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Molecular Analysis of Virulence Genes in *Proteus* Species Isolated from Chicken Meat and Human Samples in Wasit Province, Iraq

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ABSTRACT

The objective of this study was to examine the presence of the most crucial genes mrpI, ureC, and zapA associated with the virulence of *Proteus* species isolated from chicken meat and human sources, with the goal of better understanding how they spread via the food chain. Between March and September 2023, two hundred chicken meat samples were collected from local supermarkets, and 120 human urine samples were evaluated in Wasit province hospitals, Iraq. The study found that *P. mirabilis* and *P. vulgaris* were isolated at a rate of 13 and 4%, respectively, from 200 chicken meat samples. However, the isolation rate in humans was 25 and 6.6%, with 30 and 8 isolates from 120 urine samples. Data revealed that contamination with *P. mirabilis* boosts the risk by 3.40 times compared to *P. vulgaris*. Molecular analysis showed that the ureC and zapA genes were identified in both isolates at 100 and 99%, respectively. In comparison, the mrpI gene existed in all isolates derived from chicken flesh and human samples at 99%. These findings demonstrate that bacteria can cause infection and disease due to the existence of virulence genes that control mechanisms such as biofilm formation, toxin release, and tissue invasion. The discovery of these bacteria in human food emphasizes maintaining the highest health and safety standards when preparing and processing meat for human consumption.

Keywords: Chicken meat, Foodborne disease, *Proteus* species, Virulence genes

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INTRODUCTION

Animal-derived *Proteus* is a major food-borne zoonotic bacillus frequently detected in the broiler breeding sector. *Proteus spp* is a member of the Enterobacteriaceae family and an important opportunistic and foodborne pathogen, gram-negative, rod-shaped, facultatively anaerobic, non-capsulated, non-spore-forming, motile, and a significant zoonotic conditional pathogen that infects both humans and animals (Kozlovska, 2023). *Proteus* has five identified species: *Proteus mirabilis, Proteus vulgaris, Proteus penneri, Proteus hauseri,* and *Proteus myxofaciens*, as well as three unnamed *Proteus* genomospecies (Drzewiecka, 2016). *Proteus mirabilis* has been linked to a variety of infections, including urinary tract infections, respiratory tract and wound infections, burns, and digestive system infections (Odoki *et al.*,2019). Many pathogenic bacteria contribute to foodborne illnesses, causing diarrhea, vomiting, and stomach cramps, particularly when eating contaminated or undercooked foods (Hashem and Abdaljaleel, 2024; Khudhir, 2021). It can be found in various environments, including human digestive tracts, and wild and domestic animals (Avi, 2024). Meat is one of the world's most popular foods due to its nutritional value, including a high concentration of proteins, vitamins, and necessary amino acids required for body growth. As a result, care must be taken to ensure its quality and safety against all sorts of contamination, whether caused by microbes or heavy metals Chicken meat can be a source of contamination with these bacteria and others, such as *Salmonella* and *Escherichia coli*, that

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cause diseases in humans leading to multiple cases of food poisoning and gastrointestinal illnesses ،that may reach it via the air, water, soil, or manufacturing processes (Connolly and Campbell, 2023). Bacterial contamination of poultry carcasses and cuts results from improper hygienic measures. In addition, the dissemination of infection throughout plants during processing occurs in the evisceration, cooling, packaging, and transport (Hasan *et al*., 2023). Also, *proteus* in chicken droppings may be transmitted to workers who handle infected chickens directly or through fecal-contaminated poultry products (Al-Kubaisi and Al-Deri, 2022). Even if the poultry appears healthy and clean, *Proteus* strains may be present in their droppings and bodies (feathers, feet, and beaks) (Jambalang *et al*., 2017). *Proteus* species can adhere to cages, coops, feed and water dishes, hay, plants, and dirt in the birds' environment; eggshells may also become contaminated with *Proteus* via poultry droppings (poop) or the location where they are laid (Jahantigh, 2010). *E. coli* dominated the bird's intestinal content (Dakheel *et al*.,2023), and *P. mirabilis* was the second most common species found in 50% of birds (Sanches *et al.*, 2020). *Proteus* has virulence factors and enzymes, such as the protease enzyme, which helps break down protein, and urease hydrolyzes urea. It even contains toxins such as hemolysin, a hemolytic toxin, and *Proteus* toxin agglutinin (Abd Sharad, 2020). In addition, *P. mirabilis* has another virulence component involved in adhesions, flagella, and colonization and the virulence factors of urinary pathogens promote subsequent infections and adhesion to mucosal surfaces (Chakkour *et al*., 2024; Markey *et al*., 2014). The current study aimed to identify and analyze virulence genes (mrpI, ureC, and zapA) in *Proteus* species isolated from Chicken meat and human urine samples. The Introduction section should explain: The background to the study. The aims. A summary of the existing literature. The reason why the study was necessary, and the novelty must be explained.

MATERIALS AND METHODS

Ethical approval: The study was approved and carried out at the ninth session in the Council of the College of Veterinary Medicine, University of Baghdad, held on 10/1/2023, with approval No. 199 on the date 25/1/2023.

