LABORATORY DIAGNOSIS OF URINARY TRACT INFECTIONS CAUSED BY *E. COLI*

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RESEARCH ARTICLE –

Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections, with Escherichia coli being the primary etiological agent. Accurate and rapid diagnosis is essential for initiating appropriate treatment and preventing complications. This paper presents laboratory diagnostic methods used to identify E. coli in urinary infections, including microscopic examination, urine culture, biochemical tests, and molecular methods. UTIs are common conditions in medical practice, affecting individuals of all ages. Escherichia coli is responsible for over 80% of community-acquired UTI cases and a significant proportion of nosocomial infections. Laboratory diagnosis is essential for confirming the presence of the bacterium, assessing antibiotic susceptibility, and guiding treatment.

Keywords: bacterial infections, urine culture, biochemical tests

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INTRODUCTION

Escherichia coli is a bacterium that is part of the normal flora of the human and animal intestine. Most *E. coli* strains are harmless and contribute to the digestion process, but some strains can be pathogenic and cause severe infections, including urinary tract infections (UTIs), diarrhea, hemolytic uremic syndrome, and even sepsis.

E. coli infections are often caused by the ingestion of contaminated food or water, but in the case of urinary tract infections, bacteria often reach the bladder from the urethra, where they can cause inflammation and symptoms such as pain during urination, cloudy or bloody urine, and frequent urination. UTIs are more common in women due to their anatomy, but men can also be affected, especially in cases of urinary retention or prostate problems. Urine culture is a test that detects urinary tract infections (UTIs), which are inflammatory conditions caused by various microorganisms that reach the urinary system, multiply there, and cause changes in the normal functioning of the urinary tract and kidneys.

Depending on their location, UTIs can be classified as lower infections, when the urethra and bladder are affected, and upper infections, when the ureters and kidneys are also involved. From a symptomatological perspective, UTIs can be asymptomatic, when bacteriuria is present without clinical manifestations, or symptomatic, when bacteriuria is accompanied by an inflammatory response (pyuria) and clinical symptoms.

Normally, the urinary tract is sterile, except for the distal urethra, which is colonized by bacteria from the perineal skin and, in women, from the vulvar area. These bacteria can accidentally contaminate bladder urine if the urine sample is collected incorrectly.

The incidence ratio of UTIs between sexes varies with age: during the first year of life, urinary infections are more common in boys due to phimosis and paraphimosis specific to this age. The ratio tends to balance out during early childhood, after which UTIs become predominantly more frequent in females, with an increased incidence as age advances, being associated with sexual activity and pregnancy. In the elderly, the sex difference disappears, and UTI prevalence increases in men due to prostate conditions.

UTIs are favored by structural and neurological abnormalities that interfere with urinary flow, as well as systemic conditions such as diabetes or neoplasms. In elderly, immunosuppressed, mobility-impaired patients, or children, UTIs may progress without obvious symptoms or with nonspecific symptoms (fever, digestive disturbances, weight loss).

Starting in 1956, E. H. Kass established that the significant threshold for UTIs is **10^5 colony-forming units (CFU)** per milliliter of urine for Gram-negative bacilli, **5×10^4 CFU/ml** for the *Staphylococcus* genus, and **10^4 CFU/ml** for *Candida albicans*.

MATERIAL AND METHODS

For the accomplishing of the objectives proposed was used the retrospective study. The material basis of the study included the observation sheets of the patients, submitted at the archive of the hospitals, the computerized data of the two units, respectively.

The data obtained were interpreted statistically based on the determination and calculation of some series of indices.

