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Research article

Draft genome sequence of multidrug resistant Proteus mirabilis strain MAD23

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Abstract

Genome sequencing became one of the important tools for diagnosis of microorganisms. *Proteus mirabilis* is an enteric Gram-negative bacterium causes gastrointestinal and urinary tract infections in human and animals. Here, the draft sequencing of *Proteus mirabilis* (MAD23) strain was performed. The strain was collected from Chinese duck cecum suffering from diarrhea. Bacteria was identified by biochemical tests and characteristic swarming pattern on the plate. Antimicrobial susceptibility test was performed by VITEC-2 MS system. Also, draft genome sequencing was conducted using the Illumina platform NovaSeq sequencer. The genome is about 3,729,695 bp long, GC content is of 38.9%., number of contigs is 123, largest contig is 137547pb, smallest contig is 513, total of protein-coding sequences (CDSs) is 3410, and number of RNA genes is 72. Genome Pairwise comparisons of *Proteus mirabilis* MAD23 with other vs. type strain was detected based on is DDH, GC content, δ- value, genome size and number of proteins. Neighbour-Joining phylogenetic trees show relationship between *Proteus mirabilis* MAD23 and the close strains of Proteus species based on 16S rRNA sequences. In addition, analysis of the sequence reveals presence of many antibiotic resistance genes as *dfrA1, sul2, dfrA32, aadA2b, aph(3')-Ia, aadA1, aph(6)-Id, aph(3")-Ib, ere(A), aadA1, aadA2b, tet(J), tet(C), floR*, and *Cat* genes.These genes encoding the resistance to different antibiotics including Aminoglycosides, Macrolides, Aminocyclitol, Tetracycline and Amphenicol. This work is considered as an important study can help in more understanding of WGS in Proteus spp. and recognize the genes that related to antibiotic resistance in this pathogen.

Keywords: Multidrug resistant, Draft genome sequence, *Proteus mirabilis MAD23*, Antibiotic resistance genes.

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INTRODUCTION

P. mirabilis is a motile Gram-ve opportunistic pathogen that belongs to *Enterobacteriaceae* order and *Morganella* family (Mobley and Belas, 1995; Adeolu et al., 2016). This bacterium is found in water, soil and the gut of human and animals (Drzewiecka, 2016). In human, *P. mirabilis* is more frequent than other species of Proteus and may complicate with other infections in the respiratory tract, gastrointestinal tract, eyes or skin (O'hara et al., 2000; Jacobsen and Shirtliff, 2011). Also, it is common in poultry and farm animals (Chen et al., 2018; Li Z et al., 2022).

Genome sequencing is considered as common tool can be used in clinical microbiological studies. It helps in fast diagnosis of the disease and early control (Hasman et al., 2014). Also, it is able to describe the organism genome and facilitate more understanding of microbial virulence and evolution (Harris et al., 2013).

Infection with *P. mirabilis* is nearly high among people with urinary tract problems like cystitis and pyelonephritis (Eriksson et al., 1986; Rubin et al., 1986). This pathogen can adhere to the urinary tract and swarm to spread through the bladder by attachment to the urinary epithelium (Jansen et al., 2004; Jones et al., 2005; Nicolle, 2014). Incidence of the infection in the urinary tract is because of biofilm formation by this bacterium inside the catheter. The biofilm acts as protection to the bacteria against antibiotic effect and host immunity (Jacobsen et al., 2008; Kreunumkum et al.,2016; Wasfi et al., 2020). It is possible that colonization of *P. mirabilis* inside the intestine considered as a reservoir for its colonization in other organs as urinary tract (Mathur et al., 2005). However, there are some sporadic cases of *P. mirabilis* infection has caused bacteraemia and hospital acquired infection or rheumatoid arthritis (O'Hara et al., 2000; Rashid and Ebringer, 2007). *P. mirabilis* has many factors can increase its virulence as lipopolysaccharides, efflux pumps, proteins for adhesion and urease (Wasfi et al., 2020, Alsultan and Alsallami, 2022).

Antibiotic resistance in *P. mirabilis* is naturally to certain antibacterial agent such as tigecycline, polymyxin, and nitrofurantoin (Kanzari et al., 2018; Dong et al., 2019; AL-saady et al., 2022). Also, drug resistance in *P. mirabilis* has increased because of the broad-spectrum antibiotics use such as AmpC β-lactamases and carbapenemases (Luzzaro et al., 2009; Cohen-Nahum et al., 2010; D'Andrea et al., 2011; Hirabayashi et al., 2021). Moreover, biofilm formation in some organisms helps in surviving to the environmental stress and antibiotics. It was noticed that resistance to the antibiotics is increasing by 10- 1000 times in microorganisms that able to form biofilm (Hoiby et al., 2010; Tseng et al., 2013, Alsultan et al., 2023). In this study, focusing on the source of natural and acquired antibiotic resistance in *P. mirabilis* through studying the phenotype (antimicrobial resistance test) and genotype (WGS) of this organism. Also, there is an attempt to understand the compatibility between the genes that responsible for antibiotic resistance in the phenotypic and genotypic study. In addition, recognise if these genes are existing in the genome or carried on a plasmid.

