

## THE PHYLOGENETIC ANALYSIS OF NOVEL DIARRHEAGENIC *PROTEUS MIRABILIS* FROM CHICKEN CARCASSES AND HUMAN ISOLATES

<sup>1</sup> Athraa Abass Esmiel , <sup>2</sup>Huda Nsaif Jasim

<sup>1</sup> Dep. Of veterinarian hospital / Office of Agricultural Research /Ministry of Agriculture/  
Baghdad /Iraq.

<sup>2</sup> Dep. of veterinary Public Health / College of Veterinary Medicine / University of Baghdad.

**\*Corresponding author's email:**

[ath2820@gmail.com](mailto:ath2820@gmail.com) , [hoda.nj@covm.uobaghdad.edu.iq](mailto:hoda.nj@covm.uobaghdad.edu.iq)

### Abstract

*P. mirabilis* have been linked to food poisoning outbreaks, it is a pathogenic bacteria that can contaminate chicken meat not just through handling, preparation, and processing, but also by washing carcasses with polluted water. This study was conducted in the Department of Veterinary Public Health / Laboratory of Meat Hygiene from September (2020) to January (2021), where samples of chicken meat were collected under chilled conditions from Baghdad's local markets, and all health aspects were considered when transferring these samples to the laboratory .this study includes the collecting of 200 samples as well as human urine and feces samples from various sites in Baghdad city. *Proteus mirabilis* was identified from 38 of 200 samples (19%) and isolates from human (urine and feces).

We detected a strain of *P. mirabilis* isolated from chicken meat and patients' specimens (urine and feces) in Baghdad. To confirm the diagnosis API 20E kits, Vitek2 system, and ultimately the presumptive diagnosis was validated by utilizing PCR test. *Proteus mirabilis* isolates were diagnosed using PCR by 16S ribosomal RNA (16S rRNA) primers to determine their gastrointestinal pathogenicity and relationship with UTI infection experiments were performed to compare *P. mirabilis* strain (isolated from chicken meat carcasses) and *P. mirabilis* (isolated from human fecal and urine) by 16S ribosomal RNA gene, partial sequence to show genetic similarity between two strains, Also, we recorded Iraqi sequences in NCBI-Gene-Bank and DDBJ of INSDC (USA). Sequencing technology was used to diagnose *Proteus mirabilis* strain isolates were examined by (16SrRNA) genes, and the new isolates were recorded in Nucleotide/Blast and Iraqi were recorded as the first sequencing in Gene-Bank/NCBI, DDBJ, and ENA (INSDC), each sequence has Accession numbers as follows: (ID: OM811980.1, ID: OM811981.1). These are the first Iraqi isolates from chicken meat and human specimens to be recorded in the Gene Bank/USA, Also results shows genetic tree phylogenetic among *P. mirabilis* isolates in different countries in the world.

**Key word:** *Proteus mirabilis*, genetic similarity detection, (16SrRNA) genes,

### Introduction

*Proteus mirabilis* is one of the most active pathogens responsible for food poisoning outbreaks. It is a gram negative short rods, mobile, and non-spore forming bacterium that belongs to the *Proteus* genus and the family Enterobacteriaceae. *Proteus* genus members are saprophytic normal flora or opportunistic pathogens that cause many diseases when they invade locations

outside of their usual habitat (Gupta *et al.*; 2014). *P. mirabilis* is thought to be the causal pathogen in hospital cross-infection linked with urinary catheters (Hola, *et al.*, 2012). Some unique strains, on the other hand, have been documented to pose dangers to human health and may induce significant sickness in individuals (Wang *et al.*, 2010). *P. mirabilis* has been linked to an increasing number of food poisoning cases in recent years.

*P. mirabilis* is frequently isolated from the gastrointestinal system, whether it is a commensal, pathogen, or transitory organism is debatable (Janda *et al.*, 2006). The majority of *P. mirabilis* urinary tract infections (UTI) are assumed to be caused by ascend of bacteria from the gastrointestinal system, whereas some are likely to be caused by person-to-person transmission, particularly in healthcare settings (O'Hara *et al.*, 2000). Evidence suggests that some individuals with *P. mirabilis* UTI have the same strain of *P. mirabilis* in their feces, whereas others have no *P. mirabilis* in their stools (Mathur *et al.*, 2005).