Samples collection: A total of 200 random samples of Chicken meat were collected from different local supermarkets and 120 human urine samples from different clinical cases from hospitals in Wasit Governorate, Iraq from March to September 2023, for molecular analysis of virulence genes in *Proteus* isolates and to identify the mutations that enable these bacteria to cause infections and diseases. The samples were put in an ice box under complete aseptic conditions and transferred directly to the meat hygiene laboratory, Department of Public Health, College of Veterinary Medicine, University of Baghdad.

Bacterial culture media: *Proteus* species were isolated and identified from Chicken meat samples according to ISO 6579:2017(36). Twenty-five grams of each sample were weighed and combined in a sterile flask with 225 ml of buffered peptone water before being incubated overnight at 37°C for 18-24 hours. One milliliter of the suspension was mixed with nine milliliters of tetrathionate broth and incubated at 42°C for 18-24 hours. The samples were cultivated on three media: standard MacConkey agar, selective xylose-lysine deoxycholate (XLD) agar, and differential agar (Hi-chrome UTI agar). Following the purification of *Proteus* colonies, bacterial species were identified using conventional techniques. All suspected colonies were identified using conventional biochemicals, followed by PCR for further confirmation.

Molecular analysis: All confirmed *Proteus mirabilis* and *Proteus vulgaris* isolates were extracted with an Add Prep Genomic DNA Extraction kit (Korea). The DNA purity and concentration were measured using a Nanodrop (NanoPhotometer® N50 Germany), and all extracted DNA was stored at -20 ℃ until used as a template for PCR. The 16S rRNA gene was specific to *Proteus* species, and the virulence factor genes mrpI, ureC, and zapA were studied for both Chicken meat and human samples. PCR conditions were optimized according to the recommendation of

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the standard PCR amplification (Sensoquest, Germany), and the products were separated on a 0.8 % agarose gel with Red Safe stain at 80V for 45 minutes at room temperature (25°C). Table (1) lists the primers examined in the NCBI GenBank database.

Sequencing and phylogeny analysis: Six PCR products of *Proteus spp*. for specific rRNA gene identification and virulence factor genes from the tested samples were delivered to Microgen/Korea for sequencing. All six samples were analyzed using the NCBI Basic Local Alignment Search Tool (BLAST). MEGA 11 software was used for the analysis and extraction of evolutionary history, and the phylogenetic tree was constructed according to a scale, with branch lengths representing the evolutionary distances used to create the phylogenetic tree. The maximum composite likelihood approach was used to calculate the evolutionary distances based on the number of base substitutions per site.

Table 1. The sequence of primers used in the current study

Genes name	Sequence	Primer sequence 5'- - 3'	Tm (°C)	GC%	Amplicon length	Reference
6S rRNA	F	CTGCCCGATAGAGGGGGATA	64.9	60	383bp	(Zahra *et al*.,2022)
	R	GGAGTTAGCCGGTGCTTCTT	62	55		
mrpI	F	GGCGTTTAAAAGGTGGGCTT	60.7	50	265bp	(Zahra *et al*.,2022)
	R	TCTCTGCCTAAATCCGCCAG	62	55		
ureC	F	GTTATTCGTGATGGTATGGG	55	45	317bp	(Ali and Yousif, 2015)
	R	ATAAAGGTGGTTACGCCAGA	57.8	45		
zapA	F	ACCGCAGGAAAACATATAGCCC	61.5	50	540bp	(Sanches *et al*., 2019)
	R	GCGACTATCTTCCGCATAATCA	58.1	45		

Statistics: The statistical analysis was done by using SAS software (SAS, 2018). The comparison of proportions of isolation was conducted using the Chi-square test. The Odds Ratio (OR) also compares the odds of exposure among cases to the odds of exposure among controls to measure the strength of the association between exposure and outcome. Odds Ratio (OR) is calculated according to (Szumilas, 2010) by the following formula:

Odds ratio (OR)= a×d ÷ b×c

a = Number of individuals who were exposed and have the outcome (Exposed + Diseased)

b = Number of individuals who were exposed but do not have the outcome (Exposed + Healthy)

c = Number of individuals who were not exposed but have the outcome (Unexposed + Diseased)

d = Number of individuals who were not exposed and do not have the outcome (Unexposed+ Healthy).

RESULTS AND DISCUSSION

The results of the samples obtained through the culture method and confirmed by PCR showed that the total percentage of *P. mirabilis* isolation from Chicken meat samples was 13% (26 out of 200) and 25% (30 out of 120) from human samples. In comparison, the percentage of *P. vulgaris* isolation was 4% (8 out of 200) and 6% (8 out of 120) respectively, (Tables 2 and 3). These results indicate a statistically significant difference ($P < 0.05$) in the isolation rates of chicken meat and human samples. This suggests that the greater rate of human samples is statistically significant. The results of identification and isolation by cultural media methods of *Proteus* species isolated, and microscopic examination were like the phenotypic characteristics of this bacterium. The colonies of the *P. mirabilis* and *P. vulgaris* isolates showed growth on the MacConkey agar medium with a pale color because it is a non-lactose fermenter. As for growth on XLD agar, the colonies were gray with black centers (H_2S production), but they turned yellow to light brown on hi-chrome UTI agar **(**Figures 1 and 2). A microscopic examination of bacterial isolates belonging to *Proteus* species was conducted. After staining with Gram stain, the results showed that they are characterized by the shape of short rods (Samra *et al*., 1998).

