Journal of Pioneering Medical Sciences
Received: November 18, 2024 | Accepted: January 30, 2025 | Published: March 05, 2025
Volume 14, Issue 02, Pages 54-58

DOI https://doi.org/10.47310/jpms2025140209

Comparison Between Phenotypic and Genotypic to Some Virulence Factors of Proteus Mirabilis Isolation from Urinary Tract Infection

Muhammad Majbil Dawood[1*] and Zainab Hussein Mahdi[2]
[1,2]Department of Biology, College of Education Pure Science, University of Diyala, Iraq

Author Designation: [1]Master of Science, [2]Assistant Professor

*Corresponding author: Zainab Hussein Mahdi (e-mail: zainabhusseinmahdyi@gmail.com).

Abstract **Objectives:** *Proteus mirabilis* is a gram-negative facultative anaerobe with swarming motility and an ability to self-elongate and secrete a polysaccharide which allows it to attach to and move along surfaces like catheters, intravenous lines and other medical equipment. causes many diseases, whether systemic or local, as it is considered an opportunistic pathogen. It possesses many virulence factors. Phenotypic detection of virulence factors is associated with *Proteus mirabilis* possessing genes encoding for those factors, which increase pathogenicity **Methods:** The current study aimed to isolate *P. mirabilis* and detection some virulence factors, urease, protease and biofilm formation to Compare them phenotypically and genetically. **Results:** Samples of urine were collected from (150) patients complaining about urinary tract infections at Al-Karamah Teaching Hospital in Baghdad Governorate from 15 August 2022 to 15 December 2022. (90) samples from females and (60) samples from females. By used VITEK 2 to confirm the true diagnosis of isolates after culture, microscopic and biochemical methods, multiplex1PCR technique used to detect the presence of genes. Thirty isolates (27.27%) of *P. mirabilis* were obtained, The infection rate in females 23(67.2%) in comparison with males 7(23.3%). **Conclusions:** Phenotypic detection results showed the ability of all isolates (100%) to produce urease, protease, investigation of biofilm formation displays all isolates to form, 14 (46.66%) were strongly membrane-producing, while 16(53.35%) were moderately, While genetic results to detection genes which encoded for this factor consist of (Ure C, Zap A, Pmf A) genes showed the presence of these genes by (100%, 100%, 26.7%), respectively.

Key Words *Proteus mirabilis*, UreC, ZapA, PmfA, urinary tract Infection, biofilm formation

INTRODUCTION

The family Enterobacteriaceae includes Gram-negative bacteria, which are among the most important Species that affect public health and are responsible for many diseases acquired through nosocomial infection [1]. *Proteus mirabilis* is a member of this family, as it is characterized by being short bacilli that are Gram-negative, negative, motile, facultative, facultative anaerobes, non-fermenting for lactose, dimorphic, found in liquid media as broth in the form of short cells called swimmer cells which are responsible for the migration of colony members on the surfaces of solid culture media [2]. *Proteus mirabilis* pathogenic bacterium because their virulence factors which facilitates it to colonize the host and cause diseases, including flagella, fimbria and the production of many enzymes such as the urease enzyme, Hemolysin, Protease and Lipase, as well as Biofilm Formation [3]. *Proteus mirabilis* has eight genes encoding to the production of the urease enzyme, three of which are synthetic genes, UreC, which encodes for the large alpha (α) subunit and *Ure*B and *Ure*A, which encode for the beta and gamma (β,γ) subunits, respectively. These units aggregate to form an inactive tripartite complex known as the apoenzyme [4]. While gene responsible for protease *Zap* A. It is a transposon gene and is regulated in conjunction with the cell differentiation process [5]. As for PMF, it is mainly involved in the colonization of *P. mirabilis* in the urinary bladder and kidneys [6]. This fibrillation operon encodes five of five genes, namely pmfACDEF [7]. A study found *P. mirabilis* which phenotypically produces hemolysin, protease and produces biofilm possesses as the same time

genes encode to these factors genetically [18], which led to thinking about the importance of the subject and linking them together and for its future importance in increasing the pathogenicity and resistance of the isolation to antibiotics.

