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Evaluation of beta-hemolytic, metallic green sheen, and ONPG test properties *Escherichia coli* isolated from urinary tract infections

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Abstract

Introduction: Uropathogenic *Escherichia coli* strains are the most common cause of urinary tract infections in nosocomial and community-acquired infections. Phenotypic characteristics of Escherichia coli isolates in patients with urinary tract infections vary from region to region. Therefore, studying the phenotypic properties of the bacterium is very important.

Materials and Methods: In the current study, 100 strains of *Escherichia coli* were detected from urine samples of patients with urinary tract infections in Mazandaran province, Babol. This study aimed to investigate the properties of metallic green sheen, beta hemolysis, and Ortho-nitrophenyl-β-D-galactopyranoside (ONPG) test of Escherichia coli.

Results: The most common bacterium isolated from urinary tract infections was *E. coli* (68.02%). In the present study, the properties of beta hemolysis, metallic green sheen, and ONPG in uropathogenic *E. coli* were 1, 80, and 100%, respectively.

Conclusion: The results of this study showed that 20% of E. coli strains lacked metallic green sheen, which should be identified through the IMViC test and other biochemical tests.

Keywords: Uropathogenic *E. coli*, ONPG test, Metallic green sheen, β hemolysis

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Introduction

Escherichia coli (*E. coli*) is normally found in the intestines of humans and animals (1). *Escherichia coli* is the most common cause of UTI, accounting for 80 to 90 percent of community-acquired infections and 40 to 50 percent of nosocomial infections. More than 50% of women between the ages of 20 and 40 experience a urinary tract infection more than once (2). If left untreated, cystitis can accelerate ascending infections such as pyelonephritis and sepsis with kidney damage (3). *E. coli* that cause urinary tract infections are known as uropathogenic *E. coli* (UPEC) (4). Urinary tract infections are caused by bacteria ascending from around the urethra to the urethra, bladder, and other urinary tract. Colonization of the area around the urethra with pathogenic bacteria is a vital factor in the development of urinary tract infections. Women are more likely than men to get urinary tract infections due to the shorter distance between the urethra and the proximity of the urethra to the anus (5). Phenotypic tests of metallic green sheen in the culture medium of eosin methylene blue agar, hemolysis in blood agar medium, and Ortonitrophenyl beta galactoside are used in the detection of uropathogenic *Escherichia coli*. In EMBs, strong acid producers such as *E. coli* usually form green metallic-colored colonies that show a dark core. Under acidic conditions, the color of eosin Y precipitates, and due to the formation of an amide bond between eosin Y and methylene blue in the medium, dark-colored colonies usually form with a metallic green glow (6). uropathogenic *E. coli* lysis red blood cell by producing hemolysin A by creating a hole in the membrane. HlyA, encoded by the hlyCABD operon, is the most important factor in the UPEC. This toxin gives UPEC the ability to cause tissue damage, cross mucosal barriers, release host nutrients, and damage immune cells (7). The ONPG test detects lactose-negative and delayed lactose-positive bacteria. Prevalence of lactose-negative strains (0-5%) has been reported in studies (8). Given that the beta-hemolytic, metallic green sheen, and ONPG characteristics of uropathogenic E. coli have been reported differently, this study aimed to investigate these properties.

Materials and Methods

Sample collection and detection

In this cross-sectional descriptive epidemiological study, urine samples were collected from patients referred to Babol Shahid Beheshti Hospital, Mazandaran Province, Babol. A total of 1202 urine samples were collected for 5 months (from November 2020 to March 2020) under the supervision of the Medical Ethics Committee of the Islamic Azad University, Babol branch (Ethics Code 1345). Blood agar, McConkey agar, and Eosin methylene blue (EMB) agar media were used for isolation and identification of UPEC. If the number of colonies of a single microorganism in the urine sample was 10^5 colony-forming units per milliliter (CFU / ml) or more, a urinary tract infection would be considered positive. Patients who received antibiotics two weeks before sample collection were excluded from the investigation (9). Strains of *E. coli* were confirmed using the IMViC (Indole, Methyl Red, Voges-Proskauer, Citrate utilization), urease and triple sugar iron agar (TSIA), and other biochemical tests. All confirmed isolates were stored in trypticase soy broth with 15% glycerol at -80 ° C until further investigation (10).

Metallic green sheen

EMB (Eosin Methylene Blue) agar medium is a selective and differential culture medium used to isolate facultative anaerobic bacteria such as *E. coli* (11). To determine the green sheen, *E. coli* colonies are cultivated as streak plate on EMB agar medium, then are kept in an incubator at 37◦C for 24 hours. Lactose-fermenting Gram-negative bacteria acidify the environment and under acidic conditions the dyes produce a dark purple complex, which is usually accompanied by a metallic green sheen. metallic green sheen is an indicator of intense fermentation of lactose and/or sucrose. A smaller amount of acid production resulting from slow fermentation develops a brown-pink coloration of growth. Colonies of non-lactose fermenters appear as clear or pink (12).

