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**ORIGINAL ARTICLE**

# Detection of Virulence Genes of Proteus Mirabilis Isolated from Clinical Samples of some Hospitals in Nasiriyah City

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## ABSTRACT

The study aimed to detect some virulence genes of Proteus mirabilis isolates, isolation and diagnosis by biochemical tests and Vitek-2 system . The research was conducted in Nasiriyah City/Dhi-Qar, Iraq, in the period between April 2021 and January 2022, and 70 urine and 30 wound samples were obtained from patients admitted to some Hospitals . Isolates diagnosis was made based on morphological and cultural characteristics of blood agar, MacConkey agar, as well as conventional biochemical tests and Vitek-2 identification system. Depending on these criteria, out of 100 samples bacterial growth appeared in only 80 (80.0%) samples, while no bacterial growth appeared in the remaining 20 (20.0%).

only 20 (20%) isolates were obtained from P. mirabilis. chosen for genetic testing in order to detect the virulence factors (15) isolates from Proteus mirabilis, such as colonization factor antigen (CFA), extracellular protease, swarming activity, and urease, were identified. The results showed that all isolates (100%) possessed UreC gene, ZapA gene, flaA gene and mrp gene.

**Keywords:** Proteus mirabilis, UTIs, UreC, ZapA, mrp and flaA

## INTRODUCTİON

Proteus mirabilis, a gram-negative , motile bacterium belonging to the Enterobacteriaceae family , pathogen are extensively dispersed in the environment, particularly in water, soil, and human and animal digestive systems (Drzewiecka 2016). It is an opportunistic pathogen that accounts for roughly 0.005% of the human gut flora in healthy people (Yatsunenko et al. 2012).

The bacterium P. mirabilis has the capacity to infect people and is well recognized for causing urinary tract infections (UTIs) in wounds, the eye, the gastrointestinal system, and the urinary tract (Armbruster et al. 2017a). P. mirabilis is capable of producing symptomatic UTIs such as cystitis and pyelonephritis, as well as being present in asymptomatic bacteriuria, especially in the elderly (Matthews and Lancaster 2011). Bacteremia, which may progress to potentially lethal sepsis, can also be caused by this illness. Furthermore, P. mirabilis infections may result in the formation of urinary stones (urolithiasis) (Papazafiropoulou et al. 2010).

P. mirabilis is commonly isolated from the gastrointestinal system, despite being a symbiont or pathogen organism (Janda and Abbott 2006). The majority of UTIs are likely to be caused by bacterial ascent from the gastrointestinal system, whereas some are thought to be caused by person-to-person transmission, especially in hospital settings (O'Hara et al. 2000). This is reinforced by findings that certain P. mirabilis urologists have the same strain of P. mirabilis in their feces (Mathur et al. 2005).

P. mirabilis, Escherichia coli, Klebsiella, and Serretia marscens, were found in wounds, especially diabetic wounds (Gadepalli et al. 2006). It creates severe clinical infections that are difficult to remove, particularly in hosts with complicated wounds, catheters, underlying illness, or immunodeficiency conditions ( Gadepalli et al. 2006). Proteobacteria that colonize the stomach vary from those that colonize wounds in their potential to carry antibiotic resistance genes (Yah et al. 2007).

In humans, the bacterium is the most common etiological agent connected to urinary tract and wound infections. P. mirabilis was responsible for 60-90% of all Proteus species infections. The variation in prevalence rates might be attributed to organism dispersion in various environments (Feglo et al. 2010).