METHODS

Isolation and Identification of *Proteus mirabilis*

After obtaining ethical approval, (150) urine samples were collected, including (75) urine samples patients complain about urinary tract infections and (75) urine samples from patients with dialysis and divided into (90) samples for females and (60) samples for males, with ages ranging from (5-75) years, (Pregnant women, recent antibiotic users and immunocompromised patients were excluded) for the period from August 15, 2022 to December 15, 2022 from Al-Karama Teaching Hospital in Baghdad Governorate. The samples were transferred using sterile plastic bottles. For the purpose of planting on pre-prepared culture media. The diagnosis was done based on the traditional methods of culture, microscopy and biochemical reaction as mentioned in Hemraj *et al.* [8] and then confirmed by using the VITEK 2 device.

Phenotypic Detection of Protease, Urease and Biofilm in *P. mirabilis*

Detection of Protease Production: Skim milk agar was Inoculated with the bacterial suspension of the isolates Under study, then after Incubated at period of 24 hours at temperature of 37°C, the result is considered positive when a transparent halo appears and does not appear, this indicates that the result is negative [5].

Detection of Urease Production

This test used for discover the bacterial ability to produce the enzyme urease, which Produces ammonia and gas from the fractionation of urea. The medium of the urea agar was inoculated with bacterial isolates using the Stab and surface planing method, then for 24 hours incubated at 37°C. Where it changes color Middle Pink Indicates a positive test result, yellow indicates a result Negative [8].

Biofilm formation by Microtitre Plates Method

The microplate method was used to investigate biofilm formation in bacterial isolates: it was done according to Tang *et al.* [9].

Molecular Study

DNA Extraction: DNA was extracted from bacterial cells using an extraction kit prepared by the Korean company FAVORGEN and the following steps were followed according to the company's instructions

Preparation of Primers Solutions

According to the instructions of the supplying company, Macrogen (Korea study), by using nuclease free water for each primer used which is shown in Table 1 to obtain a concentration of (100) picomoles/microliter. About 10 microliters of the storage solution for each primer separately and added to 90 microliters of (NFW) microliters of (NFW) water and mixed well, then kept at (-20)°C until use.

Prepare the PCR Reaction: Equipped as Follows

- Prepare the polymerase mixture in the PCR tubes exist with the kit and containing 2 components
- The tubes were closed with careful mixing with the Vortex mixer for (5) seconds.
- Optimum conditions for thermos cycling conditions, which are represented by the processes of separation of the DNA denaturation strand, the association of primers to (annealing) and chain lengthening The Extension (DNA) as see in Table 2

Thermal Cycles Program for PCR

Polymerase reaction carried out using an applied thermo cycler PCR, Stander reaction for genes was programmed the device as shown in the Table 3. Electrophoresis of the resulting DNA was performed.

RESULTS AND DISCUSSION

Isolation of *P. mirabilis*

Bacterial growth appeared in (110) samples with a rate of (73.33%), while bacterial growth appeared in (40) samples with a rate of (26.66%). *Proteus mirabilis* was 30 (27.27%) isolates, with a rate while 80 isolates, with a rate of (72.73%), belonged to other bacterial species. In this study, *P. mirabilis* came in second place after *Escherichia coli*, as shown in Table 4. This result converged with Perween *et al.* [10], when *P. mirabilis* was isolated by 27 isolates, 17.88% out of 151% of the growing isolates, as 124 isolates, 82.11% of which belonged to other bacterial species.

Table 1: The sequence of primers used in the study

Primer	Sequence Primer sequence5'- - 3'	Size pb	References
Zap A	F*: ACCGCAGGAAAACATATAGCCC R**: GCGACTATCTTCCGCATAATC	540	Hussien and Yousif [18]
Ure C	F: CCGGAACAGAAGTTGTCGCTGGA R: GGGCTCTCCTACCGACTTGATC	533	(D'Orazio *et al.*, 1996)
PmfA	F: GGATCATCTATAATGAAACTG R: CTGATAATCAACTTGGAAGTT	563	(Sosa *et al*, 2003)

F*: Forword, R**: Reverse

Table 2: Components and volumes of reaction mix for gene amplification (Ure C, Zap A, pmf A) by PCR

Component	50 µL (Final volume)
Master mix	25 µl
Forward primer for each primer	10 picomols/µl (1 µl)
Reverse primer for each primer	10 picomols/µl (1 µl)
DNA	5µl
Distill water	14µl

