Molecular Detection of Adhesion Genes of Proteus *mirabilis* Isolated from Iraqi Patients with Urinary Tract Infection and Kidney Stones

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Abstract

Background/Aims: Urinary tract infection (UTI) is a prevalent disease of significant public health importance, affecting over 150 million individuals annually. This research aims to discover adhesion genes ZapA gene (protease) and mrpA gene (fimbriae) of P. mirabilis isolated from patients with urinary tract infection and kidney stones, and whether the presence of such virulence factors contributes to increasing its ability to evade the host's immune defences.

Materials and Methods: Two hundred and ten samples were collected from Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Governorate - Iraq, during the period from July 2023 to December 2023. The bacterial isolates that were obtained from urine samples taken from patients with UTI and kidney stones were cultured purified and then diagnosed using the Vitek-2 system. Compact - Biomerieux-France. Then, the adhesion genes were investigated using PCR technology, where the ZapA gene and mrpA gene were detected.

Results: The results of the study showed that P. mirabilis was the dominant microorganism that caused urinary tract accounting for 30% of the cases, followed by Escherichia coli (24%), Klebsiella pneumoniae (20%), Staphylococcus (16%) %), and finally mixed infections represented (10%) of cases. Gene screening results showed that mrpA and ZapA genes of P. mirabilis were positive in all the relevant samples.

Conclusions: The current study concluded that P. mirabilis is the most common bacterial species that causes urinary tract infections with kidney stones. Adhesion-related genes and immune evasion genes were positive in all samples.

Keywords: UTI, Proteus mirabilis, adhesion genes, mrpA, ZapA.

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Introduction

Studying the virulence factors of *Proteus mirabilis* bacteria isolated from clinical samples is of great importance to public health, as these bacteria constitute a major burden on health systems in the world. Studying the virulence factors paves the way for finding solutions to them, as the virulence factors represent the strengths of this bacteria that have caused an increase in its pathogenicity, and by identifying them we can find solutions in neutralizing these elements affecting over 150 million individuals annually. This widespread infection incurs substantial economic costs, with an estimated global financial burden approximating $6 billion each year. (1,2). *P. mirabilis* infection can lead to various clinical conditions, including (UTIs), wound infections, bloodstream infections and respiratory tract infections. This infection can cause significant morbidity and, in severe cases, lead to life-threatening complications. *P. mirabilis* has been increasingly associated with antimicrobial resistance, limiting treatment options for infected individuals (3). *P. mirabilis* infections acquired in healthcare settings, often referred to as nosocomial infections, can have a significant impact on patient

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outcomes and healthcare systems. These infections can lead to prolonged hospitalization, increased healthcare costs and higher morbidity and mortality rates (4,5). *P. mirabilis* infection shows a marked tendency to cause diverse clinical manifestations. Urinary tract infections (UTIs) are ranked as the most prevalent infection, with *Proteus mirabilis* accounting for a significant proportion of complex and recurrent UTIs. This infection can ascend to the urinary tract, leading to pyelonephritis and, in severe cases, urosepsis. *Proteus mirabilis* has also been implicated in wound infections, especially in immunocompromised individuals and surgical patients. Its ability to form biofilms on medical devices and indwelling catheters further complicates treatment and eradication efforts (6,7). *P. mirabilis*, a common cause of complicated urinary tract infections, especially in high-risk patients who use catheters, can also contribute to kidney stone formation. These bacteria produce an enzyme called urease that reduces the acidity of urine, creating a favorable environment for stone formation. Furthermore, the numerous fimbriae of *Proteus mirabilis* allow it to adhere firmly to the tissues of the urinary tract, facilitating the formation of stones (8). Proteases are essential not only for the virulence of *P. mirabilis* but also for its survival, especially in the urinary tract. The environment within the urinary tract is challenging for bacteria because they face powerful host defences such as antibodies and antimicrobial peptides. Among the various proteases used by *P. mirabilis*, *ZapA* (mirabilysin) is a potent metalloprotease capable of efficiently degrading many host proteins in vitro. The ability of *ZapA* to degrade a variety of host proteins in vitro confirms its adaptability and underscores its importance in disrupting the host immune response (9). The *mrpA* gene is integral to the pathogenesis of *Proteus mirabilis*. It encodes a major component of the MR/P fimbriae, which is essential for bacterial adhesion to host tissues, biofilm formation, immune evasion and persistence in the urinary tract. By facilitating these processes, the *mrpA* gene contributes significantly to the virulence of *P. mirabilis* and its ability to cause complex urinary tract infections. Understanding the role of *mrpA* in the pathogenesis of *P. mirabilis* could aid in the development of targeted therapies to prevent and treat infections caused by this bacterium (10,11). This study aims to detect genes of *P. mirabilis* that are responsible for adhesion and evasion of host immune responses.