MATERIALS AND METHODS

Bacterial isolation and growth conditions

The *P. mirabilis* strain MAD23 was collected by rectal swab from the rectum of Chinese duck suffering from diarrhoea. The bacteria were grown in 10 ml nutrient broth at 37 °C for 24 h. Then were sub cultured on MacConkey agar. Colonies that do not fermentative to the lactose were selected and grown on *Xylose Lysine Deoxycholate agar* (*XLD agar*). The bacteria were negative to Gram stain and clearly swarming pattern was noticed on the agar plate.

Biochemical tests

Biochemical identification to the isolate was performed using VITEK2 Compact Microbial Identifier.

Antimicrobial Susceptibility Test (AST)

The bacterial isolate was recognised by VITEC-2 MS system (bioMerieux, Marcy-l'Étoile, France). Resistance or susceptibility of *P. mirabilis* strain MAD23 to different antibiotics was performed.

Draft Genome Sequencing

The extraction of genomic DNA was performed according to of supplier's (Qiagen, USA).

Genome assembly was obtained from paired end sequences, read length is 151. Using TruSeq Nano DNA kit and illumina system. Also, the circular genome map of *Proteus mirabilis* MAD23 was performed using CGView Comparison Tool (Stothard et al., 2019).

Genome submissions to NCBI GenBank

The genome sequence of *Proteus mirabilis* MAD23 has been deposited at DDBJ/ENA/GenBank under the accession number JARULK000000000.

Genome assembly and annotation

The raw reads were *de novo* assembled to contigs using SPAdes 3.5 bioinformatics tool (Bankevich et al., 2012) applying settings of k-mer length of 21,33,55,77. QUAST software (Gurevich et al., 2013) was used to generate assembly statistics. The RAST server has been used to annotate of the assembled genome (Aziz *et al*., 2008). Predication of gene function was performed using The SEED tool (Overbeek et al., 2014).

Draft Genome Based Phylogenetic Tree

In order to infer the draft-genome-based phylogenetic tree of *Proteus mirabilis* MAD23 and the most closely related strains, the Type Strain Genome Server has been performed. FASTA format of the genome was uploaded then FastME 2.0 program which is integrated within the TYGS have been used to infer the tree (Lefort et al., 2015).

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

GGDH bioinformatics tool (Meier-Kolthoff et al., 2022) was used to measure *is*DDH values between *Proteus mirabilis* MAD23 and the related strains based on draft genome sequences data.

16S rRNA gene phylogenetic tree analysis

The Nucleotide Basic Local Alignment Search Tool (BLASTn) program (Altschul et al., 1990) was used to search for homology to the *Proteus mirabilis* MAD23 sequence against sequences which are available on the sequence NCBI GenBank database. The phylogenetic tree was constructed by bootstrap (100X) analysis using the MEGA-11 software (Tamura et al., 2021).

Detection of antimicrobial Resistance Genes (acquired type) within the genomes of *Proteus mirabilis* MAD23

The ResFinder 4.0 tool (Zankari et al., 2020) was used to identify the acquired antimicrobial Resistance Genes in the Genome of *Proteus mirabilis* MAD23.

Genome comparisons

The BLAST Ring Image Generator (BRIG) software (Alikhan et al., 2011) was used to align *Proteus mirabilis* MAD23 with the most related *Proteus mirabilis* strains as well as *Proteus cibi* FJ2001126-3 as different species and generate image that shows difference and similarity between *Proteus mirabilis* MAD23 genome and other bacterial sequences as a set of concentric rings.

RESULTS

Biochemical tests

Bacterial isolate was urease, citrate and utilization tests positive while, it was indole negative. Subculture of this isolate on nutrient agar showed swarming pattern. Also, it was negative to the Gram stain.

Antimicrobial susceptibility test

The antimicrobial susceptibility test results show that *Proteus mirabilis* MAD23 is susceptible to Ticarcillin, Calvulanic Acid, Ceftazidime, Cefepime, Aztreonam, Amikacin, Gentamicin and Tobramycin. While, it was resistant to Piperacillin, imipenem, Tazobactam, Pefloxacin, Minocycline, Colistin, Rifapicin and Trimethoprim as shown in Table 1.

Table 1 Antimicrobial Susceptibility Test (AST) of *Proteus mirabilis* MAD23

Antimicrobial	MIC Interpretation (MIC microgram per ml.)				
Ticarcillin	8=<	S	Amikacin	2=<	S
Ticarcillin/Calvulanic Acid	8=<	S	Gentamicin	1=<	S
Piperacillin	4=>	R	Tobramycin	1=<	S
Piperacillin/Tazobactam	8=>	R	Ciprofloxacin	2	I
Ceftazidime	1=<	S	Pefloxacin	4=>	R
Cefepime	1=<	S	Minocycline	16=>	R
Aztreonam	1=<	S	Colistin	8=>	R
Imipenem	2=>	R	Rifapicin	4=>	R
Meropenem	4=>	R	Trimethoprim/Sulfamethoxazole	320=>	R

Features of the *P. mirabilis* MAD23 genome

The genome of *P. mirabilis* MAD23 consists of a 3,729,695 bp chromosome. Number of contigs is 123, largest contig is 137547pb and smallest contig is 513. Comparing with other *Enterobacteriaceae* (chromosome length mean about 4.6 Mb), this genome is smaller. Also, total of protein-coding sequences (CDSs) is 3410, and number of RNA genes is 72. General gnome features are shown in Table 2. Also, circular genome map and genome comparison were shown in Figure 1 and Figure 2.