Necessary materials:

1. **Sterile collection container**: A special sterile container designed for urine collection for urine culture is typically used. This is available in pharmacies or at hospitals/laboratories and is designed to prevent sample contamination.
2. **Protective mask, sterile gloves**: To prevent sample contamination and to protect the staff performing the collection, it is important to wear gloves and other appropriate protective equipment.
3. **Devices for urine collection**:
    - **Collection systems for children (if necessary)**: For young patients, sterile collection bags can be used, which are applied to the genital area to collect the urine.
    - **Local washing**: It may be necessary to perform prior hygiene of the perineal area to reduce the risk of contamination with bacteria from the skin.
4. **Transport**: The urine sample should be transported as quickly as possible to the laboratory, preferably within one hour of collection, in a sealed sterile container to prevent contamination.

Urine collection method for urine culture:

1. **Patient preparation:**
    - Before collection, patients must follow a set of instructions to ensure correct and hygienic collection.
    - The patient should thoroughly wash their hands and genital area to prevent contamination with surrounding bacteria.
    - Women should clean the genital area with water and soap or antiseptic solutions, while men should retract the foreskin (if applicable) and clean the genital area.
2. **Urine collection:**
    - The urine should be collected from the midstream (the first part of the urine is discarded, and only the midstream is collected) to prevent contamination with bacteria from the urethra or skin.
    - It is important that the patient does not urinate before starting the collection to ensure an accurate sample.
    - The urine should be collected in a sterile container to avoid any external contamination.
3. **Sample transport:**
    - The urine sample should be transported to the laboratory as quickly as possible (within 1-2 hours of collection) to prevent bacterial multiplication or changes in the composition of the urine.
    - If immediate transport is not possible, the urine can be temporarily refrigerated, but it should not be stored for more than 24 hours.

RESULTS AND DISCUSSIONS

Urinary tract infections (UTIs) represent a major health problem, being among the most common bacterial infections. Accurate microbiological diagnosis is essential for initiating appropriate treatment, and isolation and identification of pathogens are performed using selective and differential culture media. Among these, Levine (EMB), MacConkey, and CLED media are frequently used due to their ability to highlight specific characteristics of bacteria involved in UTIs.

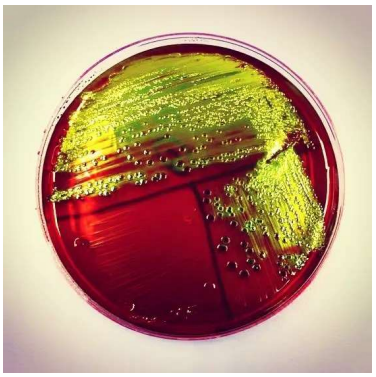

Fig.1. *Escherichia coli.* Levine. MacConkey Colonies in Eosin Methylene Blue Agar

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Fig.2. *Escherichia coli.* Agar. Pink colonies (lactose fermentation)

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Fig.3. E. coli. CLED (Cystine-Lactose-Electrolyte-Deficient) Yellow colonies (lactose fermentation)

https://agarindo-biological.com/

Tab.1. Comparison of positive colonies in urinary infections on different culture media

Bacterie / Mediu	Levine (EMB - Eosin Methylene Blue)	Agar MacConkey	CLED (Cystine-Lactose-Electrolyte-Deficient)
E. coli	Colonies with a green metallic sheen	Pink colonies (lactose fermentation)	Yellow colonies (lactose fermentation)
Klebsiella spp.	Mucoid, dark colonies	Pink, mucoid colonies	Yellow, mucoid colonies
Proteus spp.	Colorless or pale colonies	Colorless colonies (do not ferment lactose)	Blue/green colonies (do not ferment lactose)
Enterococcus spp.	Reduced growth, small colonies	Small, light pink colonies	Yellow colonies
Staphylococcus saprophyticus	Small, colorless colonies	Pale pink or colorless colonies	Yellow or cream colonies

In the study we conducted, we used the EMB (Levine) medium because it is a differential and selective medium primarily intended for the identification of Gram-negative bacilli from the Enterobacteriaceae family. Its components, eosin and methylene blue, inhibit the growth of Gram-positive bacteria, thereby promoting the isolation of Gram-negative bacteria.