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A B C

Figure 1. *Proteus mirabilis* on (A) MacConkey agar (B) XLD agar (C) Hi chrome UTI agar

A B C

Figure 2. *Proteus vulgaris* on (A) MacConkey agar (B) XLD agar (C) Hi chrome UTI agar

Furthermore, Table (4) data revealed a significant difference in the percentage of chicken meat contaminated with *P. mirabilis* 13% compared to 4% with *P. vulgaris*. These findings suggest that contamination with *P. mirabilis* poses a risk 3.40 times more than contamination with *P. vulgaris* (Table 5). In addition, Table (Algammal *et al.,*2021) demonstrates that *P. vulgaris* was isolated in 6.6% of human samples (8 out of 120), but *P. mirabilis* was isolated in 25% of the same samples (30 out of 120). The statistics reveal that *P. mirabilis* provides a much higher risk of contamination to people than *P. vulgaris*. Specifically, the risk is 4.67 times greater. This shows that *P. mirabilis* is a more worrying pathogen in human infections than *P. vulgaris*, and efforts to control and prevent infections should focus on *P. mirabilis*.

Table 2. Isolation percentage of *P. mirabilis* from different sample sources

Source of isolation	No. of samples	*P. mirabilis*	Chi-square	P-value
Chicken meat	200	26 (13%)	7.48	0.006
Human	120	30 (25%)		
Total	320	56		

Table 3. Isolation percentage of *P. vulgaris* from different sample sources

Source of isolation	No. of samples	*P. vulgaris*	Yates' chi-square	P-value
Chicken meat	200	8 (4%)	1.12	0.28 NS
Human	120	8 (6.6%)		
Total	320	16		

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Table 4. Comparison between the percentage of contamination with *Proteus spp* in raw chicken meat

Total No. of samples	*P. mirabilis%*	*P. vulgaris%*	Chi-square value	P-value
200	13% (26)	4% (8)	10.99	0.0009

Table 5. The risk factor of contamination with *Proteus spp* in raw chicken meat

Proteus spp	No. of samples	Isolation%	Odds ratio (OR)	95% Confidence Interval (CI)	P-value
P. vulgaris	200	4% (8)	Reference		
P. mirabilis	200	13% (26)	403.	1.09-10.63	30.0

Table 6. The risk factor of contamination with *Proteus spp* in human samples

Proteus spp	No. of samples	Isolation%	Odds ratio (OR)	95% Confidence Interval (CI)	P-value
P. vulgaris	120	6.6% (8)	Reference		
P. mirabilis	120	25% (30)	4.67	2.04-10.67	0.0003

Table 7. The risk factor of contamination with *P.mirabilis* isolated from different sample sources

Proteus spp	No. of samples	Isolation%	Odds ratio (OR)	95% Confidence Interval (CI)	P-value
P. vulgaris	200	26 (13%)	Reference		
P. mirabilis	120	30 (25%)	2.23	1.23-4.00	0.007

The results of molecular detection for virulence factors in *Proteus* species isolated from detected samples revealed that all these isolates possessed the three virulence genes (mrpI, ureC, and zapA) (Figures 3,4 and 5). Table (8) shows the types of polymorphisms in the mrpI gene of *Proteus mirabilis* and *Proteus vulgaris*. A transversion type change in the mrpI gene of *Proteus mirabilis* was found, where Adenine (A) was replaced by Thymine (T) at position (1499865). In *Proteus vulgaris*, a transition type change had been additionally detected, where Thymine (T) was converted to Cytosine (C) at position (3945737). The identities of both types (*P. mirabilis* and *P. vulgaris*) were 99% identical to the database's reference sequences (Sequence ID). Although chicken meat may be a source of contamination, the risk from humans is much higher, meaning that humans may be a major source of bacterial spread that increases public health concerns. Bacterial functions such as reproduction, biofilm formation, and virulence factor secretion are linked to quorum sensing, which involves gene expression based on cell density (Rouger *et al*.,2017). Many genes involved in flagellar motility and quorum sensing have been found to play regulatory roles in this process in *Proteus* (Whitehead *et al.*, 2001). Genetic alterations (polymorphisms) were discovered in all the genes that were analyzed but in varying degrees between the two types of bacteria especially in the mrpI gene of both *Proteus mirabilis* and *Proteus vulgaris*, which may affect virulence factors or antibiotic resistance. Certain genetic modifications could boost the bacteria's capacity to infect the host and cause disease (Salama *et al*., 2025). Mutations in the mrpI gene, for example, may boost *Proteus mirabilis'* ability to produce biofilms or withstand the immune system. Genetic differences may influence how bacteria interact with host cells, enhancing their ability to cling to cells or penetrate tissues. These findings also contribute to improved awareness of the genetic alterations in *Proteus* bacteria, which may be associated with enhanced virulence or adaptability to diverse settings, emphasizing the importance of following hygiene and sterilization procedures in human environments (such as hospitals or homes) to reduce infection spread. The results of the isolation of *P. mirabilis* and *P. vulgaris* from humans and chicken meat showed variation in the serotypes of *Proteus spp*. The variation in the percentages of the high prevalence of *Proteus spp*. in chicken products compared to that in humans may be due to the location of sampling, the timing of sample collection, geographic climate, age, immunity of humans or chickens, drug