In the early 1980s, sequencing of 16SrRNA genes, followed by comparison of these sequences and phylogenetic analysis, became another promising taxonomic tool; the 16STRNA gene is highly conserved, raising the question of whether this gene is appropriate to differentiate very closely related species, and several studies have described the results obtained by PCR with 16SrRNA, Its perfect diagnosis and differentiation of closely related species (Watts *et al.*, 2017) There are various advantages of 16SrRNA gene sequencing over phenotypic and biochemical identification.

Another advantage of employing this sort of investigation is the lack of studies about the significant mutations occur in this gene. When compared to biochemical and phenotypic identification techniques, 16SrRNA gene sequencing has more accurate. Because phenotypic traits in bacteria have fluctuate results owing to environmental factors, growth substrate, temperature, and pH levels, so the phenotypic characteristics are not as reliable as genotypic approaches like 16STRNA (Poria *et al.*, 2017). The molecular approach has been preferred due to its speedier, more specific, and precise results, as well as its ability to justify the diversity of microbes in the environment (Walker *et al.*, 2014).

As a result, the main objective of this study was to highlight the prevalence of these bacteria in local and imported chicken meat from various areas in Baghdad, and to demonstrate the relationship between gastrointestinal tract (GIT) infection caused by contaminated chicken meat by *p. mirabilis* and ascending infection in urinary tract infections (UTI), as well as to describe the genetic similarity between isolates.

## **Material and methods**

### **Isolation and identification of *proteus mirabilis***

Tow hindered (200) samples, these samples were taken from different places in the governorate of Baghdad, Iraq During the period of September 2020 to October 2021 .Chicken meat carcasses were taken and preserved in special sterile bags for preserving samples and taken directly to the laboratory. human samples of human feces and human urine of a case diagnosed as food poisoning in AL-Kadhimiya Hospital and Ghazi Hariri Hospital in Madenat Al Tib were transferred to the laboratory and streaked on a special culture medium in order to diagnose it with technology

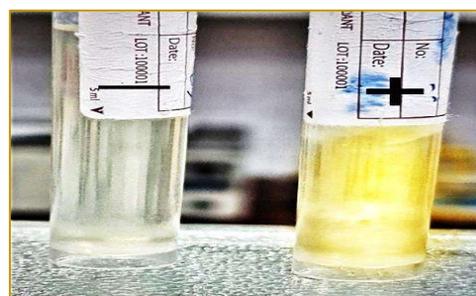
Polymerase chain reaction (PCR) to compare with bacteria isolated from carcasses of chicken meat to show the genetic similarity between the two strains. All of the above samples were inoculated on Tryptone Soy Broth (TSB) and incubated at 37 C° for 24 hours. Inoculated on the selective media of *Portus spp*, MacConkey and blood agar, the plates were incubated at 37 C° for 24 hours in order to observe the morphological features on culture media such as swarming on blood agar, non-lactose fermented growth on MacConkey. As well as the isolates were identified bacteriologically, biochemically according to the methods described by (Moyes *et al.*, 2009).

The biochemical tests for *P. mirabilis* showed the positive results for urease test, motility test and H<sub>2</sub>S production while the negative results were established for indole in SIM test and this test was also used to differentiate *P. mirabilis* due to the hydrolysis of the amino acid (tryptophan) and its conversion to indole. Figure (). This result was agreed with (Oliveira de Araujo *et al.*, 2022). Isolates that show positive interaction with TSI were identified as *Proteus mirabilis* and this is similar to the result approved by (Khayyat *et al.*, 2021).

Also Ali, (2012) isolated a negative result for the oxidase test for their inability to produce the enzyme oxidase, which were identical with (Ridha *et al.*, 2022). In addition to that all isolates respond to the catalase test and this result is similar to (White, 2021). Phenylalanine deaminase test appeared, a demineralized product of phenyl pyruvic acid forming a transient green color when ferric chloride is added which were agreed with (Armbruster *et al.*, 2017). This result illustrated in table (1), figure (1)

**Table (1): Biochemical tests of *P. mirabilis* isolates**

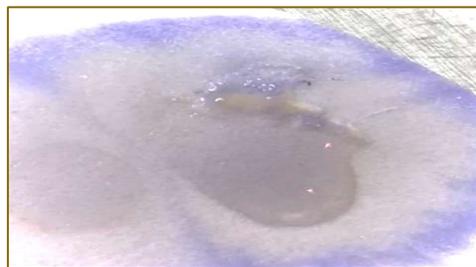
No.	Biochemical tests	Results	Reaction
1.	Gram stain	-ve	<b>bacilli occur singly or as double</b>
2.	Oxidase test	- ve	<b>No purple color</b>
3.	Catalase test	+ ve	<b>Bubble</b>
4.	Simmon Citrate test	+ ve	<b>Blue color</b>
5.	Gelatinase test	+ ve	<b>Liquefaction of gelatin</b>
6.	Methyl Green Phenylalanine Deaminase dimethyl agar	+ ve	<b>dark green colonies</b>
7.	Urease test	+ve	<b>Pink color</b>
8.	motility test	+ve	<b>growth</b>