Table 3: Multiplex PCR program for (Zap A, Ure C, Pmf A) genes

No. of cycle	Time	Temperature (°C)	Stage	N*
One cycle	3 M	94	Initial denaturation	1
35 cycle	1 M	94	Denaturation	2
	1 M	53	Annealing	3
	1 M	72	Extension-1	4
One cycle	7 M	72	Extension-2	5

N: Number, M: Minute, Tm: Melting temperature

Table 4: Bacterial species infection

Bacterial isolated	NO (Number)	Percent (%)
Escherichia coli	53	48.18
Proteus mirabilis	30	27.27
Klebsiella pneumonia	12	10.91
Enterobacter aerogene	7	6.36
Staphylococcus aureus	6	5.46
Pseudomonas aeruginosa	2	1.82
Total	110	100

Table 5: Formation of biofilms by *P. mirabilis*

	Biofilms formation		
P. mirabilis	Moderate	Strong	Total
30	16 (%53.34)	14 (%46.66)	100%

Phenotypic detection results showed that all isolated 30 (100%) of *P. mirabilis* was able to produced urease. This result agreed with the findings of Al-Duliami *et al.* [11], Urease enzyme works on the analysis of urea to ammonia and carbon monoxide case which caused to raise the hydrogen base pH and increase urine alkalinity and thus the formation of Urolithi proteins [12]. As for the production of Protease it was found that all isolates produced it and this result was agree with Al-Mayahi [13] Proteolytic enzymes, proteases, are one of the most important enzymes that contribute to the interaction of bacteria. They work on analyzing large proteins and fragmenting them into small proteins [14]. Microtiter plate was used for biofilm formation Reality has shown the ability of all isolates to form 14 isolates (46.66%) were strongly membrane-producing, while 16 were moderately membrane-producing, as shown in Table 5. This result agreed with Al-Atbi [15] and Mishu *et al.* [16], which founded about 72.97 and 75.86% of isolated were forming biofilm respectively. Biofilm is an important factor in *P. mirabilis*. A recent study showed that isolates of *P. mirabilis* have the ability to form a biofilm and spread on surfaces by 100% [17].

Multiplex PCR Detection ZaPA, ureC and PmfA Genes in *P. mirabilis*

This included the detection for three genes of virulence factors included ZaPA, ureC and PmfA and the results of the detection of the ZaPA gene responsible for the production of protease enzyme show that All 30 (100%) isolates was carried it as in Figure 1, ZaPA owns the results of the study with Hussien and Yousif [18]. And agreed with Stankowska *et al.* (2008), it was found the rate of isolates possessing the ZaPA gene was 100%. By comparing the phenotype and the molecular detection of the encoded ZaPA gene, the protease enzyme found a match between the phenotypic profiling and the molecular detection of the ZaPA gene. As all the isolates produced this factor phenotypically and genetically.

As for the ureC gene, it is one of the important structural genes for b urease and is highly preserved and replicated. It is one of the useful diagnostic features of *P. mirabilis*, the main cause of high basal urine and the formation of urinary stones [19]. The results of the current study showed that all 30 (100%) isolates were carriers of the gene as shown in Figure 1. The conclusion is the result with the findings of Al-Janahi [20] in Babel and Al-Mayahi [13]. Therefore, the investigation of this factor both phenotypically and genetically was (100%). With regard to the PmfA gene, it is one of the genes involved in the formation of biofilms and its presence in isolates leads to the formation of a high-density biofilm and thus increases antibiotic resistance. It plays a useful role in the settlement of *P. mirabilis* in the bladder and kidneys [21]. The results of the detection of the PmfA gene using the PCR technique for *P. mirabilis* bacterial isolates showed that 8 (26.7%) of them gave a positive result for this gene. While 22 (73.4%) gave a negative result, as shown in Figure 1. This result about PmfA gene differed from what Hengge (2019) [22] concluded, as all isolates (100%) carried the gene. The reason for the difference may be due to the possession of the studied isolates to other genes that stimulate and encode the formation of the biofilm such as rsbA gen [23], especially after the results of the phenotypic detection of the biofilm showed the ability of all isolates to form it [24,25].