Table 2 General genome features of *Proteus mirabilis* MAD23 generated using QUAST software

Feature	Value
Genome total length (pb)	3,729,695
Number of contigs	123
Largest contig (pb)	137547
Smallest contig (bp)	513
GC content (%)	38.9
Total of protein-coding sequences (CDSs)	3410
Number of RNA genes	72
N50	63950

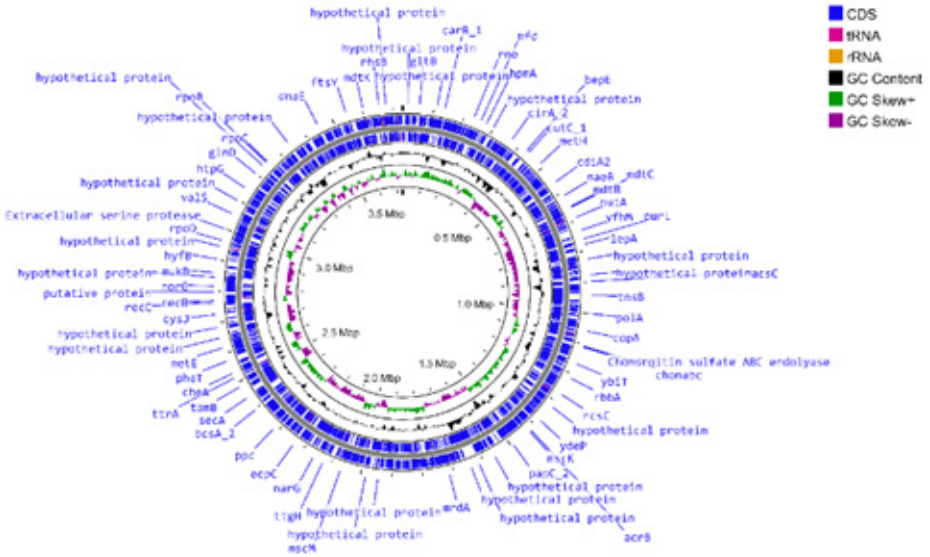

Figure 1 Circular genome representation of *Proteus mirabilis* MAD23 genome. The inner most ring represents chromosome position. The next rings represent the feature regions which are indicated in different colours

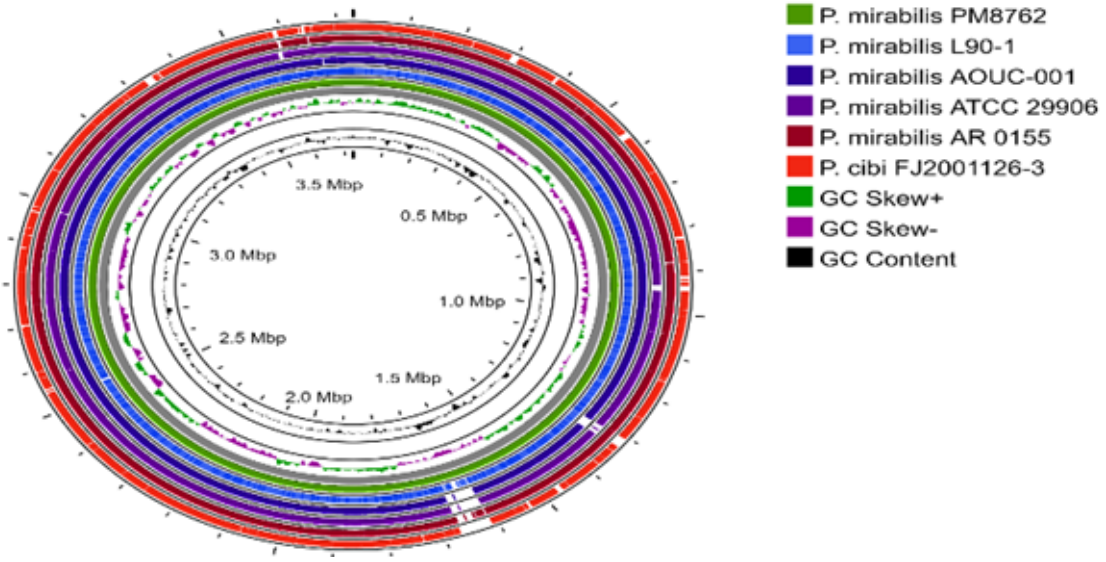

Figure 2 Shows *Proteus mirabilis* MAD23 genome compared against 5 *Proteus mirabilis* strains and one non mirabilis (*P. cibi* FJ2001126-3). The innermost gray circle represents the genome of Proteus mirabilis MAD23. The rings show GC skew (purple/green) and GC content (black). The next rings represent the genomes of other Proteus strains which are indicated in different colors. Regions without colour in the ring indicate absence of the region and the difference among the genome sequences

Draft Genome Based Phylogenetic Tree

Depending on the draft genome sequencing of the *Proteus mirabilis* MAD23, distribution of subsystem categories was inducted. Annotation of the genome reveals percentage of proteins that are included in the subsystems and the proteins that are not included in the subsystems. Also, the subsystem category distribution shows numbers of each subsystem in the genome as in Figure 3.

