

# Isolation and Detection of UreC and UreR of *Proteus mirabilis* in Gastrointestinal Samples from Patients with Crohn's Disease in Iraq

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**Abstract** *Proteus mirabilis* is a Gram-negative facultative anaerobe bacillus isolated from the human gastrointestinal tract that has recently been linked to Crohn's disease (CD) recurrence after bowel resection. CD is an inflammatory bowel disease that affects the entire gastrointestinal tract, including the mouth and anus. Inflammation, ulcers, and other damage to the digestive tract lining characterize it. In the current study, CD patients as well as controls provided 79 biopsy samples of both sexes and ages from the Teaching Hospital Gastroenterology and Hepatology, medical city, Baghdad, from October 2022 to March 2023, to detect *Proteus mirabilis* (*P. mirabilis*) bacteria, as well as the results of biochemical tests and the Vitek 2 system. *P. mirabilis* was subjected to a PCR assay using particular primers that targeted the genes ureR and Urease C (ureC), which encode for the urease enzyme, a virulence factor in *Proteus* species.

**Key Words** *Proteus mirabilis*, PCR, Crohn's disease

## 1. Introduction

*P. mirabilis* has sparked particular interest among the many bacteria that inhabit the gastrointestinal system due to their potential participation in inflammatory processes. *P. mirabilis* is a Gram-negative bacteria of the Enterobacteriaceae family known for its swarming motion and urease production, which causes urinary tract infections and kidney stone formation. Current research, however, has begun to shed light on its possible role in gastrointestinal disorders such as CD [1].

However, while these relationships have been observed, the specific function of *P. mirabilis* in the development or progression of CD is yet unknown. The link might be more complicated than a simple cause-and-effect circumstance, and the prevalence of *P. mirabilis* in CD patients could be an effect of their changed gut environment rather than a direct cause [2].

*P. mirabilis* contains many harmful components such as Fimbria, Flagella, Urease, Protease, Hemolysin, lipopolysaccharide and endotoxins [3], which help in revitalizing the pathological process of bacteria. Protease and urease enzymes are virulence factors produced by all strains of *Proteus* spp bacteria. This distinguishes this species' members from the rest of the family members [4].

CD is a complex, chronic inflammatory gastrointestinal

disease affecting millions of people worldwide. While the precise etiology is unknown, a new study suggests a possible link between the gut microbiota and the onset or exacerbation of this condition. The human gut contains a diverse and dynamic microbial ecosystem critical for homeostasis and overall health. Dysbiosis, or disruptions in this microbial balance, has been related to a wide range of gastrointestinal illnesses, including CD [3].

*P. mirabilis* infection of the intestines has been linked to developing or worsening a condition called inflammatory bowel disease in some people. Furthermore, the presence of these microorganisms may be linked to clinical aspects of CD, such as disease localization and severity. Alteration in the gut microbiota characterizes CD, a condition known as dysbacteriosis (a change in the balance of the microbial populations within the gut). The microbiota of the intestinal tract in an optimal condition comprises many different kinds of microorganisms that play significant roles in the metabolism, digestion, immunological function, and other physiological functions [5].

Iraq is a unique region in terms of CD, with an increasing frequency and little study of its underlying causes. As a result, this study aims to look at the presence and properties of *P. mirabilis* in gastrointestinal samples acquired from CD

| Gene  |   | Forward and Reverse Sequence (primer 3/-5/) | Product (bp) |
|-------|---|---|--------------|
| Ure R | F | GCGGTTTATCACGAAGGGGT                        | 359 bp       |
|       | R | TGAGTGCGAAATTGCGATGG                        |              |
| Ure C | F | CCGGAACAGAAGTTGTCGCTGGA                     | 533 bp       |
|       | R | GGGCTCTCCTACCGACTTGATC                      |              |

Table 1: The primers ureC and ureR, sequences

patients in Iraq. By identifying and characterizing *P. mirabilis* strains, we are interested in contributing to expanding the body of information about the intestinal microbiota and its potential to play a role in the development of CD in this group.