A



B



C



D

**Figure (1) shows biochemical tests of *Proteus mirabilis* A- motility test (+)B- urease(+),Phenylalanine deaminase (+),Simmons citrate (+) C- Oxidase test (-) D-catalase test (+)**

### Molecular identification

The DNA genome was recovered from bacterial isolates using the ABIO pure Extraction protocol. The Quantus Fluorometer was used to measure the concentration of extracted DNA in order to determine the quality of samples for future usage. (1 µl) of DNA was combined with (199 µl) of diluted QuantyFlour Dye. DNA concentrations were measured after 5 minutes of incubation at room temperature. The primers were lyophilized, dissolved in free ddH<sub>2</sub>O to give a final concentration of 100 pmol/l as stock solution, and kept at -20 to prepare a stock solution of 10 p mol/l concentration as work primer suspended, 10 l of the stock solution in 90 l of free ddH<sub>2</sub>O water to reach a final volume of 100 l, was investigated by IDT (Integrated DNA Technologies company, Canada) as mention in Table (2)

**Table (2) Primer of 16S rRNA gene**

Primer	Sequence	T <sub>m</sub> (°C)	GC (%)	Product size	Reference
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair	Primer design
Reverse	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1		

The Neighbor-Joining approach was used to infer the evolutionary history. The evolutionary distances were calculated using the Jukes-Cantor model, which was created in the Gene6 software to determine phylogenetic distance. (Ibrahim *et al.*, 2014). The National Center for Biotechnology

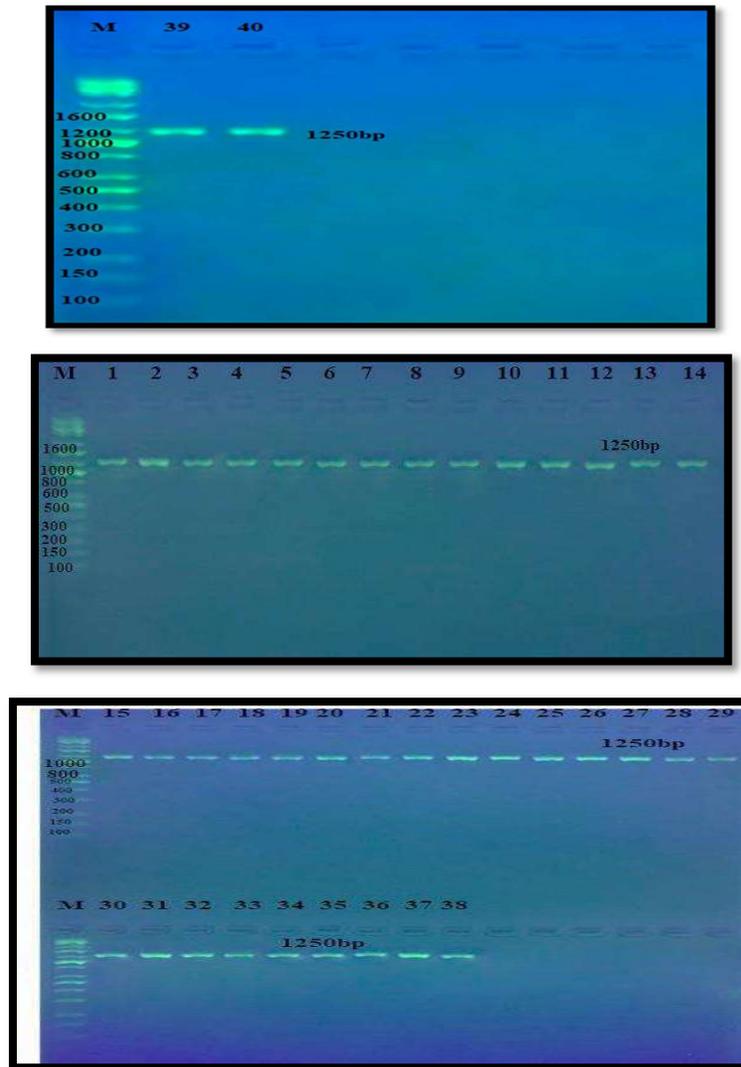
Information (NCBI) (Tamura *et al.*, 2011) is a component of the National Institutes of Health's National Library of Medicine (NLM) (NIH). The United States government has approved and sponsored it. The NCBI is headquartered in Bethesda, Maryland, and was established in 1988 as a result of legislation backed by Senator Claude Pepper. (Taubs, 2000)

### Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One and two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Chi square was also used to assess the significant differences among percentages.  $P < 0.05$  is considered statistically significant.