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consumption, and hygienic restrictions, which may be linked to the relative differences in results between different places (Dalia, 2015). The significant incidence of *Proteus spp*. could be attributed to poor hygiene practices during slaughter, scalding, de-feathering, evisceration, and carcass cutting. These processes enable the cross-contamination of healthy, clean birds with diseased or infected corpses, and eventually with humans. Inadequate veterinary control may also result in the slaughter of sick birds and the spread of illnesses (Sanches *et al*.,2020). The results of the current study agreed with Amany (2022), who said that the percentage for *Proteus mirabilis* isolation was 20.0% and *for Proteus vulgaris* was 11.0%. Also, concur with Rawnaq and Mouruj, (2016), who discovered that the proportion of isolated *P.vulgaris* was 5.0%. Additionally, Mohanad *et al*., (2015), mentioned that the percentage for *P. mirabilis* isolation was 20.0 %. Feglo *et al*., (2010), stated that the proportion for *Proteus mirabilis* isolation was (61.5%) and for *Proteus vulgaris* was (30.5%). Additionally, disagree with Ghaida'a *et al*., (2014), who said that the percentage for *Proteus mirabilis* isolation was (7.3%) and for *Proteus vulgaris* was (18.7%). *Proteus* species are more common because they are a natural flora of mammals and can contaminate water or food with faces. Contamination with *P.mirabilis* in humans poses a higher risk factor of 2.23 than in chicken meat. The elevated percentage of locally isolated may be because of the wide ability of Genus *Proteus* to invade tissues and surfaces of instruments due to their virulence factors. The potential presence of virulence genes in poultry, a source of human staple food, highlighted the necessity for vigilance in meat processing and adequate procedures to avoid meat contamination before human consumption (Alchalaby *et al*., 2025) the incorrect usage of antibiotic drugs, which generates an increased *Proteus* infection, and the contaminated urinary catheters or other indwelling devices used in an unclean environment of some hospitals (Dalia, 2015). Enteric infections are responsible for diseases that harm broilers and cause significant economic losses in the poultry industry (Mehmood *et al*.,2020). *P. mirabilis* is commonly found in the intestinal tracts of animals and humans. Animals are a key source of this pathogen's transmission to humans because contamination of chicken carcasses with intestinal flora is widespread when the carcasses are placed in a chiller for washing and cooling in the slaughterhouse (Sanches *et al*., 2021). Contamination of fresh chicken meat with different food pathogens may occur due to many improper hygiene and personal faults that occur during different slaughtering, storage, transportation, and handling processes, such as contaminated water, gastrointestinal contamination, and air, dust, sewage, and food or on food equipment, environmental surfaces (Suleman *et al*.,2022).

Table 8. Polymorphism in the mrpI gene of *Proteus* species

Gene: mrpI						
No. Of sample	Type of substitution	Location	Nucleotide	Source	Sequence ID with compare	Identities
1	Transversion	1499865	A/T	*Proteus mirabilis*	ID: CP053615.1	99%
2	Transition	3945737	T/C	*Proteus vulgaris*	ID: CP023965.1	99%

These modifications (transversion and transition) may have an impact on the mrpI gene's function, which may be related to bacterial virulence factors. Changes in the gene can modify the resultant protein, potentially impacting the bacteria's capacity to cause illness or resist antibiotics. Figure (3) shows a 265 base pairs (bp) band obtained by amplifying the mrpI gene using the PCR technique. This showed that the mrpI gene is present in the examined samples and effectively amplified. Genetic alterations (polymorphisms) were discovered in the mrpI gene of *Proteus mirabilis* and *Proteus vulgaris*. The ureC sequence of *Proteus mirabilis* was compared to the database, and it showed 100% identity (ID: CP116223.1), indicating that there were no mutations in this

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sample. In *Proteus vulgaris*, a base substitution (A↔T) caused a mutation at position 569289. This is characterized as a transversion, which indicates a structural change in this strain's gene sequence **(**Table 9). Gel electrophoresis analysis revealed a DNA band of 317 (bp), confirming that the ureC gene was successfully amplified using PCR. Lane M symbolizes the DNA ladder, which determines the size of DNA pieces. Lanes 1–6 indicate effective amplification of the ureC gene in several samples, demonstrating the existence of this gene in all examined samples (Figure 4)**.**