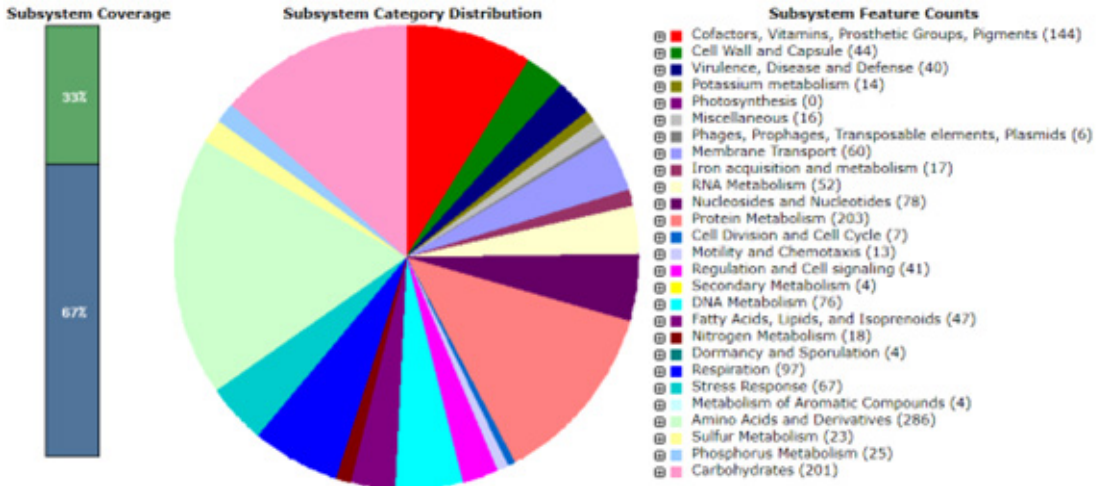

Figure 3 Subsystem category distribution statistics of *Proteus mirabilis* MAD23. The RAST server has been used to annotate of the assembled genome. SEED viewer was used to display systemic feature and subsystem coverage. As shown the pie chart, 33% of the proteins included in the subsystems (Green bar) while 76% the proteins that are not included in the subsystems (Blue bar)

Phylogenetic tree of taxonomy

The Phylogenetic taxonomy tree of *Proteus mirabilis* MAD23 shows that this strain is close related to five strains of *Proteus mirabilis* and it is not related to nine other species of Proteus as clearly appears in Figure 4 using FastME 2.0 approach based on balanced minimum evolution method (100X pseudo-bootstrap support values).