The Relationship Between Phenotypic and Genotypic Detection of the Studied Virulence Factors

The results obtained in the current study confirmed the existence of a relationship between the phenotypic detection of the virulence factor and the percentage of the presence of the gene associated with the same factor as in the Table 6, as the isolates phenotypically showed the production of protease, urease enzymes and production of the biofilm by Tissue Culture Plate Method (TCPM) was 30 (100%), At the same time, the genetic detection results showed that all of

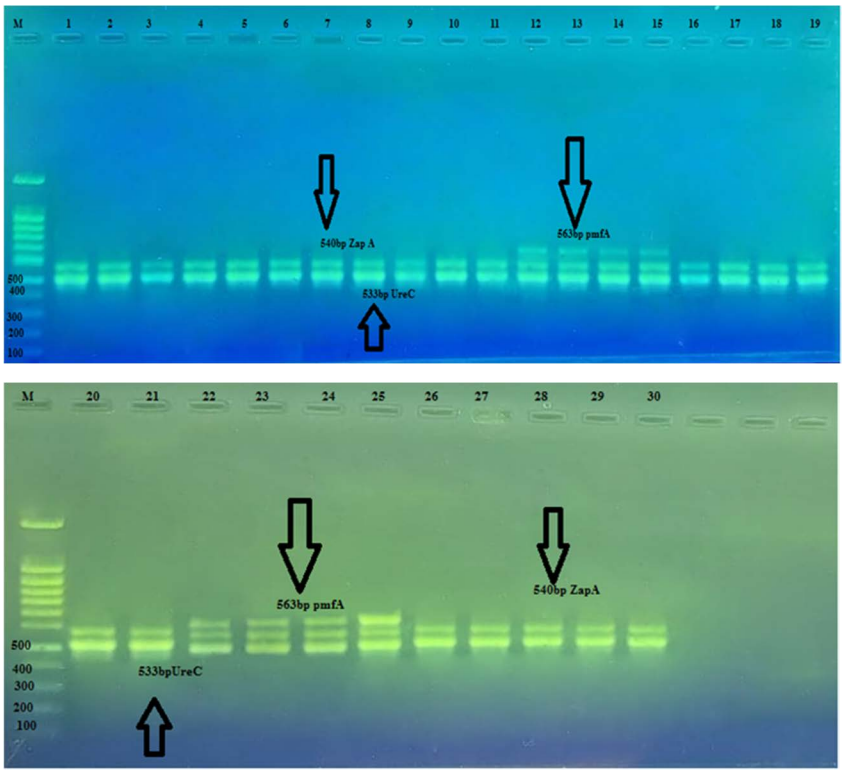

Figure 1: Electrophoresis results of the product of the Multiplex 1 PCR reaction for (ZaPA, ureC and PmfA) genes of *P. mirabilis* isolates agarose gel (1.5%) and a voltage of 5V/cm², 1xTBE buffer for 1:30 hr, where M is the Standard DNA Ladder (100 bp). Paths from number (1-30) represent isolates positive for (ZaPA and ureC) genes. Paths (25, 24, 23, 22, 15, 14, 13, 12) represent isolates positive for PmfA gene

Table 6: The relationship between phenotypic and phenotypic detection of the studied virulence factors

Phenotypic detection	Number of positive isolates (%)	Gene name	Number of positive isolates (%)
Urease enzyme	30 (100%)	UreC	30 (100%)
Protease enzyme	30 (100%)	ZapA	30 (100%)
Biofilm	30 (100%)	PmfA	8 (26%)

UreC and ZapA genes in all isolates by 30 (100%), while the percentage of PmfA gene in the biofilm was 8 (26%). The difference in results for the phenotypic detection of PmfA gene is due to the isolates having other genes that encode for biofilm production.

CONCLUSIONS

Proteus mirabilis isolates have virulence factors that increase their pathogenicity. Detecting about its product of Urease, Proteas, form biofilms, showed Outwardly they are all productive, while genetically isolates possess genes of (Ure C, Zap A, Pmf A) as (100%, 100%, 26.7%), respectively. Therefore, it is possible to focus more importantly on the nature of the link between the phenotypic and genetic presence of virulence factors of *P. mirabilis*, which affects in the future the prediction of their possession of genes that make them more pathogenic.

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