Materials and methods

A cross-sectional study was carried out in Al Qasim Green University -Iraq from July 2023 to December 2023. Two hundred and ten samples were collected from patients with UTI and kidney stones who attended Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Governorate whose ages were 10-60 years. The morphology of the bacteria was studied by examining the growth of *P. mirabilis* on blood agar and identifying *P. mirabilis* by the swarming motility characteristic of this bacterium and by microscopic examination using Gram stain. In addition to these tests some preliminary biochemical tests to identify bacterial isolates. These tests included urease production, Simmons citrate, Kligler's iron agar, and Indole tests. Following these initial tests, 20 isolates of *P. mirabilis* were definitively First the prepared samples were inoculated into specially designed test cards. These cards contain multiple wells, each containing dehydrated substrates that react with specific enzymes or metabolites produced by microorganisms. The inoculation process was carried out with precision to ensure accurate and reliable results. Then, the inoculated test cards were placed in the Vitek-2 Compact system, which provides controlled incubation conditions. During this phase, the microorganisms in the sample metabolize the substrates in the wells, leading to color changes or fluorescence. The Vitek-2 Compact system continuously monitored the test cards using advanced optical scanning technology. This technology detects and analyzes the color changes or fluorescence patterns generated by microbial reactions. The system compared these patterns to an extensive database containing the

10.33762/mjbu.2024.147583.1202

characteristics of known microorganisms, enabling rapid and accurate identification of the specific pathogens present in the sample.

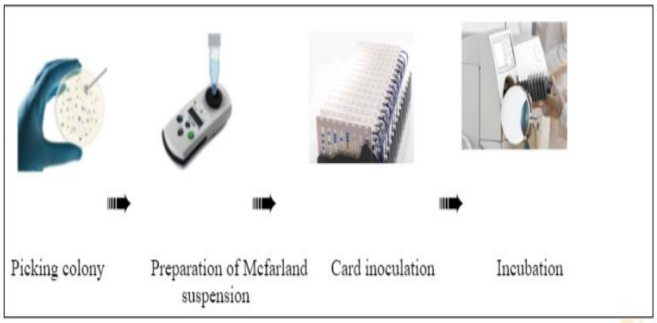

Figure 1: Process flow diagram for identification and antimicrobial susceptibility testing of bacteria directly via VITEK2

Genes responsible for adhesion and evasion of host immune responses (zapA (proteases) and mrpA (fimbriae)) of P. mirabilis were detected by using the PCR technique. The first step is to extract DNA from the bacterial sample using boiling methods, bacterial cells were lysed using heat and DNA was separated from cellular debris by centrifugation according to Mahuku et al (2004) (12). Specific primers, short DNA sequences complementary to the target gene regions, were designed in the current study. These primers bind to the target DNA and serve as starting points for DNA amplification, as shown in Table 1.

Table 1: Primer's sequence and amplicon size

Gene	Primer sequence		Product size
mrpA	F	TTTATCTGTTGTTGCGGGTTC	473 bp
	R	CGAAAGTTGCGATTGCAGTA	
ZapA	F	ACACTACTAAACACGTAGCA CAG	398 bp
	R	TGTGCGCATGCTTTCTGAAC	

The extracted DNA sample, along with the designed primers and a thermostable DNA polymerase enzyme (e.g., Taq polymerase), were subjected to a series of temperature cycles in a thermal cycler machine as shown in Table 2.

