence of Proteus mirabilis MORAY37 using the CARD database

Resistance gene	Drug class	Resistance mechanism	Predicted phenotype	Percentage identity
APH(3')-Ia	aminoglycoside antibiotic	antibiotic inactivation	neomycin, ribostamycin, kanamycin A, gentamicin B, paromomycin, lividomycin, gentamicin	100%
ANT(2'')-Ia	aminoglycoside antibiotic	antibiotic inactivation	dibekacin, sisomicin, kanamycin A, tobramycin, gentamicin	100%
aadA3	aminoglycoside antibiotic	antibiotic inactivation	spectinomycin, streptomycin	99.24%
sul1	sulfonamide antibiotic	antibiotic target replacement	sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole	100%
TEM-1	monobactam, cephalosporin, penam, penem	antibiotic inactivation	cefazolin, amoxicillin, ampicillin, cefalotin	100%
CMY-2	carbapenem, cephalosporin, cephamycin, penam	Antibiotic inactivation	cefoxitin, cefazolin, ceftazidime, ceftriaxone, ertapenem, ampicillin, cefixime, cefalotin	100%
qacE	disinfecting agents and antiseptics	antibiotic efflux	benzylkonium chloride, ethidium bromide,chlorhexidine,cetylpyridinium chloride	100%
catA4	phenicol antibiotic	antibiotic inactivation	chloramphenicol	96.77%
rsmA	fluoroquinolone antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic	antibiotic efflux	trimethoprim, chloramphenicol	92.98%
gyrB	fluoroquinolone antibiotic	antibiotic target alteration	enoxacin, ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, trovafloxacin, grepafloxacin, sparfloxacin, pefloxacin	84.58%

ATCC 29906. The isDDH value for our strain is ≥70%, above threshold based, compared with the type strain Proteus mirabilis ATCC 29906, which indicates that the two genome sequences are strongly related and might belong to the same species. Ten antibiotic resistance genes in P. mirabilis MORAY37 genome conferring resistance to aminoglycosides, fluoroquinolones, monobactams, cephalosporins, chloramphenicol, and sulfonamides were detected and more studies are required to identify the location of these genes whether on the plasmid or the chromosome and introduce possible methods that enable to eliminate multidrug-resistant P. mirabilis such as phage therapy, exposure to nanoparticles and antimicrobial peptides, and engineering CRISPR-cas system to target antibiotic resistance genes.

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