P. mirabilis has a different arrangement of cell-related factors, and these microorganisms can emit various variables, some of which have been connected to sickness causing potential and are known as harmfulness factors(Ranjbar-Omid et al. 2015), such as lipopolysaccharides (LPS), aggregation, , urease, iron acquisition systems, and proteases (Himpsl et al. 2010). The colonization and growth of infections, which are brought on by fimbriae, depend on P. mirabilis (Hasan 2020). Urease is essential for P. mirabilis pathogenesis (Ranjbar-Omid et al. 2015). In the presence of microorganisms in the host, innate and adaptive immune responses will not develop (Norsworthy and Pearson 2017). P. mirabilis has many escape strategies The metalloproteinase (ZapA) encoded by the P. mirabilis bacteria cleaves IgA1, IgA2, and IgG (Liu et al. 2015). The ZapA mutation dramatically reduces the recovery of urine, bladder, and kidney bacteria (Arciola et al. 2018). It has the ability to alter the expression of the proteins MR/P and flagellin, fooling the immune system (Zhang et al. 2015).The current study aimed to detect some virulence genes of Proteus mirabilis isolates after they were isolated and diagnosed with Vitek-2 system.

## METHODS

Sample's collection :100 (70 urine and 30 wound) samples were collected from patients with urinary tract infections (UTIs) and wounds who visited some hospital in Al-Nasiriyah City , (Dhi- Qar, Iraq) between April 2021 and January 2022 .

**Culturing and identification:** The collected swabs were streaked on blood agar and MacConkey agar plates. The plates were incubated for 24 hours at 37°C. Bacterial colonies were identified according to cultural characteristic and microscopic examination of the cells (gram stain) and Biochemical tests.then identification of Proteus mirabilis was carried out using the kit Vitek-2 system . Preparation of the suspension A sufficient number of colonies from a pure culture suspended Using a Densichek turbidity meter, calibrated to the McFarland turbidity range for gram-negative (0.50-0.63). Microbe suspensions were injected into the labels using an integrated vacuum system. A test tube containing the microorganism suspension was put on a particular rack (cassette),Incubation periods vary from 2 to 15 hours based on the growth rate of the organism. By measuring light attenuation using a scanner, the Vitek-2 programmed computer determines if each well is positive or negative. After the incubation time, the responses were automatically assessed and the identification was printed.

Table 1: Oligonucleotide primers of different genes used in this study

| Gene | Sequence [5'-3'] | | Amplicon Size (bp) | Reference |
|---|---|---|---|---|
| ZapA | F | TTTCCCATGCAATCAAGGC | 545 | (Stankowska et al. 2008) |
| | R | CTTGGAAGTGGGCGCCTGTT | | |
| UreC | F | CCGGAACAGAAGTTGTCGCTGGA | 525 | (Takeuchiet al. 1996) |
| | R | GGGCTCTCCTACCGACTTGATC | | |
| mrp | F | ACACCTGCCCATATGGAAGATACTGGTACA | 560 | (Zunino et al. 2001) |
| | R | AAGTGATGAAGCTTAGTGATGGTGATGGTGA | | |
| flaA | F | AGGTTGTATCTGGGTGCCGA | 420 | (Ali and Yousif 2015) |
| | R | GCTGACACCGCGCTGCATGCTGTCT | | |

**Genetic detection:** Only ( 15 ) P. mirabilis isolates were used for the molecular detection (PCR) ,The DNA was extracted from samples using ABIO pure extraction procedure was used to extract genomic DNA from bacterial growth . Proteus genes were revealed via PCR thermocycler amplification (Table 1), and the conditions of PCR reaction for each gene are shown in (Table 0). The reaction contents for each gene involved Taq PCR PreMix (2X) 12.5µl from iNtRON, Korea, forward primer 1 µl (10 picomols/µl ), reverse primer 1 µl(10 picomols/µl ), DNA sample was 3µl. Finally, the free nuclease water was 7.5 µl, and the final reaction volume was 25µl (Table 2). The agarose gel (2% ) was used for electrophoresis to visualize the PCR products when stained via ethidium bromide (Intron, Korea). The sequence of the products of PCR was performed by Macrogen DNA sequencing (Korea).