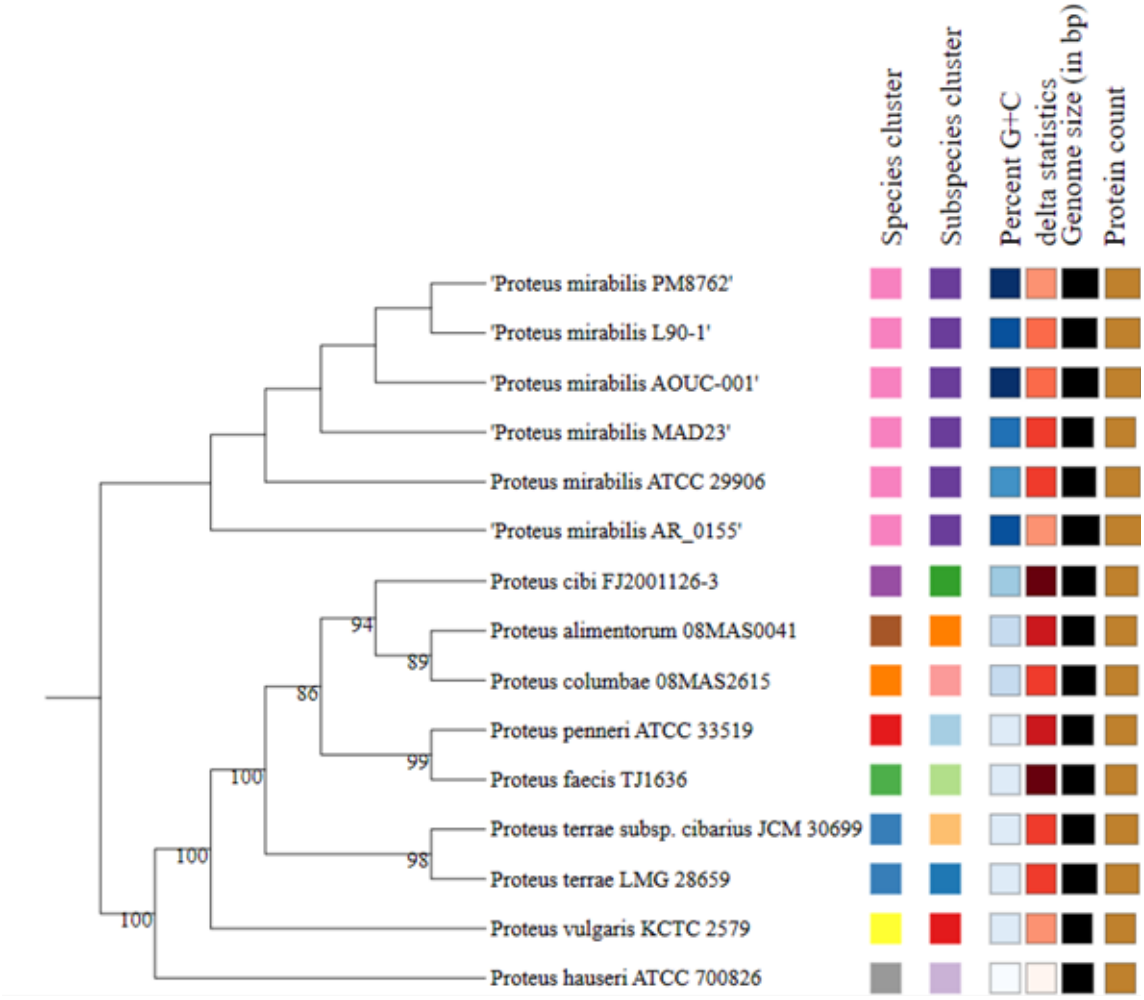

Figure 4 Phylogenetic taxonomy tree of *Proteus mirabilis* MAD23. TYGS inferred 5 closest related species and 9 non-related species genome sequences. The final tree was constructed with FastME 2.0 approach based on balanced minimum evolution method (100X pseudo-bootstrap support values). Labels on leaves are indicated by association to species and subspecies clusters, genomic GC percent, δ values, overall genome size and total number of proteins

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

The genome Pairwise comparison was used to compare *Proteus mirabilis* MAD23 strain with other 6 strains of *Proteus mirabilis* and 9 strains of different species. The comparison was depended on DNA- DNA hybridization (*is*DDH) values, GC contents, δ- value, genome size and number of proteins in each strain as shown in Table 3.

Table 3 Genome Pairwise comparisons of *Proteus mirabilis* MAD23 genome vs. type strain genomes based on *is*DDH, GC content, δ- value, genome size and number of proteins.

Proteus mirabilis **MAD23/ Type strains**	**Digital *is*DDH value (%)**	**Percent G+C (%)**	**δ-value**	**Genome Size (pb)**	**Number of proteins**	**NCBI reference sequence**
Proteus mirabilis PM8762	93.5	39.49	0.125	4,285,781	3879	NZ_CP092652.1
Proteus mirabilis L90-1	91.7	39.2	0.134	4,218,783	3865	NZ_CP045257.1
Proteus mirabilis AOUC-001	90.8	39.42	0.129	4,272,433	3955	NZ_CP015347.1
Proteus mirabilis ATCC 29906	88.7	38.6	0153	3,970,390	3812	NZ_ACLE00000000.1
Proteus mirabilis AR 0155	87.1	39.11	0.126	4,372,742	4026	NZ_CP021695.1
Proteus cibi FJ2001126-3	53.7	38.13	0188	4,004,202	3611	NZ_PENW00000000.1
Proteus alimentorum 08MAS0041	55.0	38.01	0.165	3,830,912	3426	NZ_NBVR00000000.1
Proteus columbae 08MAS2615	53.0	37.93	0.148	3,953,210	3530	NZ_NGVR00000000.1
Proteus penneri ATCC 33519	56.0	37.81	0.169	3,771,888	3410	NZ_PHFJ00000000.1
Proteus faecis TJ1636	54.8	37.78	0.202	3,880,280	3446	NZ_PENZ00000000.1
Proteus terrae subsp. *cibarius* JCM 30699	57.0	37.81	0.142	3,922,849	3512	NZ_PGWT00000000.1
Proteus terrae LMG 28659	54.0	37.85	0.147	4,098,142	3729	NZ_PENS00000000.1
Proteus vulgaris KCTC 2579	53.4	37.79	0.117	3,639,158	3251	NZ_PHNN00000000.1
Proteus hauseri ATCC 700826	49.1	37.4	0.066	3,783,512	3343	NZ_LXEV00000000.1

16S rRNA gene of *Proteus mirabilis* MAD23 was used to find the neighbour and related strains. The phylogenetic tree was indicated using MEGA-11 software with a scale length of 0.0020. The tree shows that *Proteus mirabilis* MAD23 strain locates in the same branch with 5 other strains (*Proteus mirabilis* ATCC 29906, *Proteus mirabilis* AR 0155, *Proteus mirabilis* L90-1, *Proteus mirabilis* PM 8762 and *Proteus mirabilis* AOUC-001). While, other strains of different species of Proteus locate in different branches of the tree as in Figure 5. Also, the similarity percentages of these strains with the *Proteus mirabilis* MAD23 are mentioned in Table 4.