### Results and discussion

PCR technique has been used to amplify 16SrRNA diagnostic genes of genomic DNA of all isolates of *P. mirabilis*. Genomic DNA were extracted from all isolates and visualized as show in figure (1). The results of all isolates diagnosis by PCR technique for detection 16SrRNA Clarified that 38/38(100%) isolates of *P. mirabilis* produced correct products of 16SrRNA genes figure (2).



**Figure (2) PCR product the band size. The product was electrophoresis on 1.5% agarose at 5 volt/cm<sup>2</sup>. 1x TBE buffer for 1:30 hours. M: DNA ladder (100),the band number 1-38 *proteus mirabilis* chicken meat samples , 39-40 bands for human *proteus mirabilis* (isolates from urine and feces).**

The PCR method is faster, more sensitive and more specific to detect bacterial strains. Moreover, it allows detection of dead cells and viable but non-cultivable cells, Nowadays, PCR-based methods, are used primarily to identify and quantify either pathogens or beneficial populations, including, based on the 16S rRNA genes or their specific functional genes (**Postollec et al., 2011**) Compared with the genetic markers 16S rRNA, the housekeeping gene that is preserved in almost all bacterial species, one may speculate molecular method should be more specific and discriminating in the detection of *P. mirabilis* which were illustrated by (**Zhang et al., 2013**). In this study, a PCR test was used for more accurate diagnosis *Proteus mirabilis* and results of PCR amplification to 16S rRNA primers and primers indicated positive results reported in humans, and chicken meat which were in agreement with (**Takahashi et al., 2017**)

Diagnosis the gene 16S rRNA that of *Proteus mirabilis* is identical to the researchers (**Algammal et al., 2021**) which were showed that 16S RNA sequences in the diagnosis of *Proteus mirabilis* from clinical cases, while (**Wachinoet al., 2006**) found that the 16S RNA gene sequence is responsible for the resistance that *Proteus mirabilis* has against antibiotics from the aminoglycoside group. Diagnosis of *Proteus mirabilis* according to 16S RNA gene was agreed with (**Kumar & Das, 2016**) In addition, several research employed PCR sequencing to show that *P. mirabilis* had variable virulence islands (**Soliman et al., 2017**). Two clinical isolates of *P. mirabilis* harboring Salmonella genomic island (SGI1) variations, SGI1-PmABB and SGI1-W, were reported for the first time in Africa.

rRNA sequence analyses of these bacteria would aid in evaluating the extent to which this interesting physiological and behavioral adaptation is phylogenetically distributed. PCR has been used in the current study to amplify genes of (16SrRNA) of genomic DNA of all isolates *p. mirabilis* Genomic DNA were extracted from all isolates and visualized. The current results of all isolates diagnosed by PCR technique for detection 16SrRNA clarifying that all isolates (100%) of *p. mirabilis* produced specific segmental 16SrRNA genes figure (3)

#### **Sequencing analysis/ Alignment of *p. mirabilis* Genes**

Sequencing and Alignment of *Proteus mirabilis* 16S ribosomal RNA gene shows Microbial genomes are subject to variability due to mutation or. The sequence variability within particular genes can be used in molecular typing schemes to determine the relatedness of bacteria. An increasing number of truly complete bacterial genomes are being placed in the International Nucleotide Sequence Database Collaboration, a public database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome>). This method is also acquiring practical implications for the identification and typing of microorganisms (**Ranjbar et al., 2014**)

The results of DNA sequencing should be firstly examined to confirm the nucleotide sequences and closed relationship with others world strains, test used to confirm was through using NCBI-BLASTn nucleotide. *P. mirabilis* isolates were examined by sequencing technology to diagnosis

of isolates and record it by (16SrRNA) genes. All isolates were successful in processing of a good running of sequencing by a Company DNA Macrogen/ Korea. The results were revealed the first Iraqi isolates after compared with data in Gene Bank/ BLAST which is available at the NCBI online, using Nucleotide/Blast and recorded as the first sequencing in Gene-Bank/NCBI, DDBJ and ENA (INSDC). 16SrRNA genes were successfully amplified using specific PCR primers as mentioned in table (1) *p. mirabilis* isolates which observed of results in () which showed PCR amplification for 16SrRNA genes, which have a specific products (1250) bp .