Among the pathogens of UTIs (urinary tract infections), *Escherichia coli* stands out the most on this medium, forming colonies with a green metallic sheen, characteristic of intense lactose fermentation and acid production. This appearance allows for quick identification and differentiation from other enterobacteria. *Klebsiella spp.* showed mucoid and dark-colored pigmented colonies, while lactose non-fermenting bacteria, such as *Proteus spp.*, appeared as colorless or pale colonies.

Regarding MacConkey agar, it is another differential and selective medium used for isolating and identifying Gram-negative bacteria, especially from the Enterobacteriaceae family. It is based on lactose fermentation, differentiating bacteria into two main categories: lactose fermenters (*E. coli*, *Klebsiella spp.*), which form pink colonies due to acid production that causes a color change in the pH indicator (neutral red), and non-lactose fermenters (*Proteus spp.*, *Pseudomonas spp.*), which appeared as colorless colonies.

The MacConkey medium is particularly useful in the diagnosis of urinary tract infections (UTIs) because it allows for the rapid differentiation of etiological agents and highlights possible infections with opportunistic bacteria.

Additionally, the CLED medium is preferred in UTI diagnosis due to the absence of electrolytes that inhibit the swarming characteristic of Proteus species. This is crucial because Proteus spp. can cover the entire surface of other media, preventing the development of other bacteria and complicating the diagnosis.

Depending on the ability to ferment lactose, bacteria are differentiated into lactose fermenters (E. coli, Klebsiella spp.) forming yellow colonies and non-lactose fermenters (Proteus spp., Pseudomonas spp.), which appear as blue/green colonies.

The CLED medium is, therefore, a preferred medium for UTI (urinary tract infection) diagnosis, providing good differentiation of pathogenic bacteria and preventing the overgrowth of Proteus spp. Although culture media provide essential information regarding the characteristics of bacterial colonies, correct interpretation must be made in correlation with clinical data and complementary investigations, such as urine analysis and antibiogram.

Thus, the presence of E. coli with characteristic colonies on EMB and MacConkey, and yellow colonies on CLED, suggests a typical UTI. In contrast, the identification of Proteus spp. or Pseudomonas spp. on CLED (blue/green colonies) and MacConkey (colorless colonies) may suggest a complicated infection, requiring targeted antibiotic treatment.

The study titled "Escherichia coli O157: H7 Infection in Humans" reveals that infection with E. coli O157:H7 manifests through a wide range of clinical presentations, including asymptomatic carriers, diarrhea without blood, hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. Not only is E. coli O157:H7 an important agent of hemorrhagic colitis, but it is also one of the main causes of bacterial diarrhea. Patients at the extremes of age are at higher risk of infection and associated complications. E. coli O157:H7 is primarily transmitted through the foodborne route. Undercooked meat is the most common source of infection, but secondary person-to-person transmission is also significant. This bacterium produces at least two Shiga-like toxins, which differ antigenically, physicochemically, immunologically, and in their biological effects. These toxins are believed to play a direct role in the pathogenesis of E. coli O157:H7 infection. The diagnosis of infection is made through positive culture from stool samples, identification of Shiga toxins, or both methods. Timely stool collection (within 7 days of disease onset) is essential for a high rate of bacterial detection. Treatment is mainly supportive and focuses on managing complications if necessary. Antibiotic therapy has not been proven to be beneficial. Important public health measures include educating the population about the dangers of consuming undercooked meat, raising awareness among physicians about E. coli O157:H7 infection, and mandating the reporting of cases.

CONCLUSIONS

The use of selective and differential culture media such as EMB, MacConkey, and CLED is essential for the rapid and accurate diagnosis of urinary infections. Each medium offers specific advantages.

The EMB medium is excellent for highlighting E. coli due to its characteristic greenish metallic sheen.

As for the MacConkey medium, it allows for the rapid differentiation of lactose fermenters and non-fermenters.

Additionally, the CLED medium is optimal for identifying a broad spectrum of urinary pathogens while preventing the spread of Proteus spp.

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