Table 2: Reaction volume and components of PCR

| Components | Volume1 Sample |
|---|---|
| Master Mix | 12.5 µL |
| Forward primer | 1µL |
| Reverse primer | 1µL |
| Nuclease Free Water | 7.5 µL |
| DNA | 3µL |
| Total volume | 25µL |

Table 3: PCR thermal cycler programs

| Gene | Initial denaturation | | Denaturation | | Annealing | | Extension | | Final extension | | Cycle |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ˚C | Time | ˚C | Time | ˚C | Time | ˚C | Time | ˚C | Time | |
| ZapA | 95 | 01:00 | 94 | 00:30 | 53 | 00:30 | 72 | 01:00 | 72 | 05:00 | 35 |
| UreC | 94 | 03:00 | 94 | 01:00 | 63 | 01:00 | 72 | 01:00 | 72 | 07:00 | 30 |
| mrp | 94 | 03:00 | 94 | 01:00 | 40 | 00:30 | 72 | 01:00 | 72 | 05:00 | 30 |
| flaA | 95 | 03:00 | 95 | 00:30 | 54 | 01:00 | 72 | 00:30 | 72 | 05:00 | 30 |

## RESULTS AND DISCUSSION

**Identification of Proteus mirabilis Isolates:** 100 (70 urine and 30 wound) samples were taken and cultured on different culture media, where bacteria growth appeared in only 80 samples. Only 20 isolates of P. mirabilis were found, and the diagnosis was performed using cultural characteristics of blood agar and MacConkey agar and biochemical test. in addition to the Vitek-2 identification system. The results of our study converged with Zuhir and Alaubydi (2016) and Wang et al.(2014) who observed as 19% and 20% isolates of P. mirabilis, respectively. Identification of P. mirabilis by Vitek-2 system give highly accuracy arranged from 87% to 99%, therefore 11/20 (55%) samples could identify by this system. This corresponds to Nimer et al.(2016) who showed the 25/40 (59.8%) of microorganisms were correctly identified (Table 4).

Table 0: Frequency and percentage of identification of P. mirabilis by classical test and Vitek-2 system

| NO | Name of test | N(20) | % |
|---|---|---|---|
| 1 | Phenotype and biochemical tests | 9 | 45.0 |
| 2 | Vitek-2 system | 11 | 55.0 |
| Total Isolates | | 20 | 100 |

the bacteriological culture are shown in Table 5. According to the types of bacteria diagnosed, out of 100 samples bacterial growth appeared in only 80 (80.0%) samples, while no bacterial growth appeared in the remaining 20 (20.0%). Most common bacteria are E.coli (34.0%) and P. mirabilis (20.0%) in urine and wounds.

Table 5: Distribution of the results of culture

| Culture Results | Number Isolates | % |
|---|---|---|
| No growth | 20 | 20.0 |
| Bacterial growth | 80 | 80.0 |
| P. mirabilis | 20 | 20.0 |
| P. vulgaris | 11 | 11.0 |
| P. penneri | 4 | 4.0 |
| E. coli | 34 | 34.0 |
| bacteria Other | 11 | 11.0 |

Our results found a large proportion of culture outcome (34.0%) yields growth of E.coli, the result of this study was agreement with many studies as well as with study documented by Sabri and Kareem (2020), in Baghdad city, who recorded that E. coli was caused UTIs with 36.8%, where it was proved that E. coli is the main cause of UTIs. While 20.0% yields P. mirabilis, 11.0% yields P. vulgaris growth, these results get an approach with Feglo et al.(2010) who mentioned that the percentage for P. mirabilis isolation was 19.5%, P. vulgaris 12.5% . These results also were in agree with Al-Khateeb (2014), demonstrate that P. mirabilis is more common than P. vulgaris in clinical infections because P. mirabilis is a component of the normal flora of mammals, including humans, which can cause contamination of water or food with faces. In contrast, P. vulgaris and other Proteus species are not a component of the normal flora of mammals, including humans, so they have a lower rate of infection (Wassif et al. 1995).