This present study results shows that PCR product the band size 1250 and the product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours, DNA ladder (100), the band number (1-11) *proteus mirabilis* isolates from imported chicken meat samples, while the bands number (12-38) *proteus mirabilis* isolates from local chicken meat samples and bands number (39-40) human isolates (isolates from urine and feces) These are the first Iraqi isolates from chicken meat and human recorded in Gene Bank/USA.

***Proteus mirabilis* strain D91 16S ribosomal RNA gene, partial sequence**

Sequence ID: [OK310567.1](#) Length: 1077 Number of Matches: 1  
 Range 1: 17 to 914 [GenBankGraphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
<b>1698 bits(883)</b>	0.0	893/898(99%)	0/898(0%)	Plus/Plus
Query 1	CATGCAAGTCGAGCGGTAACAGGAGGAAGCTTGCTTTCTTGCTGACGAGCGCGGACGGG			60
<b>Sbjct</b> 17	..... <b>A</b> .....			76
Query 61	TGAGTAATGTATGGGGATCTGCCCGATAGAGGGGATAACTACTGGAAACGGTGGCTAAT			120
Sbjct 77	.....			136
Query 121	ACCGCATAATGTCACGGACCAAGCAGGGGCTCTTCGGACCTTGCACTATCGGATGAAC			180
Sbjct 137	.....			196
Query 181	CCATATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCTCTAGCTGG			240
Sbjct 197	.....			256
Query 241	TCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGAC'TCC'TACGGGAGGCA			300
Sbjct 257	.....			316
Query 301	GCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAG			360
Sbjct 317	.....			376
Query 361	AAGGCCTTAGGGTCGTAAAAGTACTTTCAGCGGGAGGAGGTGATAAGGTTAATACCCCTT			420
<b>Sbjct</b> 377	..... <b>T</b> .....			436
Query 421	ATCAATTGACGTTACCCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCCGGTAA			480
Sbjct 437	.....			496
Query 481	TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGCGGTCAAT			540
Sbjct 497	.....			556
Query 541	TAAGTCAGATGTGAAAGCCCCGAGCTTAACCTGGGAATTGCATCTGAAACTGGTTGGCTA			600
Sbjct 557	.....			616
Query 601	GAGTCTTGTAGAGGGGGTAGAATCCATGTGTAGCGGTGAAATGCGTAGAGATGTGGAG			660
Sbjct 617	.....			676
Query 661	GAATACCGGTGGCGAAGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTG			720
Sbjct 677	.....			736
Query 721	GGGAGCAAACAGGATTAGATACCCTGGTTGTCCACGCTGTAAACGATGACGATTTAGAGG			780
<b>Sbjct</b> 737	..... <b>A</b> ..... <b>T</b> .....			796
Query 781	TTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACCGCTTAAATCGACCGCCTGGGGAGTGC			840
<b>Sbjct</b> 797	..... <b>A</b> .....			856
Query 841	GGCCGCAAGGTTAAACTCAAATGAATTGACGGGGCCCGCACAAAGCGGTGGAGCATG			898
Sbjct 857	.....			914