Ortho-nitrophenyl-β-D-galactopyranoside test

Lactose permease and beta-galactosidase enzymes are needed to break down lactose. β-galactosidase permease exists in the cytoplasmic membrane in which transports lactose into the cell, but cytoplasmic beta-galactosidase hydrolyzes lactose into glucose and galactose. There are strains of E. coli that ferment

lactose with delay. Some bacteria lack β-galactoside permase enzyme but have β-galactosidase (13). In these strains, there is an insertion sequence between the genes of beta-galactosidase and lactose permease, which may cause a decrease in the expression of lactose permease. To identify delayed lactose fermenting bacteria, the ONPG test is used (14). ONPG is structurally similar to lactose and colorless and enters the cell more than lactose. Inside the cell, ONPG is cleaved by β-galactosidase to o-nitrophenol, which has a yellow color. To perform the test, one ONPG disc is add to a sterile test tube containing 0.1 ml of sterile 0.85% w/v sodium chloride solution (physiological saline). Emulsify the desired colony in the tube containing the disc. Incubate the tube at 37-35 degrees Celsius. Observe the tube at 1- to 6 hour intervals to detect active lactose fermenters. Incubate the tubes for 24 hours to detect late lactose fermenters. If beta-galactosidase is positive, the fluid and disc will turn yellow (15).

Hemolysis on blood agar

Certain bacteria produce extracellular hemolysin, which hemolyzes red blood cells and releases the hemoglobin contained in them. three types hemolysis create on blood agar medium. Alpha hemolysis creates a green halo around the colony. The hemolysis is produced by oxidation of oxyhemoglobin (Fe^{+2}) to non-oxygen-binding met-hemoglobin (Fe^{+3}) through hydrogen peroxide (16). Beta hemolysis, the complete destruction of red blood cells, shows a clear area around the colony. Gamma hemolysis indicates the absence of hemolysis around the colony. Blood agar culture medium is usually prepared from trypticase soy agar or Columbia agar base with 5% sheep blood. Pure colonies were inoculated using streak plate method on blood agar and then, were kept at 37°C for 24 hours.

Results

In urine culture, 147 individuals had significant urinary tract infections caused by Gram-negative bacteria. The average age of patients was 58.2 years. The prevalence of urinary tract infections caused by *E. coli* strains was 100 (68.02%). Out of 100 *Escherichia coli* isolates, 80% showed a metallic green sheen but 20% did not have a green gloss (Figure 1).

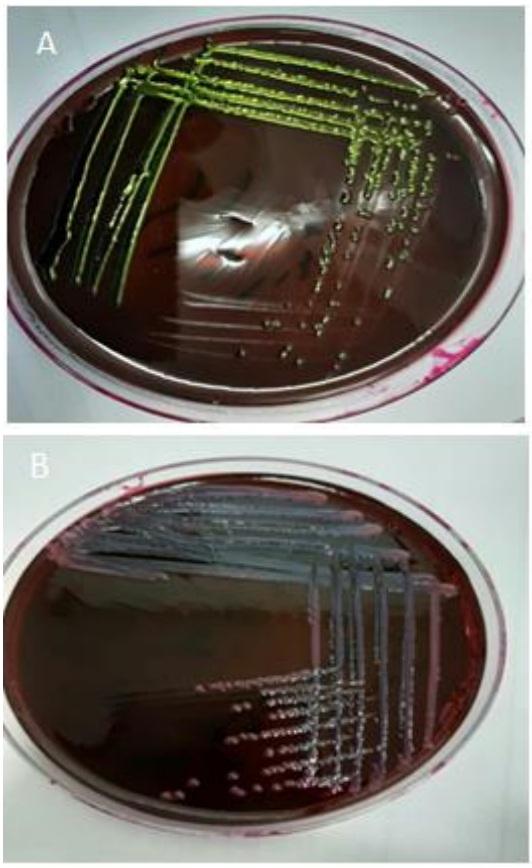
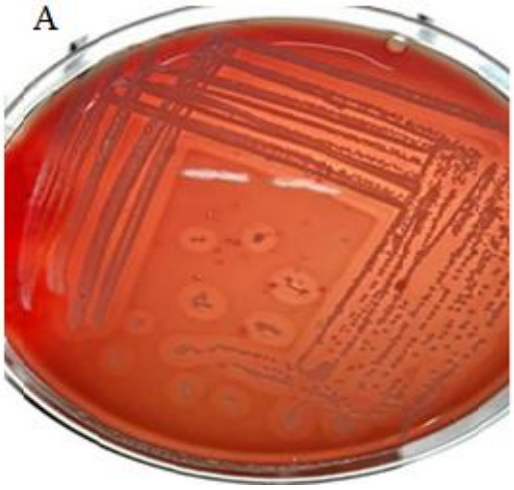


Figure 1. A. Metallic green sheen, B. No green sheen.