**Virulence Factors of P. Mirabilis:** Only 15 Proteus mirabilis isolates were chosen for genetic testing in order to detect the virulence factors. The existence of the genes UreC, ZapA, mrp and flaA was screened using universal primers and the expected sizes for the gene fragments were 525 bp, 545 bp, 560 bp, and 420 bp, respectively.

The PCR results for the P. mirabilis isolates revealed that the UreC gene (100%) was present in( Figure 1) ,The findings are consistent with several researchers (Al-Salihi 2012, , Al-Ataby 2013, , Ali and Yousif 2015), who discovered that (100%) of P. mirabilis.

Urease is one of the most important components in the pathogenesis of P. mirabilis. In vitro (on urea agar), urea is hydrolyzed by urease to produce alkaline ammonia and carbon dioxide, which raises pH and turns the phenol red indicator pink (Friedrich et al. 2005). However, in vivo (inside the human body), this enzyme leads to the formation of kidney and bladder stones as well as the encrustation or obstruction of indwelling urinary catheters (Coker et al. 2000).

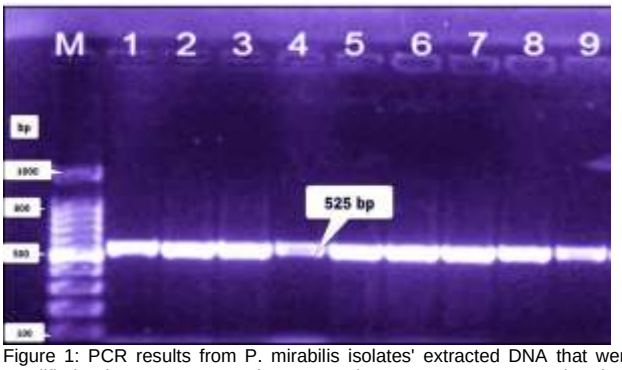


Figure 1: PCR results from P. mirabilis isolates' extracted DNA that were amplified using UreC gene primers are shown on an agarose gel stained with ethidium bromide. DNA molecular size marker lane (M) (100-1500 bp ladder). The isolates in lane between 1 and 9 shows UreCgene

The ability of P. mirabilis to produce extracellular protease ,15 isolates were analyzed for the presence of the ZapA gene, which was positive for all 15 (100%) samples (Figure 2). Our results were agree with by (Coker et al. 2000, Stankowska et al.2008), who reported that 100% of P. mirabilis is capable of producing proteases. However, our results differed from those obtained by Al-Salihi (2012) and Al-Duliami et al.(2011), who reported lower rates of positivity for substantial category of

proteolytic enzymes discovered in Proteus isolates are metalloproteases . This P. mirabilis produces a metalloprotease that is a member of the zinc protease serralysin family and is encoded by the ZapA gene (Wassif et al. 1995). These proteases can break down host proteins, releasing amino acids as fuel. They may also break down proteins like IgA, which are implicated in both host defense and tissue injury (Jost and Billington 2005).

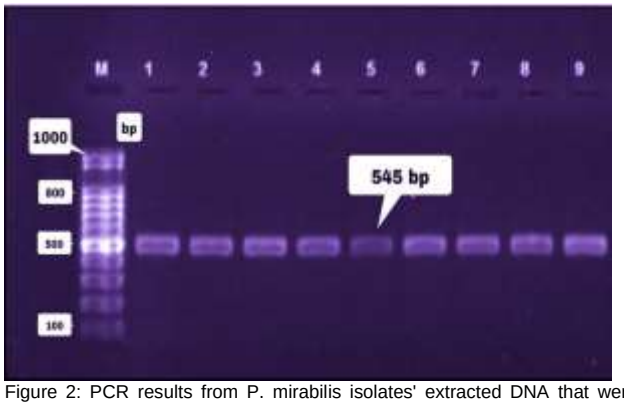


Figure 2: PCR results from P. mirabilis isolates' extracted DNA that were amplified using ZapA gene primers are shown on an agarose gel stained with ethidium bromide. DNA molecular size marker lane (M) (100-1500 bp ladder). The isolates in lane between 1 and 9 shows ZapA gene

showed that the flaA gene was present in every isolates (Figure 3). This result is consistent with earlier studies conducted in Iraq (Mohammed 2014, Ali and Yousif 2015). They reported flaA gene presence in Proteus isolates in two different percentages: 100% and 86.66%, respectively.

