

Molecular detection of some virulence gene in *Proteus mirabilis* isolated from chicken and human

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Abstract

The goal of this significant study is to isolate *Proteus* spp from both human and broiler sources and to compare the presence of the most significant genes responsible for virulence factors like *zapA*, *rspA*, and *ureC*. This research, conducted from September 2023 to February 2024, collected twenty-five samples from the internal organs of broilers (heart, lung, liver, air sac, meat) and twenty-five samples from urinary tract infections in humans. The standard bacteriological method was used for bacterial identification, and PCR confirmed the *Proteus* spp isolates. Further sequencing and phylogenetic analysis of *zapA* were conducted. The study revealed 23 isolates of *P. mirabilis* from a total of 25 broiler samples, at a rate of 92%, while the isolation rate in humans was 88%, with 22 isolates from 25 urine samples. Molecular examination showed that all *zapA*, *rsbA* and *ureC* genes were found in both human and poultry isolates. The phylogenetic analysis shows that all *Proteus* isolates aggregate in one clade. In conclusion, our findings underscore the potential danger of virulence genes in poultry, a human staple food. This highlights the crucial need for caution in meat processing and the use of effective methods to prevent infection and sterilize meat before human consumption.

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Introduction

The most widespread bacteria from the Enterobacteriaceae family that are capable of causing diseases in humans and birds is the *Proteus* spp (1). Two types of *Proteus* bacteria can cause disease, and *Proteus mirabilis* is considered the most virulent type (2). *Proteus mirabilis* can produce urease and is characterized by a movement that resembles a bull's eye on the center of the agar plate (3). These bacteria are responsible for causing multiple infections in humans, like wounds, urinary system infections, and respiratory infections, as well as causing many cases of gastroenteritis (4). Recently, due to the resistance of bacteria isolated from poultry to most antibiotics, great concern and fears have begun to be created that these animals will become a reservoir for transmitting resistance to humans (5). *P. mirabilis* is found in the

environment, and recently, an increasing number of infections have been recorded in poultry fields. Hence, studying their relationship to human infections and their transmission in different ways began after the increased consumption of white meat in most developing countries (6). *P. mirabilis* has many virulence factors that contribute to causing infection, like uroepithelial cell adhesin, fimbriae enabling bacteria to attach to host cells (7), and *zapA*, breaking down proteins. It contributes to evading the immune system (8). also, HpmA and HlyA hemolysins cause pores on the host cell (9). While the bacterium obtains iron from the host cells through the IreA siderophore receptor (10). *P. mirabilis* also possesses the *rsb* gene, which is responsible for producing a protein that works to form biofilms in addition to polysaccharides and serves as a device to control the movement of bacteria, as it acts as a sensor in environmental conditions (11). The enzyme urease breaks

down urea and produces ammonia, which has an essential role in diseases and injuries of the urinary tract and is secreted by the *ure C* gene (12).

Due to the importance of the *ure C*, *rsb*, and *zap A* genes in the spread of bacteria and the contamination of poultry meat with them, and the danger they cause to the life of the consumer because white meat has become more widely consumed, our study aimed to inspect the presence of some virulence genes between poultry meat and the human consumer.

Materials and methods

Data collection permit

The certificate with the number UM.VET 2023. 063 on 15/8/2023, which was given by the Commission of Scientific Morals used for collected data and provided the moral cover to carry out the research in the College of Veterinary Medicine.

Samples

Twenty-five samples were collected from the internal organs of chickens (five samples from each heart, lung, liver, air sac, and meat) and from cases of urinary tract infection in human females. All samples were placed in sterile glass bottles and transmitted to the microbiology laboratory. All the processing and tests related to conventional and molecular isolation techniques were done in department of the Microbiology, College of Veterinary Medicine, University of Mosul (13,14).

Bacterial isolation and culture media

The samples were cultured on MacConkey and brain heart infusion agar. After purifying the *Proteus* colonies, a

diagnostic examination was performed using traditional methods to determine the species of bacteria (15). All suspected colonies were further diagnosed using conventional biochemicals, followed by the Vitek 2 test for more confirmation. All positive isolates were confirmed by using PCR techniques (16).

DNA extraction

All confirmed *Proteus* spp. Bacterial isolates were subjected to molecular conformation to confirm species and detect antibiotic virulence genes. According to company instructions, the DNA extraction was done using AddPrep Genomic DNA Extraction (Korea). The DNA concentration was determined using nanodrop (NanoPhotometer® N50/ Germany), and all extracted DNA was stored at -80 °C till used (17,18). The 25µl PCR mixture (1 µl from each forward and reverse primer, 10 µl of GoTaq® G2 Green Master Mix (Promega, USA), 8 µl of Danase-free water, and 5 µl of extracted DNA) was used to amplification 16S rRNA gene specific for detection of *P. mirabilis*, farther *reb A*, *zap A* and *ure C* virulence genes were also detected for both human and animals isolate. All primer sequences and amplification cycles are listed in tables 1 and 2. Amplification was done using conventional PCR (Sensoquest, Germany).