The characteristic of beta hemolysis from 100 tested *Escherichia coli* isolates on Blood agar medium showed that only one (1%) of them had beta hemolysis and the rest (99%) did not have hemolysis (Figure 2).



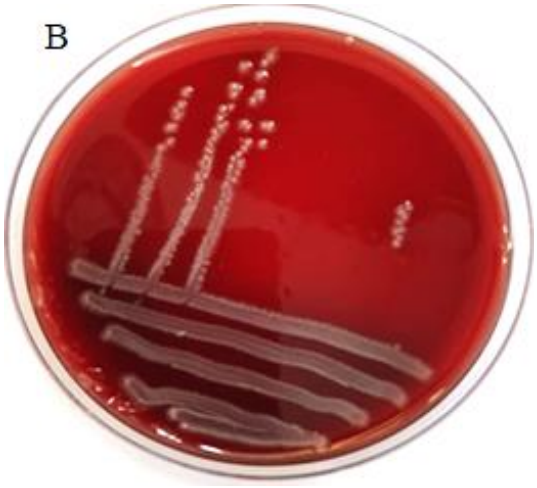


Figure 2. A. Beta hemolysis, B. Gamma hemolysis.

The ONPG test of all *E. coli* strains showed that 100% of the strains had positive reaction (Figure 3).

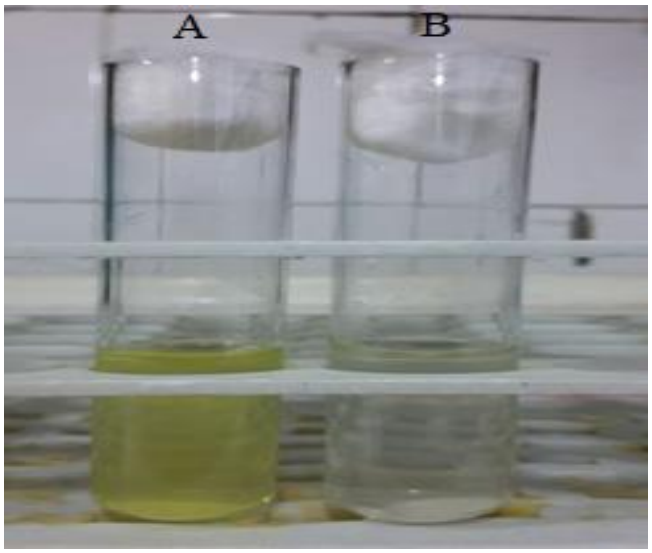


Figure 3. A. Positive ONPG, B. Negative ONPG

Discussion

Escherichia coli is the most prevalent bacterial agent that causes urinary tract infections (UTIs), mainly in women. In our research, the uropathogenic *E. coli* was responsible for 68.02% of urinary tract infections. In a study by Michael W. Dunne and colleagues, it was shown that *E. coli* caused 75.6% of UTIs (17). In another study, was determined that 60.5% of UTI was caused by *E. coli (18).* The results of these studies show that UTIs caused by E. coli have almost the same prevalence. In the current research, 80% of uropathogenic *E. coli* showed metallic green sheen in an EMB agar medium. In the study conducted by Jain et al., all strains of *E. coli* separated from the urine had a metallic green sheen (19). The presence of metallic green sheen has been reported in various studies, but the prevalence of green sheen caused by uropathogenic *Escherichia coli* has not been determined definitively. In our study, only one percent of uropathogenic *E. coli* showed beta hemolysis, but the rest did not cause hemolysis. In the study carried out by Sayan Bhattacharyya, only 10% of uropathogenic E. coli were hemolytic (20). In the study conducted by Sonal Jindal, 34% of uropathogenic E. coli produced hemolysin and 66% of the remaining isolates did not show hemolysis (21). Noha Mahmoud showed that 15 (30%) uropathogenic *E. coli* isolates were β-hemolytic while 35 isolates (70%) were non-hemolytic (22). The results of these studies show that the production of hemolysin in uropathogenic *E. coli* is different in different regions, which is a common occurrence due to mutations in the gene. Based on our results, 100% of uropathogenic *E. coli* isolates were ONPG positive. In a study by C. LONGHI and et al., 70.9% of uropathogenic *E. coli* presented ONPG positive (23). In another study conducted by Mahshid Deldar Abad Paskeh , all 91 isolates of uropathogenic *E. coli* were ONPG positive (24). The results of these investigations are almost consistent with the present study.

Conclusions

In conclusion, all uropathogenic *E. coli* don't produce metallic green sheen, so other biochemical features must be considered. A small percentage of uropathogenic *E. coli* cause beta hemolysis, so this test can be used to identify *E. coli* in the laboratory. Almost all uropathogenic *E. coli* are ONPG positive, so this test is not mandatory.

Author contribution

MA designed research, analyzed the data, wrote the manuscript, and performed the interpretation of the results; **RM** wrote the manuscript and collected the specimens; **HKGh** performed the practical experiments and collected the samples.; All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest in this manuscript.

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