Table 2: PCR steps and conditions of the current study

Gene	Pre-Denaturation	Denaturation	Annealing	Extension	Final Extension
Cycle No.	1 cycle	35 cycles			1 cycle
mrpA	95 C° for 5 min	95 C° for 30 sec	57 C° for 30 sec	72 C° for 45 sec	72 C° for 8 min
ZapA	95 C° for 5 min	95 C° for 30 sec	59 C° for 30 sec	72 C° for 45 sec	72 C° for 8 min

Finally, the amplified DNA products were visualized using agarose gel electrophoresis done according to Mishu et al (2022) (13), where they were separated based on size. The presence of specific DNA bands of the expected size indicates the presence of the target genes. Statistical analysis

Statistical analysis was done using SPSS software 25 and Microsoft Excel.

Results

The bacterial isolates were diagnosed with preliminary biochemical tests, as shown in Figure 2 and P. mirabilis swarming growth was recognized on blood agar with the gram-negative rod appearance on microscopical examination, as shown in Figure 3, then 20 isolates of P. mirabilis were diagnosed with the VITEK 2 system. The study showed that Proteus mirabilis was isolated

10.33762/mjbu.2024.147583.1202

from 63 samples, representing 30% of the cases, thus was the predominant microorganism, followed by Escherichia coli (50 samples; 24% of the cases), Klebsiella pneumoniae (42 samples; 20% of cases), Staphylococci (34 samples; 16% of cases) and mixed bacterial infections (21 samples; 10% of the cases), as shown in figure 4. The study also showed a significant statistical association between the studied bacteria, with a Chi-square value of 40.32 and a p-value less than 0.0001.

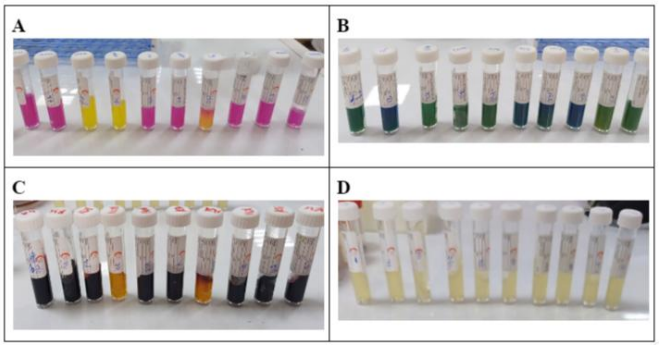

Figure 2: Biochemical tests of some isolates in the current study. A: Urease production test, B: Simmons citrate test, C: Kligler's iron agar test, and D: Indole test

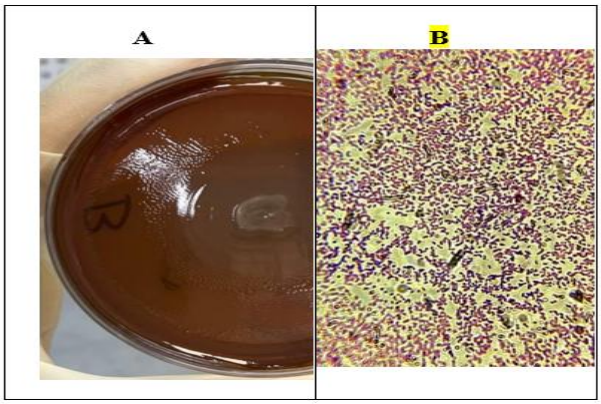

Figure 3: P. mirabilis colonies that show swarming growth on A- blood agar media, B- P. mirabilis by microscopical examination using oil immersion lens