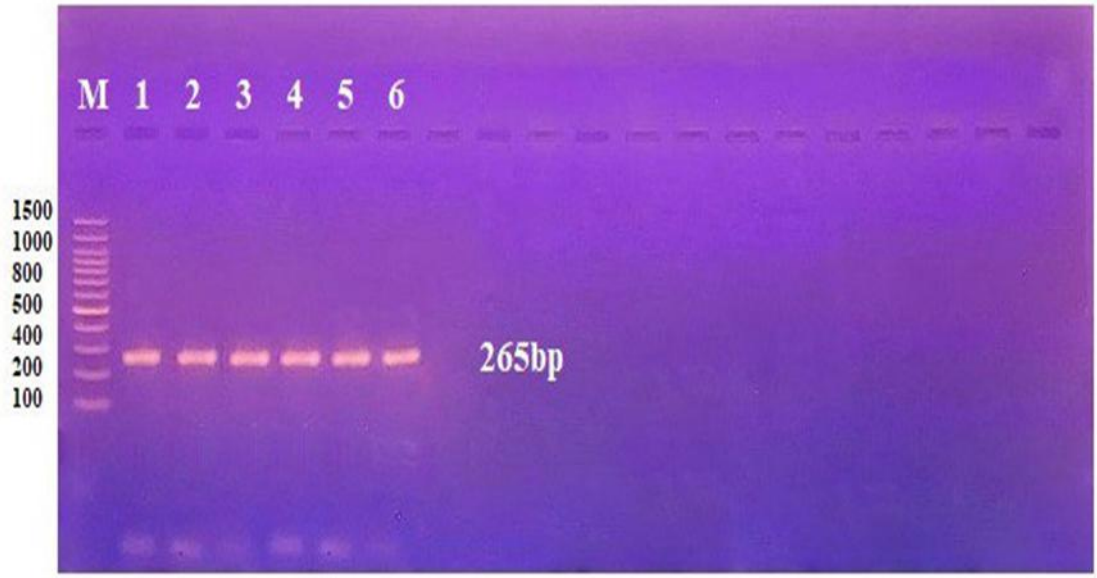

Figure 3. DNA product analysis of a 265 bp of the mrpI gene from *Proteus spp* using 0.8% agarose gel electrophoresis at 80V for 45 minutes at room temperature (25°C).

The *Proteus mirabilis* zapA gene showed no polymorphism (substitution). It shared 100% similarity with the reference sequence, indicating no genetic variation in the zapA gene. A transversion type substitution occurred in the zapA gene of *Proteus vulgaris*, where Adenine (A) was replaced by Cytosine (C) at position (2981349), also, a transition type in Adenine (A) was converted to Guanine at position (2981376). The sequence had a 99% match to the reference sequence (ID: CP104121.1), indicating some variance. *Proteus vulgaris* revealed 99% identity, showing a modest genetic difference caused by the transversion at the previously identified location (Table 10).

Table 9. Polymorphism in ureC gene of *Proteus* species

No. Of sample	Type of substitution	Location	Nucleotide	Source	Sequence ID with compare	Identities
1	-------	------	----	*Proteus mirabilis*	ID: CP116223.1	100%
2	Transversion	569289	A/T	*Proteus vulgaris*	ID: CP047344.1	99%

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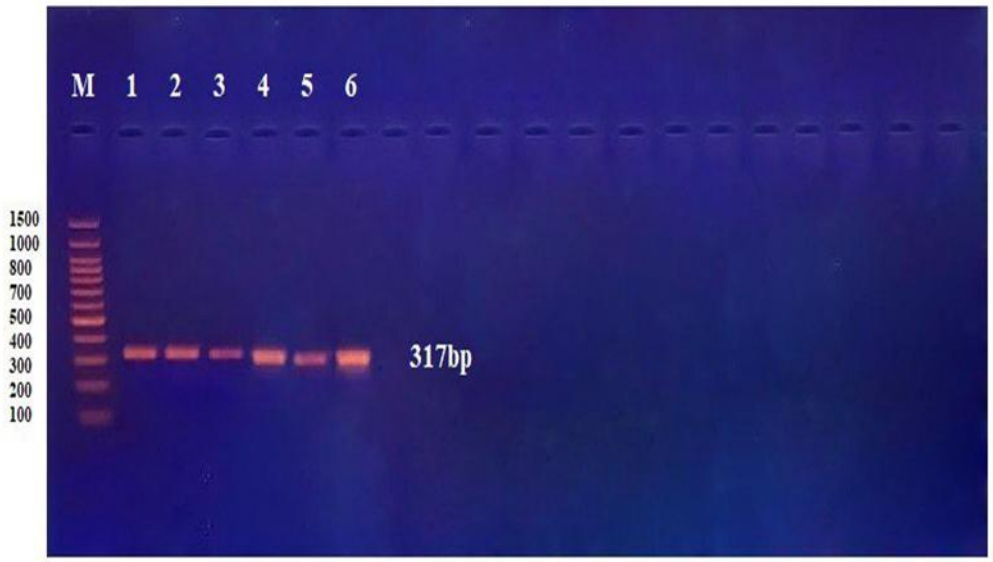

Figure 4. DNA product of a 317 bp of the ureC gene from *Proteus spp* using 0.8% agarose gel electrophoresis at 80V for 45 minutes at room temperature (25°C).analysis

Table 10. Polymorphism in zapA gene of *Proteus* species

Gene : zapA						
No. Of sample	Type of substitution	Location	Nucleotide	Source	Sequence ID with compare	Identities
1	-------	------	----	*Proteus mirabilis*	ID: CP053616.1	100%
2	Transvertion	2981349	A\C		ID: CP104121.1	
	Transition	2981376	A\G	*Proteus vulgaris*		