Bioinformatic and phylogenetic tree construction

Four *Proteus mirabilis* isolates positive for the *zap A* gene (two from animal origin, two from human origin) were subjected to Sanger Sequencing (Microgen/ Korea); all four samplings underwent nblast analysis by using NCBI nblast, and phylogenetic tree construction was done using Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 1000 re-samplings.

Table 1: Primers used in this study

Primer	Sequence (-)	Product	PCR product size	References
16S rRNA	F R	AGAGTTTGATCCTGGCTCAG TACGGTTACCTTGTACGACTT	1500 bp	19
<i>rsb A</i>	F R	TTGAAGGACGCGATCAGACC ACTCTGCTGTCCTGTGGGTA	467 bp	20
<i>zap A</i>	F R	ACCGCAGGAAAACATATAGCCC GCGACTATCTTCCGCATAATCA	540 bp	21
<i>ure C</i>	F R	GTTATTTCGTGATGGTATGGG GTAAAGGTGGTTACGCCAGA	317 bp	21

Table 2: The amplification programs used for PCR and multiplex PCR

Type of PCR	Initial denaturation (°C/min)	Cycle numbers 35 (°C/min)			Final extension (°C/min)
		Denaturation	Annealing	Extension	
16SrRNA	94/5	94/0.3	60 /0.3	72/1	72/7
<i>rsb A</i>	95/5	94/1	58/0.45	72/1	72/7
<i>zap A</i>	95/2	94/0.30	59/0.30	72/1	72/5
<i>ure C</i>	95/2	94/0.30	56.2/0.30	72/0.30	72/5

Results

Samples and conventional isolation of *Proteus* spp.

Twenty-five samples were collected from the internal organs of chickens (heart, lung, liver, air sac, meat), and Twenty-five samples were collected from cases of urinary tract infection in humans. The period extends from September 2023 to February 2024. The outcome of this study showed that conventional isolation revealed that the percentage of isolation of *P. mirabilis* in cases of urinary tract infection in humans was 88% (22 from 25), While the percentage of its isolation in poultry was 92% (23 from 25), the heart, liver and meat show 100 % isolation rate while lung and air sac show 80% isolation rate. All the confirmation by conventional biochemical tests (triple sugar iron, indol, methyl red, Voges–Proskauer, citrate, nitrate reduction test, urea, and production of swarming phenomena) gives positive results to all 45 *P. mirabilis* isolate tested as appears in figure 1, Vitek 2 compact system gave a positive result for the 45 *P. mirabilis* isolate from both broiler or human females with purity percentage reach to 99.9%.



Figure 1: Shows the biochemical tests for *Proteus mirabilis* and the swarming phenomenon in brain heart infusion agar: test from left to right triple sugar iron, indol, methyl red, Voges-Proskauer, citrate, nitrate reduction test, urea, and production of swarming phenomena

Molecular diagnosis of *Proteus mirabilis* isolates

The molecular identification of 16S rRNA specific for *P. mirabilis* species that gives amplicon 1500 bp shows that all culture-positive *Proteus* isolates from humans and animals belong to *P. mirabilis* (Figure 2). Both humans and animals *P. mirabilis* give positive results to the *rsb A*, *zap A*, and *ure C* virulence genes, respectively, which means they carry more than one resistance gene (Figures 3-5).

Bioinformatic and phylogenetic tree contraction

All *zap A* sequence samples from humans and animals show 100% compatibility with *P. mirabilis* isolated in NCBI isolated from China while showing 99% identity with Iraqi *P. mirabilis* isolated. our samples were recorded in NCBI with Accession Number (PQ181567, PQ181568, PQ181569, PQ181570) for animals and human respectively (Table 3).

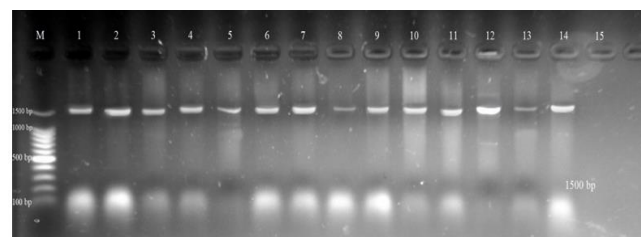


Figure 2: the specific 16S rRNA gene amplification for *P. mirabilis*, M= 100 kb marker, 1-8 = animal isolates, 9-14= human isolate, 15= negative control.