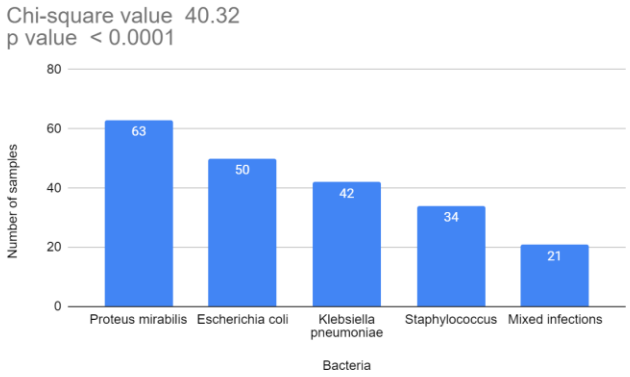

Figure 4: Distribution of bacterial samples

Molecular analysis of the studied samples revealed that both mrpA (fimbriae) and ZapA (protease) genes of P. mirabilis were present in all the tested samples as shown in Figure 5.

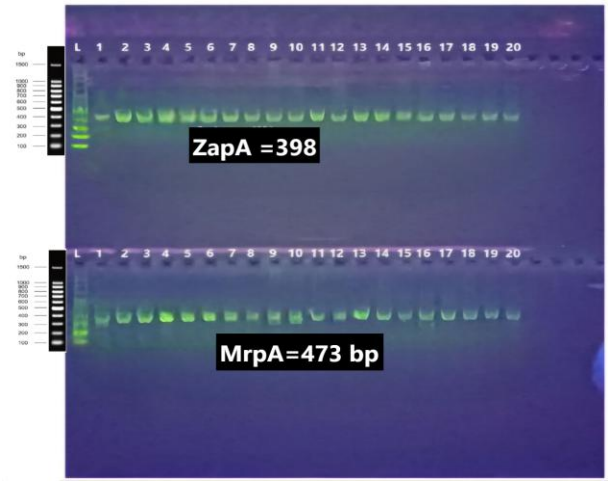

Figure 5: Gel electrophoresis showing PCR amplification of *mrpA* and *ZapA* genes of *P. mirabilis*

10.33762/mjbu.2024.147583.1202

Discussion

This study was carried out to detect genes responsible for adhesion and evasion of host immune responses of P. mirabilis in patients with UTI and kidney stones in Al-Diwaniyah Governorate - Iraq, and provides a comprehensive overview of the bacterial distribution within the examined samples, along with their respective percentages. Notably, P. mirabilis emerged as the most prevalent bacteria representing 30% of the cases, followed by E. coli (24% of cases) and K. pneumoniae (20% of cases). Additionally, Staphylococcus aureus was present in 16% of the cases, while mixed infections were found in 10% of the cases. The statistical analysis revealed a significant relationship among the different bacterial types, as indicated by a chi-square value of 40.32 and a p-value of less than 0.0001. The emergence of such results can be attributed to various factors, including environmental conditions, host factors and microbial interactions (14). Factors such as antibiotic usage, hospitalization and immune status of the host could influence the prevalence of certain bacterial species. Moreover, the transmission dynamics of these bacteria within healthcare settings or communities may contribute to their varying prevalence rates (15).

Previous studies investigating similar topics have shed light on the epidemiology and prevalence of P. mirabilis infections. For instance, a study conducted by Tumbarello et al (2012) (16) examined the distribution of P. mirabilis in clinical samples collected from hospitalized patients. Their findings corroborated our results, highlighting the prominence of P. mirabilis and E. coli in clinical settings. Furthermore, their study emphasized the importance of infection control measures and antibiotic stewardship programs in mitigating the spread of multidrug-resistant bacteria.

Both the mrpA and ZapA genes of P. mirabilis were identified in all 20 isolates, indicating a 100% prevalence rate. The presence of these genes may contribute to the pathogenicity and virulence of the bacterial strains (17). The mrpA gene, for instance, has been associated with factors such as adhesion, biofilm formation, and resistance to host defences (18), while ZapA is known for its role in protease activity, which can facilitate tissue invasion and immune evasion (8).