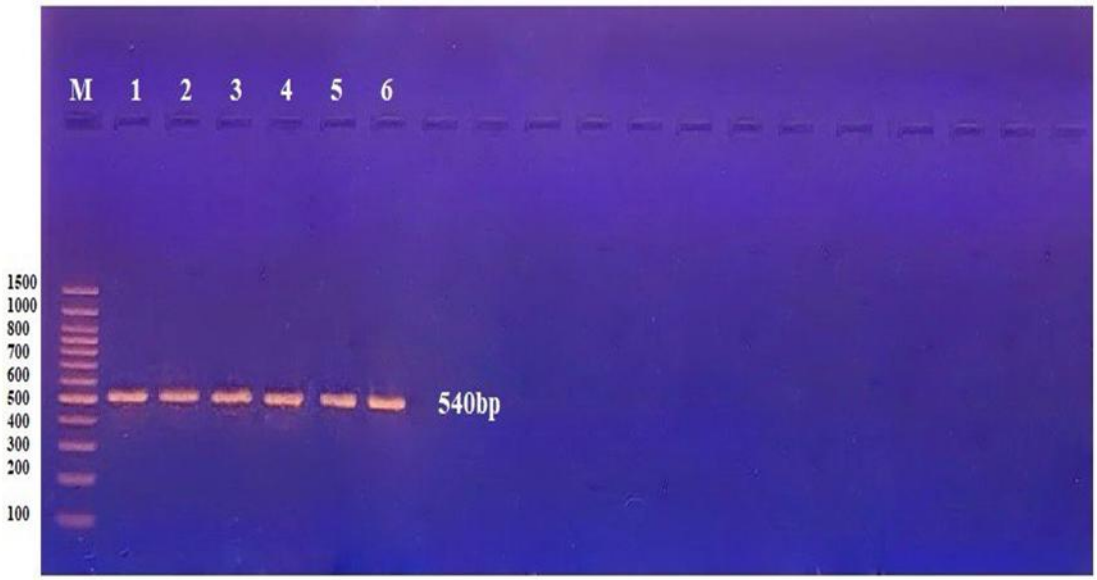

Figure 5. DNA product analysis of a 540 bp of the zapA gene from *Proteus spp* using 0.8% agarose gel electrophoresis at 80V for 45 minutes at room temperature (25°C)

Transversion (A/C) in zapA gene of *Proteus vulgaris* suggests genetic diversity. This modification can influence the gene's function, influencing virulence, antibiotic resistance, and other bacterial properties. The zapA gene was successfully amplified, confirming its presence in the investigated *Proteus* species (Figure 5). The nucleotide sequencing for virulence factors showed one nucleotide substitution in the ureC virulence factor for *P.*

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vulgaris, which was Adenine A\T to Thymine (Table 9); also, one nucleotide substitution in the mrpI virulence factor for *P. mirabilis* was Adenine A\T to Thymine and one nucleotide substitution in the mrpI virulence factor for *P. vulgaris* was Thymine T\C to Cytosine (Table 8). In comparison, in the zapA virulence factor, two nucleotide substitutions for *P. vulgaris* were Adenine A\C to Cytosine and Adenine A\G to guanine (Table 10). Figure (6) showed a comprehensive phylogenetic tree of mrpI gene sequences from the tested samples compared with sequences from the NCBI database. The sequences of *Proteus mirabilis* isolates showed high similarity with reference sequences as 100% with sequences from China (CP053615.1), USA (CP021852.1), Italy (CP045538.2) and Singapore (CP044134.1). The sequence of *Proteus vulgaris* from Iraq (PP477473.1) showed 99.52% similarity with a reference sequence from the USA (CP023965.1). A phylogenetic tree of ureC gene sequences from *Proteus mirabilis*/ Iraq (PP477474.1) showed 100% similarity with a reference sequence from China (CP116223.1). Also, the sequences showed 99.63% similarity with a reference sequence from the USA (CP020052.1), while the sequence of *P. vulgaris* from Iraq (PP477475.1) showed 99.62% similarity with a reference sequence from China (CP047344.1), 98.08% from Italy (CP063314.1) and 89.27% from the USA (CP023965.1) (Figure 7). The ureC gene of the *Proteus spp* is one of the basic structural genes responsible for producing the urease enzyme, and it has been well-maintained and duplicated. Urease production is a defining feature of the *Proteus* genus and plays a significant role in its pathogenicity, which is an important virulence factor (Mohammed *et al*., 2014). *Proteus mirabilis* produces urea-inducible urease, which breaks down urea into ammonia and carbon dioxide. Raising the local pH causes the precipitation of normally soluble calcium and magnesium ions, which can grow to enormous sizes to form bladder and kidney stones, a characteristic of *Proteus spp*. (AL-Oqaili et al., 2017). According to (Dumanski *et al*., 1994) the buildup of hazardous levels of ammonia induced by the urease-mediated breakdown of urea during *Proteus* UTIs is harmful to tissues, including renal epithelia. The phylogenetic tree of the zgpA gene showed that sequences of Proteus mirabilis from Iraq (PP477476.1) had a strong genetic similarity of 100% with two strains from China (CP053616.1) and (CP053894.1). In addition, the similarity is 100% identity with isolates from different countries (Brazil, Singapore, Poland, and France). The sequence of P. vulgaris Iraqi isolate (PP477477.1) showed 100% similarity with a reference sequence from Germany (CP083628.1).