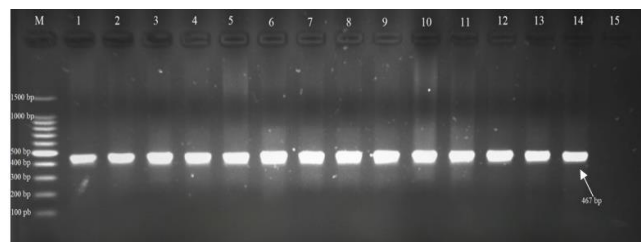


Figure 3: the *rsb A* gene amplification for *P. mirabilis*, M= 100 kb marker, 1-8 = animal isolates, 9-14= human isolate, 15= negative control.

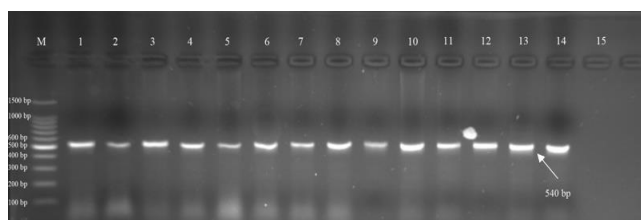


Figure 4: the *zap A* gene amplification for *P. mirabilis*, M= 100 kb marker, 1-8 = animal isolates, 9-14= human isolate, 15= negative control.

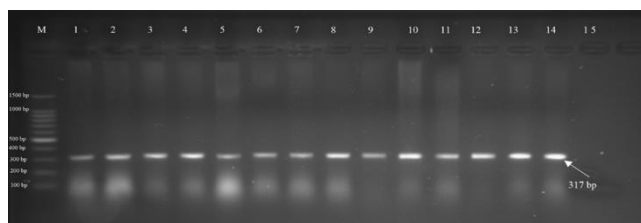
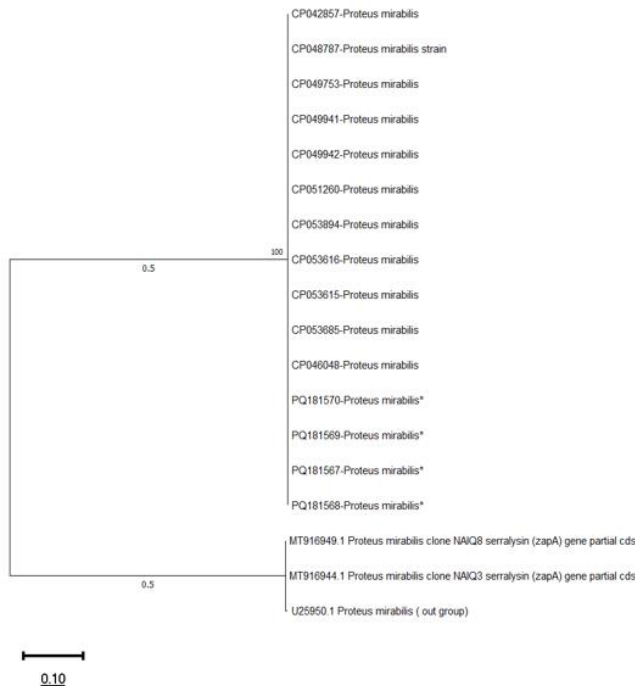


Figure 5: the *ure C* gene amplification for *P. mirabilis*, M= 100 kb marker, 1-8 = animal isolates, 9-14= human isolate, 15= negative control.

The phylogenetic tree was constructed and shows that all *zap A* *P. mirabilis* genes were collected in one clade with other *P. mirabilis* from other sites in the world (China and Singapore) with distance differ from other clade that contain Iraq isolated as shown in figure 6.

Table 3: Percentage distribution of *Proteus mirabilis* on partial ZapA according to nblast of NCBI

Accession Number	Query Cover %	Identic Number %	GenBank accession number	Country
PQ181567 PQ181568 PQ181569 PQ181570	100	100	CP046048	China
	100	100	CP053685	China
	100	100	CP053615	China
	100	100	CP053616	China
	100	100	CP053894	China
	100	100	CP051260	China
	100	100	CP049942	China
	100	100	CP049941	China
	100	100	CP049753	Brazil
	100	100	CP042857	China
	100	100	CP048787	China
	100	100	CP044135	Singapore
	99	100	MT916949.1	Iraq
	99	100	MT916944.1	Iraq
	100	99.8	U25950	USA

Figure 6: Phylogenetic tree of *Proteus mirabilis* from Iraq. Partial DNA sequences of the ZapA gene were used as input data.