## 2. Materials and Methods

The study comprised 79 patients who visited the Teaching Hospital Gastroenterology and Hepatology, Medical City, Baghdad. Over a six month period (1<sup>st</sup> October 2022 to 1<sup>st</sup> march 2023). Samples were taken from colon biopsies and divided into two parts: the first part was stored in preservation media prior to culture in Blood Agar Medium, MacConkey Agar Medium, and Nutrient Agar. Blood agar medium was prepared by dissolving 40 g of blood agar base in 1000 mL DW, sterilizing at 121 °C for 15 min, then cooling to 50 °C, and 5% human blood was added. 51.5 g of MacConkey agar powder was suspended in 1000 mL of DW and then boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 121 °C for 15 minutes at 15 psi. Nutrient Agar Suspended 28g in 1000ml of distilled water. Boil until the medium is completely dissolved to taste and sterilize by steam sterilization at 121 °C for 15 minutes. Then transfer them to sterile Petri dishes and incubate at 37 °C for 24 hours. To give a more accurate result, Urea Base Media was used by dissolving 2.5 gm of urea base agar in 95 ml of dry water; after autoclave sterilization, 5 ml of sterile urea solution was added, then poured into sterile tubes obliquely, incubated at 37 degrees Celsius for 24 hours, as well as the use of Vitak test and biochemical tests. And the second part was placed in a sterile plastic container for PCR investigation. The DNA was extracted using a (gSYNCTM DNA Extraction Kit) (cell & tissue), the biopsy sample was digested with 200ul of PBS, and the tissue was homogenized by grinding. A 533-bp segment of the *P. mirabilis* ureC gene was amplified using specialized forward primer F1(5- CCGGAACAGAAGTTGTCGCTGGA-3) and reverse primer R2(5- GGGCTCTCCTACCGACTTGATC-3). Furthermore, a 359-bp fragment of the *P. mirabilis* ureR gene was amplified using specific forward primer F1, (5-GCGGTTTATCACGAAGGGGT-3) along with particular reverse primer R2 (5-TGAGTGCGAAATTGCGATGG-3) (Table 1, 2).

### Statistical Analysis

The data analysis was conducted using SPSS-22 (Statistical Package for the Social Sciences), a widely recognized software for statistical analysis. The descriptive statistics, particularly basic frequency counts and percentages, were

| NO | Content of reaction Mixture | The amount of reaction mixture in a single tube |
|----|-----------------------------|---|
| 1  | Green master mix            | 12.5 $\mu$ l                                    |
| 2  | DNA template                | 6 $\mu$ l                                       |
| 3  | Forwar primer(10 Picomol)   | 1 $\mu$ l                                       |
| 4  | Reverse primer(10 Picomol)  | 1 $\mu$ l                                       |
| 5  | Nuclase free water          | 4.5 $\mu$ l                                     |
|    | Total volume                | 25 $\mu$ l                                      |

Table 2: Polymerase Chain Reaction Mixture

employed to succinctly represent the data. For assessing the significance of differences in percentages, which is a key aspect of quality data analysis, the Chi-square test ( $\chi^2$ ) was utilized. The criterion for establishing statistical significance was set such that the P-value obtained from the analysis had to be equal to or less than 0.05. This threshold indicates the point at which results are considered statistically significant for the purposes of relevance checking

## 3. Results and Discussion

### Isolation and Diagnosis *P. mirabilis*

Our research participants' ages ranged from 14 to 75 years old and included both sexes, reflecting the wide demography impacted by CD. It is important to investigate if age and gender influence the prevalence of *P. mirabilis* in this environment. The current research found that 33 isolates (41.8%) of 39 samples belonging to the genus *P. mirabilis* were collected from Crohn's patients by colonoscopy and examination of some cultures. The following are the results of the character and biochemical tests. MacConkey's agar colonies were light in color and lactose-free. The odor of bacterial development, which smells like decaying fish, also exists; on the blood agar, which is the key diagnostic formula for this particular bacteria, a rippling or crowding movement appeared. The isolated bacterial cells are short bacilli Gram-negative and do not form spores, according to microscopic analysis of the results. The findings of the biochemical testing were also approved [6]. The data showed complementary features of the first *P. mirabilis* diagnosis. *P. mirabilis* isolates are oxidase and indole negative but catalase and urease positive. This study's findings are compatible with those of [3], who isolated *P. mirabilis* from CD patients.

Dysbacteriosis (microbial population imbalance) may be caused by environmental factors such as nutrition and antibiotic exposure, which can affect the microbial cosmetics of the colon. In response to these conditions, *P. mirabilis* may thrive or decline, or alter the structure of the microbial community, triggering immune responses that are innate via pattern recognition receptors such as Toll-like receptors, causing the production of cytokines that are pro-inflammatory, leading to intestinal inflammation, and potentially modifying its effect on Crohn's disease [7]. The current study agreed with [8], [9]

The proliferation is particularly noticeable in the ileum, rectum, and sigmoid areas, which are commonly affected by lesions. This phenomenon can be attributed to the marked decrease in the percentage of *Escherichia coli*, which may be affected by the secretion of *P. mirabilis* substances that hinder the reproduction of some bacteria, such as productive