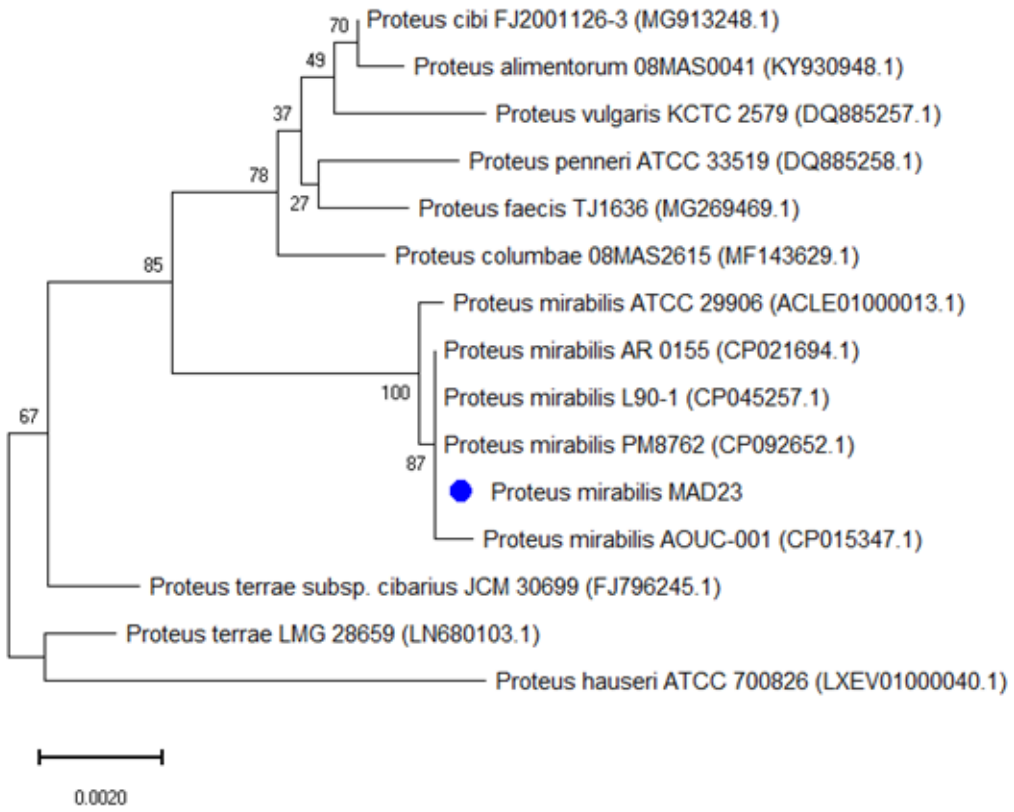

Figure 5 Neighbour-Joining phylogenetic trees showing the relationship between *Proteus mirabilis* MAD23 (indicated in blue circle) and the related strains of *Proteus* species based on 16S rRNA sequences using MEGA-11 software with a scale length of 0.0020. The percentage of replicate trees in which the associated strains clustered together in the bootstrap test (100 replicates) are shown next to the branches

Table 4 Most related *Proteus* species with their accession numbers that show homology with *Proteus mirabilis* MAD23 retrieved from NCBI database

Species name	Strain name	Accession No.	Similarity (%)
Proteus mirabilis	PM8762	CP092652.1	99.89
Proteus mirabilis	L90-1	CP045257.1	99.8
Proteus mirabilis	AOUC-001	CP015347.1	99.7
Proteus mirabilis	ATCC 29906	ACLE01000013.1	99.6
Proteus mirabilis	AR 0155	CP021694.1	99.78
Proteus cibi	FJ2001126-3	MG913248.1	99.30
Proteus alimentorum	08MAS0041	KY930948.1	99.20
Proteus columbae	08MAS2615	MF143629.1	99.20
Proteus penneri	ATCC 33519	DQ885258.1	99.13
Proteus faecis	TJ1636	MG269469.1	99.20
Proteus terrae subsp. *cibarius*	JCM 30699	FJ796245.1	99.25
Proteus terrae	LMG 28659	LN680103.1	99.10
Proteus vulgaris	KCTC 2579	DQ885257.1	99.04
Proteus hauseri	ATCC 700826	LXEV01000040.1	98.57

Acquired antibiotic resistance genes in the genome of *Proteus mirabilis* MAD23

The acquired antibiotic resistance genes of *Proteus mirabilis* MAD23 strain were identified in the genome after sequencing using ResFinder 4.0 tool with ID ≥90%. The results reveal that there are about 15 different resistance genes responsible for the resistance to many antibiotics as mentioned clearly in Table 5.

Table 5 Antibiotic resistance genes detected in the genome sequence of *Proteus mirabilis* MAD23 by ResFinder 4.0 tool with ID ≥90%

Resistance class	Resistance gene	Identity	Predicted phenotype	PubMed ID	Accession No.
Folate pathway antagonist	dfrA1	100.0	trimethoprim	6308574	X00926
	sul2	100.0	sulfamethoxazole	11600347	AY034138
	dfrA32	100.0	trimethoprim	19921331	GU067642
Aminoglycoside	aadA2b	99.8	spectinomycin, streptomycin	85862590	D43625
	aph(3')-Ia	100.0	neomycin, kanamycin, lividomycin, paromomycin, ribostamycin	6270337	V00359
	aadA1	100.0	spectinomycin, streptomycin	22486636	JQ480156
	aph(6)-Id	99.8	streptomycin	2653965	M28829
	aph(3")-Ib	100.0	streptomycin	12029529	AF321551
Macrolide	ere(A)	99.8	Erythromycin	unpublished	DQ157752
Aminocyclitol	aadA1	100.0	spectinomycin, streptomycin	22486636	JQ480156
	aadA2b	99.8	spectinomycin, streptomycin	85862590	D43625
Tetracycline	tet(J)	98.9	doxycycline, tetracycline	Unpublished	ACLE01000065
	tet(C)	99.6	doxycycline, tetracycline	11591134	AF055345
Amphenicol	floR	98.1	chloramphenicol, florfenicol	10339826	AF118107
	cat	98.7	chloramphenicol	3900035	M11587