The flagellin protein FlaA, which is encoded by flaA, makes up the majority of the filamentous part of the P. mirabilis flagellum (Manos and Belas 2006). The flagellin-determinant gene (flaA, flaB, and flaC) on the Proteus mirabilis genome, however only one of its three copies is expressed (O'May 2008). Normally, flaB is quiet while the flaA allele is expressed. These genes can, however, unite again to create flagella that are antigenically different(Manos and Belas 2006). Because flagellin is a highly immunogenic protein, it has been proposed that this recombination may aid immune evasion during infections (Chilcott and Hughes 2000).

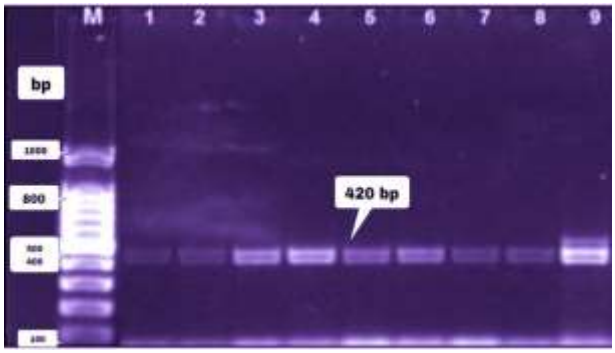


Figure 3: PCR results from P. mirabilis isolates' extracted DNA that were amplified using flaA gene primers are shown on an agarose gel stained with ethidium bromide. DNA molecular size marker lane (M) (100-1500 bp ladder). The isolates in lane between 1 and 9 shows flaA gene

All of the P. mirabilis isolates have CFA production , According to the findings of an amplification of its region (Figure 4) and the sequencing of all of the isolates, every single one of them had the mrp gene. This result is in line with the findings of previous research, such as that conducted by Jabur et al. (2013) and Mohammed (2014), who found that all Proteus isolates possessed CFA respectively. Although Mishra et al.(2001) found that 73/116 (62.93%) of the P. mirabilis isolates showed positive CFA findings. Similar to the findings of other studies ( Barbour et al. 2012, Mohammed 2014), we found that both P. mirabilis and P. vulgaris contain the mrp gene to the same extent. In addition, our findings were comparable to those published by Al-Duliami et al.(2011), who discovered that 96.0 % of P. mirabilis isolates tested positive for CFA.

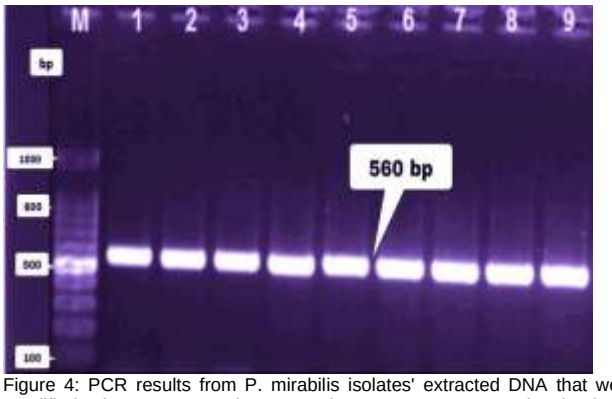


Figure 4: PCR results from P. mirabilis isolates' extracted DNA that were amplified using mrp gene primers are shown on an agarose gel stained with ethidium bromide. DNA molecular size marker lane (M) (100-1500 bp ladder). The isolates in lane between 1 and 9 shows mrp gene

## CONCLUSİONS

P. mirabilis isolates possess the capacity for many virulences gene (genetic), including urease, extracellular proteases, adhesion factors and swarming activity.

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