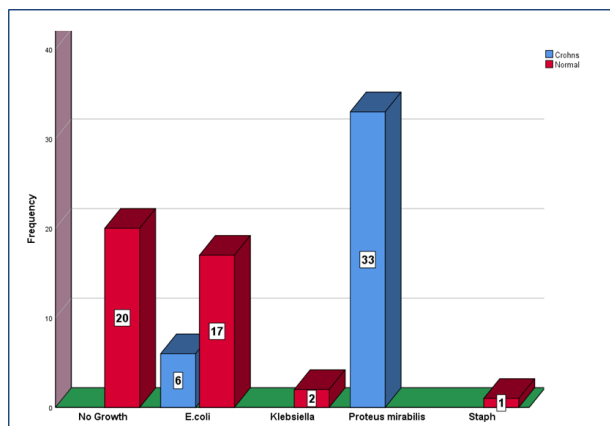


Figure 1: Distribution of bacterial associated with Crohn's Disease and healthy status



Figure 3: *Proteus mirabilis* on Nutrient agar

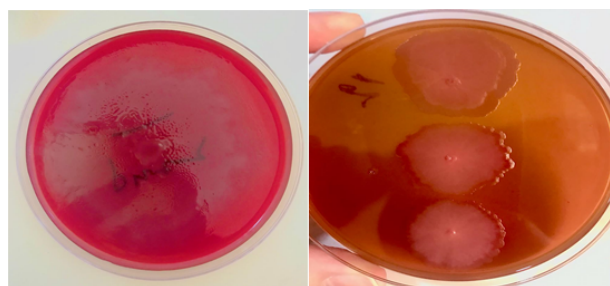


Figure 2: *Proteus mirabilis* on blood agar medium and MacConkey Agar

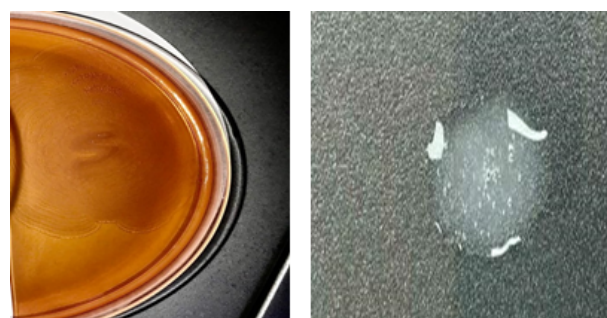


Figure 4: Biochemical tests for Catalase test positive of *Proteus mirabilis*. ( on MacConkey agar and On slide)

microorganisms for ammonia. These changes in the microbial environment can lead to changes in pH levels and urease activity [9]–[12]. the mucosa-associated *E. coli* population shows little infection in individuals diagnosed with (CD). *E. coli* populations observed at these sites showed a diminished presence of virulence markers, suggesting their potential irrelevance in pathogenesis with CD (Figure 1) [13], [14].

*P. mirabilis* is known for its swarming motion, which is the collective movement of bacteria cells across surfaces. This swarming activity can weaken the barrier made up of intestinal epithelial cells and make it more permeable. The CD is distinguished by increased intestinal permeability, which can translocate bacterial products and antigens into the mucosa, resulting in an inflammatory reaction. Urease, which can contribute to generating ammonia and other metabolites in the gut, is another virulence factor produced by *P. mirabilis*. Ammonia can harm the intestinal epithelium while also increasing the production of pro-inflammatory cytokines. Chronic inflammation can aggravate or cause CD to develop [15], as show in Figures 2 and 3.

*P. mirabilis* is characterized by being catalase positive as it transforms hydrogen peroxide ( $H_2O_2$ ) (3%) was used for the detection of bacteria which can produce catalase enzyme. This test was done on both MacConkey agar and on the slide (Figure 4).

According to current research, the presence of *P. mirabilis*

in the gastrointestinal tract is linked to (CD) and can cause inflammation in colitis cells (9, 16). Urease is an essential component of *P. mirabilis* pathogenesis and can be produced in huge quantities, hydrolyzing urea to ammonia ( $NH_3$ ) and raising the urea concentration [16], [17].