\DISCUSSION

Genome sequencing of microorganisms is considered as a precise and effective way for diagnosis. It is also important for detection and control of different diseases and restriction of outbreaks (Hasman et al., 2014). The enteric Gram-negative *P. mirabilis* is one the main causative agent of urinary and digestive tract infection. It is opportunistic bacteria and use urease enzyme to catalyse urea and obtain the nitrogen as a source of protein. This makes the pH between 8-9 and causes ion precipitation with increase probability of bladder and kidney stone formation (Griffith et al., 1976; Jones and Mobley, 1988; Burall et al., 2004). Because of the extensive use of antibiotics, the resistance to different drugs was improved in many pathogens leading to multidrug resistance problem (Somrup et al., 2018; He et al., 2021; Rubic et al., 2021).

In the present study, draft genome sequence of *P. mirabilis* MAD23 was reported. The strain was collected from Chinese duck cecum. The duck was suffering from diarrhea. Identification of bacterial species was by biochemical tests. Also, antibiotic susceptibility test was performed using VITEK. Our results showed that *Proteus mirabilis* MAD23 strain is susceptible to Ticarcillin, Calvulanic Acid, Ceftazidime, Cefepime, Aztreonam, Amikacin, Gentamicin and Tobramycin. This could be because of the rare use of these antibiotics in treatment of enteric bacteria. However, same strain (MAD23) was resistant to other antibiotics as Piperacillin, imipenem, Tazobactam, Pefloxacin, Minocycline, Colistin, Rifapicin and Trimethoprim. The resistance to the mentioned antibiotics could be associated with using of some antimicrobial drugs frequently to control the enteric and urinary tract infections in veterinary field as nitrofuran. Also, *Proteus mirabilis* is resistant to many antimicrobial agents such as colistin (polymyxins), nitrofuran, tigecycline, tetracycline and imipenem (Stock, 2003; Girlich et al., 2020; He et al., 2021).

Results of the draft genome sequencing showed that the general size of *P. mirabilis* MAD23 genome is smaller than the genome of other strains of same bacteria. For example, *Proteus mirabilis* HI4320; 4.063 Mb (Pearson et al., 2008), *Proteus mirabilis* CriePir 89; 4,292,030 bp (Shelenkov et al., 2020). Also, the strains WF2978, WF3225, WF3338, WF3430 and WF4035 were (4,197,626, 4,246,169, 4,157,273, 4,042,859 and 4,320,254 bp, respectively) in genome size (Liu et al., 2023). This difference in the genome size might be because of genome sequencing was performed by illumine sequencing as a draft sequence and has many contigs.

Based on the draft genome sequencing we discussed distribution of some subsystem categories in to 27 subcategories depending on proteins that exist or absent in the genome. Also, number of each feature in the genome was mentioned as in Figure 3. In addition, the phylogenetic tree of the taxonomy of *Proteus mirabilis* MAD23 results suggest that MAD23 strain is high similar to other strains of same species and different from other species of Proteus bacteria as in Figure 4.

However, some references mentioned that antibiotic resistance genes in *P. mirabilis* is not a carried-on plasmid (Armbruster et al., 2018). Other references demonstrated that some Proteus species may transfer plasmids with antibiotic resistance genes (Qin et al., 2015; Leulmi et al., 2019). The

interesting feature in our results is presence of about 15 resistance genes in the genome of *Proteus mirabilis* MAD23 encoding the resistance to different antibiotics including; trimethoprim, spectinomycin, neomycin, streptomycin, sulfamethoxazole, kanamycin, lividomycin, paromomycin, ribostamycin, erythromycin, doxycycline, tetracycline, chloramphenicol, florfenicol. This similar to the results that mentioned in other researches such as some antibiotic resistance genes were identified in multidrug-resistant ESBL-producing *P. mirabilis* (CriePir89 strain) chromosome as *bla*$_{CTX\text{-}M\text{-}15}$ and *sul1* (Shelenkov et al., 2020).

We hypothesized that antibiotic resistance genes in *P. mirabilis* genome might disseminate by horizontal transfer or plasmids. Also, number of resistance genes in *P. mirabilis* are carried on class II integrons (Yang et al., 2023). Although, this study is including one strain, it reveals that antibiotic resistance genes are existed within the genome of *P. mirabilis* in addition to the plasmid as previous studies mentioned (Shelenkov et al., 2020). We suppose that the identification of this isolate will help investigations of multidrug resistance in *P. mirabilis* and facilitate new treatment strategies.