*Proteus mirabilis* strain NG-MAK-B-90 16S ribosomal RNA gene, partial sequence

Sequence ID: [OL661607.1](#) Length: 1435 Number of Matches: 1  
 Range 1: 8 to 905 [GenBankGraphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1704 bits(886)	0.0	894/898(99%)	0/898(0%)	Plus/Plus
Query 1	CATGCAAGTCGAGCGGTAACAGGAGGAAGCTTGCTTCTTGCTGACGAGCGGCGGACGGG	60		
<b>sbjct</b> 8	..... <b>A</b> .....	67		
Query 61	TGAGTAATGTATGGGGATCTGCCCGATAGAGGGGGATAAECTACTGGAAACGGTGGCTAAT	120		
Sbjct 68	.....	127		
Query 121	ACCGCATAATGTCTACGGACAAAGCAGGGGCTCTCGGACCTTGCACTATCGGATGAAC	180		
Sbjct 128	.....	187		
Query 181	CCATATGGGATTAGCTAGTAGTGGGGTAAAGGCTCACCTAGCGGACGATCTCTAGCTGG	240		
Sbjct 188	.....	247		
Query 241	TCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCA	300		
Sbjct 248	.....	307		
Query 301	GCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAG	360		
Sbjct 308	.....	367		
Query 361	AAGACCTTAGGGTTGTAAAGTACTTTCAGCGGGGAGGAGGTATAAGGTTAATACCCCT	420		
<b>sbjct</b> 368	..... <b>G</b> .....	427		
Query 421	ATCAATTGACGTTACCCGAGAAGAAGCACCCGGCTAACTCCGTGCCAGCAGCCGGTAA	480		
Sbjct 428	.....	487		
Query 481	TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGCGCGTCAAT	540		
Sbjct 488	.....	547		
Query 541	TAAGTCAGATGTGAAAGCCCCGAGCTTAACTTGGGAATTGCATCTGAAACTGGTGGCTA	600		
Sbjct 548	.....	607		
Query 601	GAGTCTTGCAGAGGGGGTAGAATCCATGTGTAGCGGTGAAATGCGTAGAGATGTGGAG	660		
<b>sbjct</b> 608	..... <b>T</b> .....	667		
Query 661	GAATACCGGTGGCGAAGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCCGAAAGCGTG	720		
Sbjct 668	.....	727		
Query 721	GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAACGATGACGATTTAGAGG	780		
Sbjct 728	.....	787		
Query 781	TTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGTTAAATCGACCGCTGGGGAGTGC	840		
Sbjct 788	.....	847		
Query 841	GGCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGCAAGGGGTGGAGCATG	898		
<b>sbjct</b> 848	..... <b>C</b> .....	905		

**Figure (3) Sequence analysis and Alignment statistics of *Proteus mirabilis* strain NG-MAK-B-90 16S ribosomal RNA gene, partial sequence, (ID: [OL661607.1](#) Length: 1435 Number of Matches: 1, ID: [OK310567.1](#) Length: 1077 Number of Matches: 1).**

Recording of Iraqi sequences in NCBI-Gene-Bank and DDBJ of INSDC (USA), Sequencing technology was used to diagnose *Proteus mirabilis* strain isolates were examined by (16SrRNA) genes, and recorded the new isolates in Nucleotide/Blast and Iraqi were recorded as the first sequencing in Gene-Bank/NCBI, DDBJ, and ENA (INSDC), each sequence have **Accession numbers as following: (ID: OM811980.1, ID: OM811981.1)**. These are the first Iraqi isolates from chicken meat and human recorded in Gene Bank/USA.

for Recording of Iraqi sequences in NCBI-Gene-Bank and DDBJ of INSDC (USA), Sequencing technology was used to diagnose *Proteus mirabilis* strain isolates were examined by (16SrRNA) genes, and recorded the new isolates in Nucleotide/Blast and Iraqi were recorded as the first sequencing in Gene-Bank/NCBI, DDBJ, and ENA (INSDC), each sequence have Accession numbers as following: (ID: OM811980.1, ID: OM811981.1), 16SrRNA, genes sequence submitted to Gene bank. The results of these sequences were analyzed and examined by professional staff in Gene bank/NCBI, DDBJ and ENA (INSDC). All these sequences accepted in Gene bank and each sequence take accession number. These results recorded and published in the International Nucleotide Sequence Database Collaboration (INSDC). This location contains the database of National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ) and European Nucleotide Archive (ENA).

**The genetic similarity between *Proteus mirabilis* isolates from chicken meat and human.**

The result of genetic similarity between *Proteus mirabilis* isolates from chicken meat and human shows that both of isolates exhibited several genotypic similarity Identities to (99%) *Proteus mirabilis* strain D91 16S ribosomal RNA gene, several strains from chicken meat showed close genetic similarity to those from UTI, feces, thus, chicken meat may be an important source of the dissemination of *P. mirabilis* responsible for causing UTIs in the community. And that agreed with (Sanchez *et al.*, 2021) and non-agreed with (Yu *et al.*, 2021) were found high strain heterogeneity was present in isolates from broilers and human stool in Belgian. Partial sequence of *Proteus mirabilis* strain (D91) 16S ribosomal RNA gene shows many substitution, the type of substitution (Transition, Transversion) for *Proteus mirabilis* strains between chicken meat strains and human strains illustrated in table (3)

