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

Fadhela Nafaa Kafe1,*, Essam Mohammed Abdullah1 and Issam Abdulkriam Selman1

1 University of Anbar, College Of Medicine, Department of Microbiology, Iraq.

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 [5].

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.
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 (1st October 2022 to 1st 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 of distilled water, 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. 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 oxidase and indole negative but catalase and urease positive. The following are the results of the characteristic aspect of quality data analysis, the Chi-square test (χ²) was utilized 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 (χ²) 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 altering 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward and Reverse Sequence (primer 3/-5/)</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ure R</td>
<td>F: GCGGTTTATCACGAGGGGOT, R: TGAGTTGCAAAATGGCGATGG</td>
<td>359 bp</td>
</tr>
<tr>
<td>Ure C</td>
<td>F: CCCGAACAGAAGTTGTGGTAAG, R: GGGCTCTCCATCCGCGTGATCC</td>
<td>533 bp</td>
</tr>
</tbody>
</table>

Table 1: The primers ureC and ureR, sequences

<table>
<thead>
<tr>
<th>Content of reaction Mixture</th>
<th>The amount of reaction mixture in a single tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Green master mix</td>
<td>12.5 µl</td>
</tr>
<tr>
<td>2. DNA template</td>
<td>6 µl</td>
</tr>
<tr>
<td>3. Forward primer (10 Picomol)</td>
<td>1 µl</td>
</tr>
<tr>
<td>4. Reverse primer (10 Picomol)</td>
<td>1 µl</td>
</tr>
<tr>
<td>5. Nuclase free water</td>
<td>4.5 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

Table 2: Polymerase Chain Reaction Mixture
Kafe et al.: Isolation and Detection of UreC and UreR of Proteus mirabilis in Gastrointestinal Samples from Patients with Crohn’s Disease in Iraq

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

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

Figure 3: Proteus mirabilis on Nutrient 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 [13], [14].

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 ureC 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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Positive Samples Detected by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture UreC PCR</td>
</tr>
<tr>
<td>Positive</td>
<td>30 (76.9%)</td>
</tr>
<tr>
<td>Negative</td>
<td>9 (23.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (100%)</td>
</tr>
</tbody>
</table>

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. 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 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.

References
Kafe et al.: Isolation and Detection of UreC and UreR of Proteus mirabilis in Gastrointestinal Samples from Patients with Crohn’s Disease in Iraq