In this study, they examined both the phenotypic and genotypic characteristics of *P. mirabilis* infections to determine these virulence factors' presence and potential role. Phenotypically, we assessed the ability of clinical isolates to produce urease enzyme using standard biochemical tests such as Christensen's urea agar test. Genotypically, we performed PCR amplification and sequencing of the *ureR* and *uraC* genes to identify any mutations or variations that could affect their function. PCR results for *P. mirabilis* isolates are presented. 30 (76.9%) of the 39 CD samples were positive for the *UreC* gene, while the remaining nine patients (23.1%) did not have this exact genotype. *UreR* is an AraC-family transcriptional regulator with urea- and DNA-binding domains. *UreDABCEFG* is duplicated in the other direction; *ureR* binds to the *ureR* and *ureD* promoters. The results showed that 36 isolates (79.5%) had the *ureR* gene presented in Table 3. While the remaining eight patients (20.5%) did not have this specific genotype, this result is similar to [11].

Duplex PCR for *ureR* and *ureC* amplification revealed that

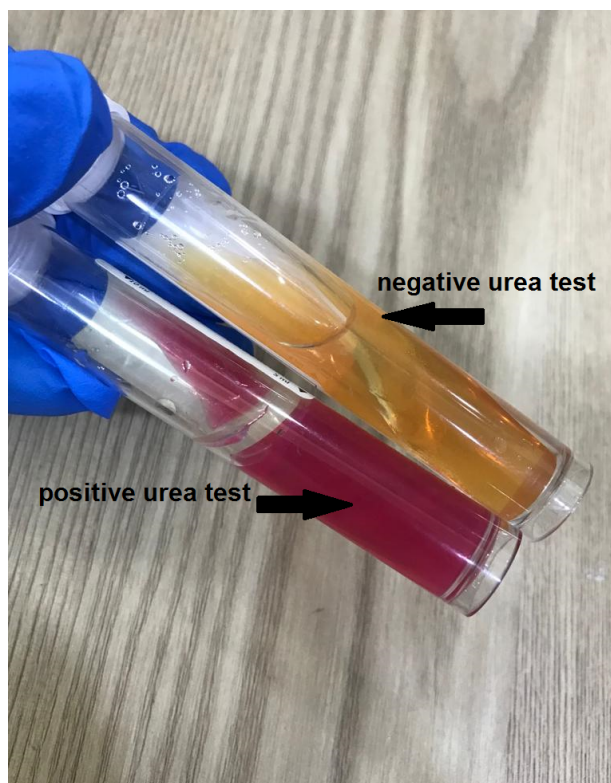


Figure 5: *P. mirabilis* can change medium together with in 24 h in Urea Base Media, as a result of *P. mirabilis* being gram negative

| Sample No. | Positive Samples Detected by PCR |                   |          |
|------------|----------------------------------|-------------------|----------|
|            | Culture Ure C PCR                | Culture Ure R PCR | Result   |
| Positive   | 30 (76.9%)                       | 31 (79.5%)        | Positive |
| Negative   | 9 (23.1%)                        | 8 (20.5%)         | Negative |
| Total      | 39 (100%)                        | 39 (100%)         | Total    |

Table 3: Performance of microscopy and ure C and ure R PCR of culture for *Proteus mirabilis* specimens for diagnosis of Crohn's disease

90% of the isolates have both ureR and ureC as evidenced by amplicons with molecular weights of 359 and 533 bp when electrophoresed on an agarose gel stained with bromide ethidium., As shown in Figures 6 and 7.

Urease is crucial in the pathological process of *P. mirabilis*. Urease hydrolyzes urea to create alkaline ammonia and carbon dioxide, raising the pH and coloring the phenol red indicator pink. As shown in Figure 5 [18]–[21]. Polymerase Chain Reaction was used to study some of the genes important for virulence in *P. mirabilis*. Oligonucleotides are DNA fragments with limited nucleotides that serve as primers for virulence genes present in *P. mirabilis*, such as ureC and ureR. The ureC genes produce the urease enzyme, which is used to identify *P. mirabilis* bacterium [22]. The study revealed isolates prolific for the ureC gene, which is also responsible for urease enzyme synthesis.

*P. mirabilis* urease enzyme is active to a greater extent

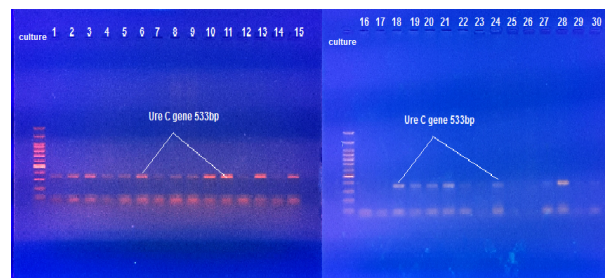


Figure 6: The genotype of the ureC (533bp) genes from *Proteus mirabilis*, isolated from a Crohn's disease patient, was shown on a 1% agarose gel using PCR. L : DNA Ladder ( 1500 bp ) , Lane (1,2,3,4,5,6,7,8,9,10,11,13,15,18,19,20,21,24,28,30) ureC gene positive . Lane (12,16,17,22,23,25,26,27,29) ureC negative gene.