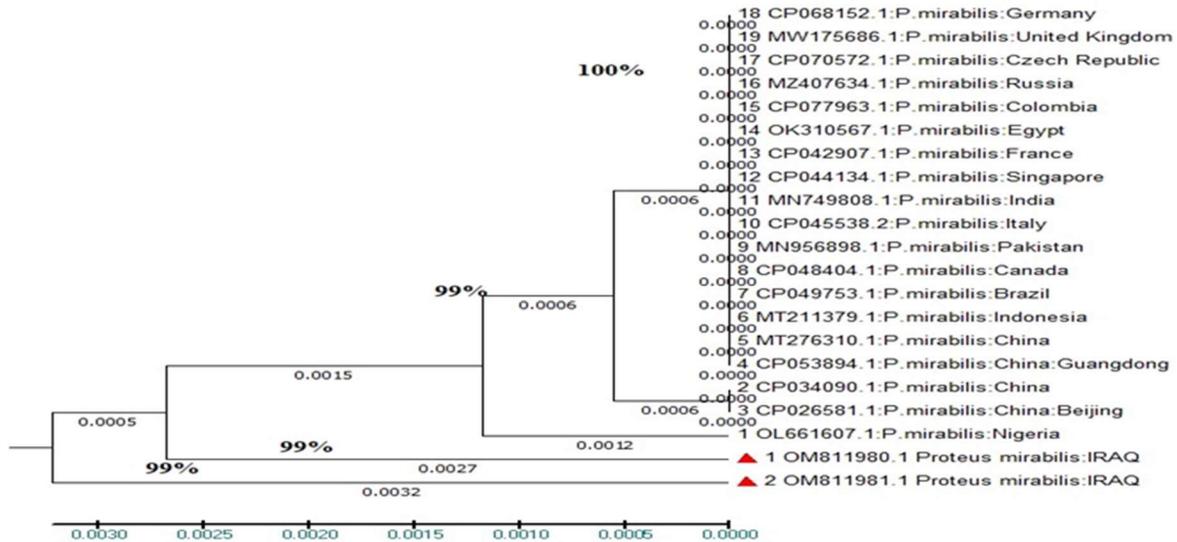
**Table (3) Type of substitution for *Proteus mirabilis* strain D91 16S ribosomal RNA gene (between *Proteus mirabilis* strain chicken meat and human)**

Gene: 16S ribosomal RNA gene

No.	Type of substitution	Location	Nucleotide	Sequence with compare	ID	Sequence with Submissions	ID	Source
1	Transition	42	A\G	ID: <a href="#">OK310567.1</a>		ID: OM811980.1 (chicken)		<i>Proteus mirabilis</i>
	Transition	390	T\C					
	Transversion	765	A\T					
	Transversion	785	T\A					
	Transition	855	A\G					
2	Transition	33	A\G	ID: <a href="#">KF471508.1</a>		ID: OM811981.1 (human)		<i>Proteus mirabilis</i>
	Transition	371	G\A					
	Transition	616	T\C					
	Transversion	884	C\G					

Dendrogram of phylogenetic analysis revealed the diversity of all isolates in the Iraq and world. The percentage level of similarity clearly showed that the isolates examined by species were

distinct cluster numbers, in addition to some single isolates, that clustered at a similarity level of (99%). In current study we compare between Iraqi chicken meat isolates and the other isolates in world from different sources to clarify the homogeneity and heterogeneity between isolates. And phylogenetic analysis showed that the *P. mirabilis* isolates from human urine and feces samples may have close relatedness with that from chicken meat. Figure (4)



**Figure (4) comparing *Proteus mirabilis* isolates in different countries in the world with the Iraqi isolate**

### Conclusions:

1. This research revealed that the prevalence of *Proteus mirabilis* from chicken meat samples are conferring important public health concern.
2. Phylogenetic analysis showed that the *P. mirabilis* isolates from human sources may have close relatedness with that from animals.
3. This research may indicate that *P. mirabilis* is successfully transmitted between species, particular from avian to mammalian hosts.
4. This study shed a light on giving greater importance to food poisoning cases caused by *P. mirabilis* bacteria because it is possible to develop to cause the ascending infection with UTI.

### References

1. Gupta, A. K., Rastogi, G., Nayduch, D., Sawant, S. S., Bhonde, R. R., & Shouche, Y. S. (2014). Molecular phylogenetic profiling of gut-associated bacteria in larvae and adults of flesh flies. *Medical and Veterinary Entomology*, 28(4), 345-354.
2. Hola, V., Peroutkova, T., & Ruzicka, F. (2012). Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections. *FEMS Immunology & Medical Microbiology*, 65(2), 343-349.
3. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution*, 28(10), 2731-2739.
4. Taubs, G. (2000). Sense from sequences: Stephen F. Altschul on bettering BLAST. *Science*

Watch, 11, 3-4.

5. SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.
6. Postollec, F., Falentin, H., Pavan, S., Combrisson, J., & Sohier, D. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food microbiology*, 28(5), 848-861.
7. Zhang, W., Niu, Z., Yin, K., Liu, P., & Chen, L. (2013). Quick identification and quantification of *Proteus mirabilis* by polymerase chain reaction (PCR) assays. *Annals of microbiology*, 63(2), 683-689.
8. Takahashi, H., Saito, R., Miya, S., Tanaka, Y., Miyamura, N., Kuda, T., & Kimura, B. (2017). Development of quantitative real-time PCR for detection and enumeration of Enterobacteriaceae. *International journal of food microbiology*, 246, 92-97.
9. Algammal, A. M., Hashem, H. R., Alfifi, K. J., Hetta, H. F., Sheraba, N. S., Ramadan, H., & El-Tarabili, R. M. (2021). atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. *Scientific reports*, 11(1), 1-15.
10. Wachino, J. I., Yamane, K., Shibayama, K., Kurokawa, H., Shibata, N., Suzuki, S. & Arakawa, Y. (2006). Novel plasmid-mediated 16S rRNA methylase, RmtC, found in a *Proteus mirabilis* isolate demonstrating extraordinary high-level resistance against various aminoglycosides. *Antimicrobial agents and chemotherapy*, 50(1), 178-184.
11. Kumar, M. S., & Das, A. P. (2016). Molecular identification of multi drug resistant bacteria from urinary tract infected urine samples. *Microbial pathogenesis*, 98, 37-44.
12. Soliman, A. M., Ahmed, A. M., Shimamoto, T., El-Domany, R. A., Nariya, H., & Shimamoto, T. (2017). First report in Africa of two clinical isolates of *Proteus mirabilis* carrying Salmonella genomic island (SGII) variants, SGI1-PmABB and SGI1-W. *Infection, Genetics and Evolution*, 51, 132-137.
13. Ranjbar, R., Karami, A., Farshad, S., Giammanco, G. M., & Mammina, C. (2014). Typing methods used in the molecular epidemiology of microbial pathogens: a how-to guide. *New Microbiologica*, 37(1), 1-15.
14. Sanches, M. S., da Silva, C. R., Silva, L. C., Montini, V. H., Barboza, M. G. L., Guidone, G. H. M. & Rocha, S. P. D. (2021). *Proteus mirabilis* from community-acquired urinary tract infections (UTI-CA) shares genetic similarity and virulence factors with isolates from chicken, beef and pork meat. *Microbial Pathogenesis*, 158, 105098.
15. Yu, Z., Joossens, M., Van den Abeele, A. M., Kerkhof, P. J., & Houf, K. (2021). Isolation, characterization and antibiotic resistance of *Proteus mirabilis* from Belgian broiler carcasses at retail and human stool. *Food Microbiology*, 96, 103724.
16. Oliveira de Araujo, J. F., Lopes da Silva, A. L., Acioly de Omena, I. C., Alvino, V., Todaro, A. R., & Bastos, M. L. D. A. (2022). *Proteus mirabilis* resistant to carbapenems isolated from a patient with a venous leg ulcer: a case report. *Journal of Wound Care*, 31(5), 460-464.

17. Khayyat, A. N., Abbas, H. A., Mohamed, M. F., Asfour, H. Z., Khayat, M. T., Ibrahim, T. S. & Hegazy, W. A. (2021). Not only antimicrobial: Metronidazole mitigates the virulence of *Proteus mirabilis* isolated from macerated diabetic foot ulcer. *Applied Sciences*, 11(15), 6847.
18. Ali, O. A. U. (2012). Prevention of *Proteus mirabilis* biofilm by surfactant solution. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, 4(1), 1-8.
19. Ridha Abbas Al-Fahham, H., & Raoof Kareem, K. (2022). Molecular Study of Urease ureR Gene of *Proteus mirabilis* Isolated from Urinary Tract Infections, Najaf, Iraq. *Archives of Razi Institute*, 77(3), 1257-1260.
20. White, A. N. (2021). Contribution of Catalase to *Proteus mirabilis* Biofilm Development and Pathogenesis (Doctoral dissertation, State University of New York at Buffalo).
21. Armbruster, C. E., Smith, S. N., Johnson, A. O., DeOrnellas, V., Eaton, K. A., Yep, A. & Mobley, H. L. (2017). The pathogenic potential of *Proteus mirabilis* is enhanced by other uropathogens during polymicrobial urinary tract infection. *Infection and immunity*, 85(2), e00808-16.