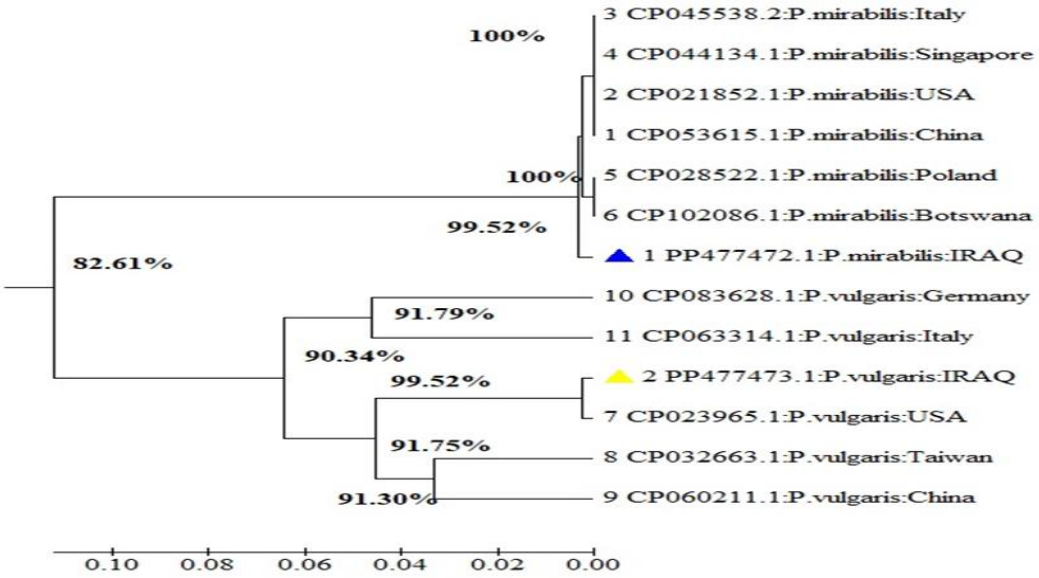

Figure 6. The comprehensive phylogenetic tree of the mrpI sequences from the NCBI database and sequences from the tested samples

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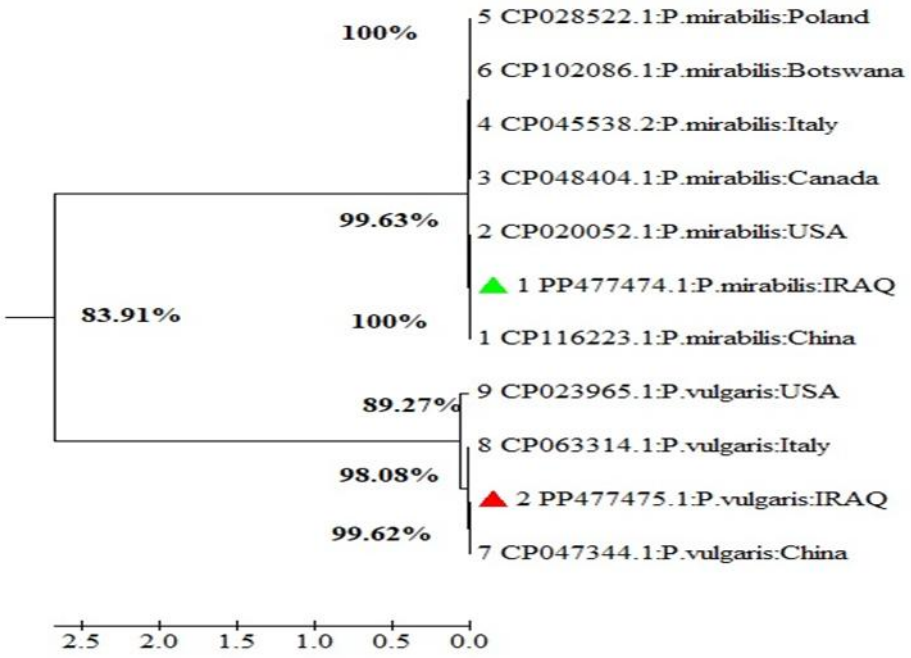

Figure 7. The comprehensive phylogenetic tree of the ureC sequences from the NCBI database and sequences from the tested samples

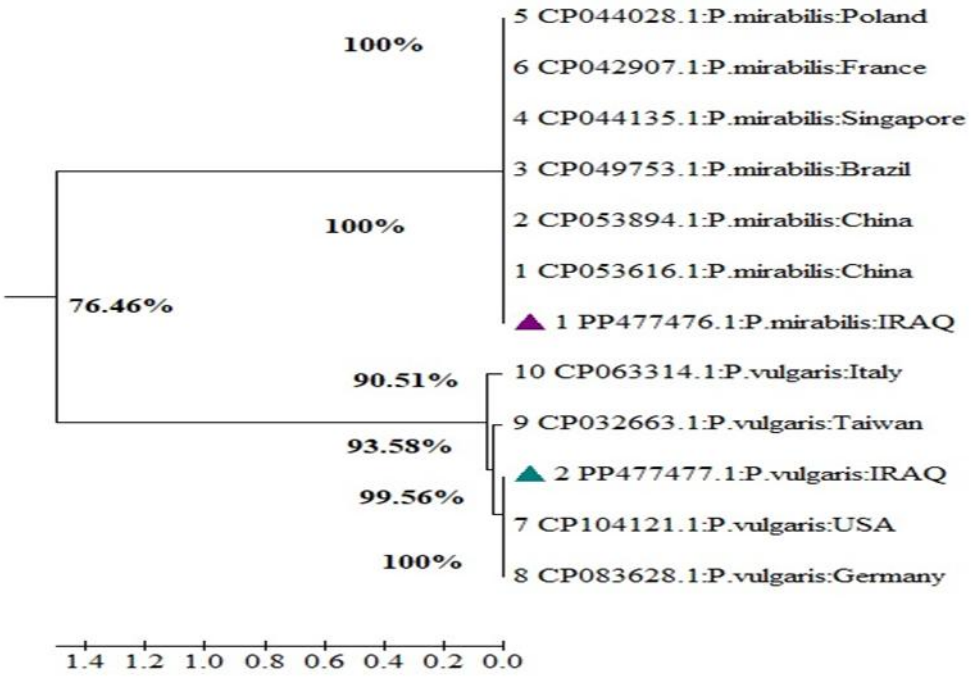

Figure 8. The comprehensive phylogenetic tree of the zapA sequences from the NCBI database and sequences from the tested samples