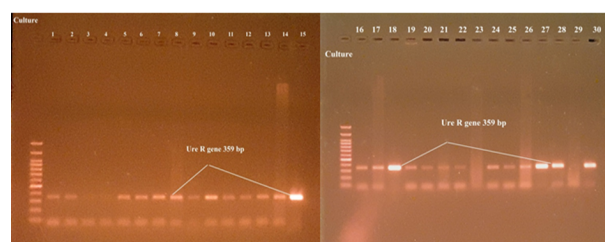


Figure 7: The genotype of the ureR (359bp) genes from *Proteus mirabilis*, which were isolated from a Crohn's disease patient, were shown on a 1% agarose gel using the polymerase chain reaction (PCR) technique. L : DNA Ladder ( 1500 bp ) , Lane (1-2,2-15, 16-22,24-28, 30 ) ure.R gene positive . Lane (3,4, 23,2 ) ureR negative gene.

than urease enzyme generated by other bacteria. It works by modifying the PH to basic, leading to the accumulation of calcium and magnesium phosphate in the biofilm formed, which leads to the development of Crystalline biofilm, which is the more complex type of biofilm because it works to close the urinary catheter and protect the bacteria from antibiotics, resulting in treatment failure. Urease production is frequently linked to the ability to live and thrive in specific settings. The human gut microbiota includes urease-producing bacteria. As a result, ammonia produced by urease activity can influence gut pH while contributing to the overall balance of microbial communities [11].

Urease is a genetic complex consisting of three structural genes (ureA, ureB, and ureC) and four helpers genes (ureD, ureE, ureF, ureG, and ureR). Urease apoenzyme is a trimer complex comprising three separate copies of each subunit. The presence of nickel electrons in the metal nucleus of ureC is required for the urease enzyme to operate. Support proteins such as ureR aid in integrating a nickel electron into the active site of the urease enzyme, synchronizing its activation [23].

UreC is the major component responsible for urease production in *P. mirabilis* and is highly prevalent throughout every species, resulting in a *P. mirabilis* diagnostic feature.

However, ureR is required for basal urea activity in an absence of urea, urea activation of Urease, and *P. mirabilis* pathogenicity [24], [25]. Several investigations have indicated that ureR and ureC are widely distributed in *P. mirabilis*.

Furthermore, Mobley and Chippendale (1990) demonstrated that all *P. mirabilis* isolates from different clinical sources produced significantly more Urease than other bacteria as well, and there was indeed a relationship among both the phenotype and the molecular determination of urease activity. UreR is a transcription regulator in the AraC family with DNA and urea-binding domains [26].

The input of nitrogen into the gut microbiota by urease may have a significant role in the formation of dysbiotic bacteria in (IBD). The prevalence of the sulfur relay system in the microbiome of the gut of CD patients highlights the potential role of nitrogen absorption in the formation of the microbiota in the intestinal tract. Urease-mediated nitrogen influx in the gut microbiota is critical in the formation of CD dysplastic microbiome. Furthermore, a link was found between CD severity in these patients, fecal amino acid contents, and bacterial taxa associated with dysplasia. Because of its involvement in bacterial nitrogen outflow, ammonia generation, and subsequent incorporation of ammonia into bacterial amino acids, this led to the notion that Urease's enzymatic activity plays a role in the creation of decomposing microorganisms [24].

Some negative results were shown for Crohn's patients when using PCR, these results consistent with [27], [28] as it was not demonstrated that UreR impacts the expression of any additional factor outside the gene group. Because our findings show that the absence of an UreR-regulatory protein inhibits urease expression, this protein could be a viable target for the treatment or eradication of *P. mirabilis*. Some mutations in the amino acid sequences ureC and ureR affected susceptibility to antibiotics as well as WGS resistance gene expression for many antibiotic classes [29].

#### 4. Conclusion:

The current study found that *P. mirabilis* has a close link with Crohn's illness due to its presence in Crohn's patients' gastrointestinal. And that the urease enzyme plays an important role in influencing the pH of the intestine via specific genes of the *P. mirabilis* bacteria, in which the UreC gene encoding a large subunit that oversees the production of the *P. mirabilis* urease enzyme, and and it is very highly common among all species, so it is considered a trait Diagnostic for *P. mirabilis*. In this work, however, it is regarded a virulence factor identified through PCR in along with ureR.

#### Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

#### Authors Contribution

All authors contributed equally in this paper.

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