Discussion

P. mirabilis have been isolated in a high percentage from broiler chicken meat, as they are considered part of the broiler microbiota (22). The presence of bacteria in high

proportions cannot be neglected, especially since they contain dangerous virulence factors that are pathogenic to humans (23). *Proteus spp.* is responsible for most cases of UTI, especially in females, which isolated approximately 92% (24), agreeing with the current study (88%) in high isolated rate from the humans. By examining the polymerase chain reaction, we found that the same genes, *zap A*, *rsb A*, and *Ure C* genes, were present in human and poultry isolates (25). These results are similar to those of our study. Recently, pathogenic biofilm-forming bacteria found in meat have become a major problem to consumer health (26,27). The accumulated membranes that form layers of substances (biofilm) secreted by bacteria *rsb A* gene make it constantly vulnerable to meat contamination with the strain that produces (28-30).

The presence of membrane aggregates led to a high prevalence of *Proteus spp.* in meat, resulting in a high infection rate of these bacteria being recorded according to previous studies on chicken meat and a high probability of their transmission to the consumer and those dealing in poultry farming fields (31-33). The high percentage of bacteria forming isolated membrane aggregate biofilms in meat prompts researchers to study and develop a strategy to eliminate these aggregates due to their danger to the meat industry (34-36). The problem is exacerbated when isolates cause infections between humans and animals (zoonotic) because these aggregates (biofilm) help evade the immune system and also produce resistance to antibiotics, which allows continued infection (37,38). All human and poultry isolates showed the presence of the *ZapA* gene, which works to produce IgA-degrading enzymes. Studies have shown that this gene is associated with bacteria that cause diseases and plays a significant role in evading the immune system, which indicates its effective relationship in diseases and pathogenesis (39,40).

Studies have indicated that the effect of the enzyme secreted by this gene is not limited to IgA but rather affects the action of IgG (41,42). A high percentage of this gene indicates that it is prevalent in strains found in chicken meat and in infections of the human urinary system (43). The *ure C* gene is responsible for the production of urease, which decomposes the urea and produces ammonia, leading to high urine acidity and the formation of stones; this explains its presence in human urinary system infections in our study (44). The occurrence of the *ure C* gene in isolates taken from poultry meat confirms its relationship to pathogenicity. It raises controversy about the possibility of these strains being transmitted to humans during consumption (45). The sequences of our isolate confirm that both animals and humans were similar and have identical sequences. Further phylogenetic analysis reveals confirmed sequencing results and shows that our isolate belongs to one clade, which gives strong evidence that our isolate can transfer from animals to humans.

Conclusion

This study concluded a strong correlation between the transmission of virulence factors between animals and human isolates, so caution was required when processing the meat and using methods to prevent infection and sterilize meat before human consumption.

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Conflict of interest

There is no conflict of interest.

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التشخيص الجيني لبعض جينات جراثيم المتقلبات الرائحة المعزولة من الدواجن والإنسان

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الخلاصة

تناولت دراستنا عزل بكتيريا المتقلبات في الإنسان ودجاج التسمين مع مقارنة وجود أبرز الجينات المسؤولة عن عوامل الفوعة مثل *zapA*, *ureC*, *rspA* بين الإنسان والحيوان. للفترة من سبتمبر ٢٠٢٣ إلى فبراير ٢٠٢٤ تم جمع خمس وعشرون عينة من الأعضاء الداخلية لفروج اللحم وهي (القلب والرئة والكبد والحوبيصلات الهوائية واللحم) وخمسة وعشرون عينة من حالات التهاب المسالك البولية عند الإنسان. تم تحديد المستعمرات وفقاً للطرق البكتريولوجية القياسية. استخدام تفاعل البلمرة المتسلسل لعزلات المتقلبات للتأكيد. أظهرت الدراسة عزل ٢٣ عزلة من بكتيريا المتقلبات الرائحة من أصل ٢٥ عينة من فروج اللحم بنسبة ٩٢%، بينما بلغت نسبة العزل في الإنسان ٨٨%، بواقع ٢٢ عزلة من إجمالي ٢٥ عينة إدرار. أظهرت نتائج الفحص الجزيئي وجود جينات عامل الضراوة *ZapA* و *rsbA* و *ureC* في جميع عزلات الإنسان والدواجن. وفي الختام يتضح ما ظهر من خطورة وجود جينات الفوعة في الدواجن التي تعتبر غذاء متكاملًا للإنسان. وهذا مؤشر على وجود علاقة قوية بين انتقال العوامل وظهورها في العزلات البشرية. وهذا يتطلب الحذر واستخدام طرق الوقاية من العدوى وكيفية تعقيم اللحوم قبل استهلاكها البشري.