Previous studies investigating similar genetic markers in bacterial pathogens have provided valuable insights into their role in infection and disease. For example, a study by Pathirana et al (2018) (19) examined the prevalence of mrpA and ZapA genes in clinical isolates of P. mirabilis and their association with urinary tract infections. Their findings corroborated our results, highlighting the widespread presence of these genes and their potential significance in urinary tract pathogenesis.

Similarly, many studies concerning the relationship between P. mirabilis and UTI were performed in Iraq. For example, a study by Al-khalidy and Aburesha (2023) detected some virulence genes of P. mirabilis isolated from UTI patients in Iraq and mentioned that these pathogenic bacteria possess virulence factors that enable them to resist the host's immunity and resist antibiotics (20). Another study, conducted by Al-obaidi et al (2023) also succeeded in the detection of mrpA gene in P. mirabilis isolated from UTI Iraqi patients and discussed the importance of mrpA gene in bacterial adherence to host tissues (21). Conclusion: The study identified a diverse array of bacterial pathogens within the examined samples, with P. mirabilis, E. coli and K. pneumoniae being the most prevalent species (respectively) in UTI Iraqi patients with kidney stones. This underscores the importance of surveillance and monitoring efforts to track the prevalence of bacterial infections in healthcare settings. The genetic analysis revealed a high prevalence of the mrpA and ZapA genes across all tested isolates of P. mirabilis, indicating potential genetic determinants of virulence and pathogenicity. Understanding the genetic makeup

10.33762/mjbu.2024.147583.1202

of bacterial pathogens is crucial for developing targeted therapeutic interventions.

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الكشف الجزيئي عن جينات الالتصاق في بكتريا Proteus mirabilis المعزولة من مرضى التهاب المسالك البولية وحصلى الكلى في العراق

خلفية / أهداف: التهاب المسالك البولية (UTI) هو مرض شائع ذو أهمية صحية عامة كبيرة، حيث يؤثر على أكثر من ١٥٠ مليون فرد سنويًا. الأهداف: يهدف هذا البحث إلى اكتشاف) جينات Zapو (mrpAفي P. mirabilis المعزولة من التهاب المسالك البولية وحصى الكلى، وهل يسهم وجود هكذا عوامل ضراوة زيادة قدرتها على التهرب من الدفاعات المناعية للمضيف.

الطرق: تم جمع العينات من مستشفى الديوانية التعليمي في محافظة الديوانية - العراق، خلال الفترة من تموز ٢٠٢٣ إلى كانون الاول ٢٠٢٣. تم زرع وتنقية العزلات البكتيرية التي تم عزلها من عينات البول المأخوذة من مرضى التهاب المسالك البولية ومن ثم تشخيصها باستخدام (نظام Vitek-2 Compact - Biomerieux-France). ثم تم التحري عن جينات الالتصاق باستخدام تقنية PCRحيث تم التحري عن جين ZapA (بروتياز) وجين mrpA(الألياف).

النتائج: أظهرت نتائج الدراسة أن P. mirabilisهو الكائن الدقيق السائد الذي يسبب التهاب المسالك البولية بنسبة (٣٠٪)، تليه Escherichia coliبنسبة (٢٤٪)، ثم Klebsiella pneumoniae شكلت (٢٠٪)، اما Staphylococcusنسبة (١٦٪)، وأخيرا العدوى المختلطة تم العثور عليها بنسبة (١٠٪). أظهرت نتائج فحص الجينات في P. mirabilis نتائج ملفتة للنظر حيث كان جين mrpA و ZapAإيجابيًا في كل العينات.

الاستنتاجات: استنتجت الدراسة الحالية إلى أن P. mirabilis هو النوع البكتيري الأكثر شيوعًا الذي يسبب التهاب المسالك البولية مع وجود حصى في الكلى. بالنسبة للجينات المتعلقة ب الالتصاق وجينات التهرب المناعي كانت إيجابية في جميع العينات.

الكلمات الرئيسية: التهاب المسالك البولية، بروتيوس ميرابيليس، جينات الالتصاق، mrpA، ZapA.

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