This study demonstrated that all isolates showed inhibitory activity against *A. hydrophila*, a common bacterial pathogen in striped catfish in the Mekong River of Vietnam (Figure 3). These findings are in agreement with previous studies, where it was reported that actinomycete isolates exhibited prohibitory activity against bacterial species in aquaculture (Peng et al., 2022). The findings by Garcia-Bernal et al. (2015) indicated that 5/31 isolated actinomycete strains from marine sediments in Cuba showed antimicrobial activity against three *Vibrio* species. The research by Nabila and Kannabiran (2018) pointed out that about 18% of the actinomycetes isolates revealed antibacterial activity against the selected fish and shellfish bacterial pathogens. In Vietnam, the results of Huong et al. (2020) showed that 8/15 endogenous actinomycetes isolated from medicinal plants in Vietnam were active with tested microorganisms, such as *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Mycrosporum gypseum*. Many studies have shown that actinomycetes have antibacterial activity because they produce many antibiotics during growth, such as erythromycin, streptomycin, rifamycin, and gentamycin (Mahajan and Balachandran, 2012). Also, previous studies revealed that bacteriocins or bacteriocin-like substances were produced by *Streptomyces* species and had antimicrobial activity against many pathogenic bacteria (Farris et al., 2011; Lee et al., 2014; Hernández-Saldaña et al., 2020; Kurnianto et al., 2021).

Previous studies revealed that nutrient medium, incubation time, temperature, pH, and NaCl are important factors affecting the growth and production of antimicrobial agents by actinomycetes (Gao et al., 2009; Oskay et al., 2011; Akond et al., 2016; Elabbasy et al., 2021). In the study, two isolates, BCA1.2 and BCA1.5, could grow well in ISP2-9 medium (Table 4). This finding is consistent with the study of Nabila and Kannabiran (2018), who found that the isolate *Streptomyces* sp. VITNK9 grew in large numbers on the ISP-3 and ISP-4 agar media. In our investigation, we showed that for two isolates, a pH range of 7.0–8.0 produced the best growth (Table 5). Similarly, Akond et al. (2016) found that the pH ranges of 6.5–8.0 for the isolate *Nocardia* JUBM–35–NS-2 and 6.0–6.5 for the isolate *Streptomyces* JUBM–35–NS-1

produced the best results for growth. However, Kontro et al. (2005) reported that ten *Streptomyces* spp. grew over a broad pH range between 4.0–5.5 and 10.0–11.5 but grew optimally at pH = 7.0 and above. Both actinomycete isolates in this investigation had maximum growth at a NaCl concentration of 3–7% and could survive at a NaCl concentration of 10% (Table 5). At 10% NaCl, however, the growth of isolates BCA1.2 was better than that of isolates BCA1.5 (Table 5). In contrast to strain RL8, which required more than 0.6% salt content in the culture media to develop, strains *Streptomyces* N7 and V4 thrived at salt concentrations ranging from 0 to 10%, according to research by Bernal et al. (2015). The research by Aouar et al. (2020) indicated that all strains were positive for growth in 4% NaCl. However, two *Streptomyces* strains, SO2 and SB1, could grow at 7% NaCl, while no growth was observed for the strain *Streptomyces* SO1.

Besides antimicrobial activity, numerous previous studies demonstrated that actinomycete strains could produce a wide range of extracellular hydrolytic enzymes, such as glucanase, chitinase, protease, lipase, and amylase (Roopan et al., 2019; Al-Dhabi et al., 2020). These findings illustrated that isolate BCA1.2 and BCA1.5, were positive for hydrolytic enzymes, including chitinase, protease, lipase, and amylase (Table 7). Earlier, *Streptomyces* sp. strain A from unexplored mangrove soils was found to have amylase (0.59 mol/ml/min), chitinase (1.356 mol/ml/min), and protease (0.248 mol/ml/min) activities, according to Deepthi et al. (2012). Research by Al-Dhabi et al. (2020) isolated *Streptomyces* sp. Al-Dhabi-49 for the simultaneous production of lipase and protease. Moreover, the production of protease and lipase was influenced by the incubation period, pH values, and culture medium temperature. Recently, according to research by Elabbasy et al. (2021), *Streptomyces canescens* MH7 has been found to be able to produce the extracellular hydrolytic enzymes consisting of glucanase, chitinase, lipase, and protease. The production of various lytic enzymes in this study showed the potential application of two isolates, BCA1.2 and BCA1.5, due to their antifungal activity in the future.

CONCLUSIONS

P. mirabilis is a Gram-negative anaerobic pathogen belongs to *Morganella* family. It is facultative, maltose positive and lactose negative bacteria. However, it is one of the normal florae in the digestive tract of animals and human. Resistance of microorganisms to the antibiotics is developed worldwide these days. This resulted in emerging of multidrug resistant pathogens, which can be considered as a real threat to the public and animal health. Antibiotic resistance in bacteria could be natural or acquired. In *P. mirabilis* antibiotic resistance genes are either carried on plasmid or found within the chromosome. In the current study, the draft genome sequencing of *P. mirabilis* MAD23 was reported. General feature of the genome and the acquired antibiotic resistance genes were identified. Also, phytogenic comparison between MAD23 strain and the related bacteria was demonstrated depending on the WGS and *16S rRNA* sequence. Finally, this work can improve our understanding about the important features of *P. mirabilis* genome and focusing on the genes responsible for antibiotic resistance.

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AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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