The results demonstrate that *Proteus* bacteria, particularly Proteus vulgaris, exhibit high genetic conservation, indicating the gene's critical role in bacterial function. However, some genetic variations, especially in *Proteus mirabilis*, suggest evolutionary adaptations or mutations that could impact virulence **(**Figure 8). Pathirana *et al*., (2018) found the gene present in 45.8% of all isolates. Mannose-resistant *Proteus*-like Fimbriae (MR/P) play a crucial role in *Proteus mirabilis* infection, including biofilm formation, auto-aggregation, and bladder and kidney colonization (Jiang *et al*., 2020; Sun et al.,2020). In addition, all human and poultry meat isolates carried the zapA gene. Based on the results of this study,

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this gene may link to the virulence of bacteria and, therefore can play a key function in evading the immune system, showing an effective linkage with diseases and their causes. Also, the current results were consistent with the research conducted by (Algammal *et al.*, 2021) which showed that investigated isolates contained the zapA gen 100% in all *Proteus* isolates. In vitro, the metalloprotease zapA can catalyze the degradation of numerous host proteins. According to Belas *et al*., (2004), protease enzymes can cleave IgA, IgG, secretory components, and antimicrobial peptides, reducing their antibacterial efficacy. ZapA could potentially aid *Proteus mirabilis* in avoiding the innate immune response when infected. When this gene is present, the organism becomes more pathogenic and resistant to antibiotic mediated healing. In addition, this study showed *Proteus spp* has a high degree of genetic similarity with reference sequences revealed in the phylogenetic tree of virulence gene sequences. The similarity in reference sequences may also suggest that the bacteria follow similar genetic patterns in different geographical regions, possibly reflecting their transmission across borders or the presence of shared strains (Li *et al.*, 2022). However, some genetic changes may indicate the emergence of new strains or mutations. These findings emphasize the necessity of monitoring genetic changes in *Proteus spp*. to understand their dissemination and evolution, leading to better prevention and treatment techniques (Oliveira *et al.*, 2021).

CONCLUSION

Finally, this study concluded that genetic variations in *Proteus* bacteria resulted in differences in molecular features, serological classification, and virulence markers. It also revealed genetic differences in virulence factors in *Proteus* bacteria, which could be crucial in determining their severity. In addition to their ability to resist medications and adapt to different environments, they can create varying infection levels in the host.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS'DECLARATION

The authors declare that this manuscript is original, has not been published previously, and is not currently under consideration by any other journal. All figures and tables are original and prepared by the authors. Any material obtained from third parties has been included with the required permissions. All authors have read and approved the final manuscript.

AUTHORS' CONTRIBUTION STATEMENT

All authors made equal contributions to the study design, methodology, experimental work, data analysis, and manuscript writing. All authors reviewed and approved the final version of the manuscript.

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التحليل الجزيئي لجينات الضراوة في بكتريا *Proteus* المعزولة من لحوم الدجاج والعينات البشرية في محافظة واسط، العراق

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المستخلص

هدف هذه الدراسة التحري عن الجينات mrpI وureC و zapA المرتبطة بضراوة بكتريا *Proteus spp* التي تم عزلها من لحوم الدجاج النيئة والمصادر البشرية وطرق انتشارها عبر السلسلة الغذائية. خلال الفترة من اذار ولغاية أيلول/ 2023، تم جمع مائتي عينة من لحوم الدجاج النيئة من الأسواق المحلية و120 عينة بول حالات مرضية بشرية في مستشفيات محافظة واسط بالعراق. أظهرت الدراسة أن نسبة عزل *P. mirabilis* و*P. vulgaris* كانت بمعدل 13 و4٪ على التوالي من 200 عينة من لحوم الدجاج النيئة، بينما بلغت نسب عزلهما في العينات البشرية 25 و6.6٪ على التوالي من 120 عينة بول. كشفت البيانات أن خطر التلوث بـ *P. mirabilis* يزيد بمقدار 3.40 مرة عند مقارنته بـخطورة *P. vulgaris* وكشف التحليل الجزيئي عن وجود جينات ureC وzapA في جميع العزلات بنسب 100 و99% على التوالي، في حين كان جين mrpI موجودًا في جميع العزلات المشتقة من لحوم الدجاج والعينات البشرية بنسبة 99%. نستنتج أن هذه البكتيريا قادرة على إحداث العدوى والتسبب بالأمراض بسبب امتلاكها جينات الضراوة التي تتحكم في آليات مثل تكوين الأغشية الحيوية، إفراز السموم، وغزو الأنسجة. كما ان اكتشاف هذه البكتيريا في الأغذية البشرية يسلط الضوء على أهمية الالتزام بأعلى معايير الصحة والسلامة أثناء تحضير اللحوم ومعالجتها لضمان سلامة الاستهلاك البشري.

الكلمات المفتاحية: لحم الدجاج؛ الأمراض المنقولة بالغذاء؛ أنواع البروتيوس؛ عوامل الضراوة.