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PRODUCTION OF CHROMOGENIC CULTURE MEDIA FOR URINARY TRACT INFECTION

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ABSTRACT:

Background: Urinary tract infections (UTIs) occur through the invasion of tissues, including the urethra, bladder, ureters, and kidneys. Pathogens such as Escherichia coli, Klebsiella pneumoniae, and Enterococcus faecalis are common causes of these infections.

Objective: To develop a three-plate selective chromogenic culture medium for the rapid identification of Escherichia coli, Klebsiella pneumoniae, and Enterococcus faecalis in urinary tract infections.

Methods: A basic solution was prepared and supplemented with four types of sugar and three types of pH indicators to create the chromogenic culture medium. The medium was tested for its ability to induce colour changes specific to the bacteria under study.

Results

Klebsiella pneumoniae: Induced a colour change in the inoculated medium.

Enterococcus faecalis: Induced a colour change in the inoculated medium.

Escherichia coli: Formed colourless colonies in the inoculated medium.

Conclusion: The developed three-plate selective chromogenic culture medium successfully differentiated Klebsiella pneumoniae and Enterococcus faecalis based on colour changes, and Escherichia coli based on the formation of colourless colonies. Further tests with additional indicators are recommended to refine the medium for enhanced bacterial identification.

KEYWORDS: Urinary Tract Infection; Fermentation; Bacteria; Chromogenic Culture Medium.

INTRODUCTION:

The urinary tract is the second most common site of infection in intensive care patients. This bacterial infection can have mild, moderate, or severe degrees depending on the degree and

time of exposure of the patient. It is necessary to identify them by isolating them, present in the urinary tract of patients, to contribute to treatment and direction together with the correct therapeutic antibiotics. Urinary tract infections (UTIs) affect areas such as the urethra, bladder, ureters, and kidneys. Gram-negative pathogens, such as Escherichia Coli and Klebsiella pneumonia, and Gram-positive pathogens, such as Enterococcus faecalis, can cause urinary tract infections (Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024).

E. coli is common in urinary infections, originating from the gastrointestinal tract when there is a possibility that K. pneumoniae, also from the gastrointestinal tract, causes infections in patients with weakened immune systems. Even E. faecalis, which normally resides in the gastrointestinal tract, can cause urinary tract infections when it migrates into the patient's body. In the laboratory routine, manual methods involving substrates, biochemical tests, pH indicators, and sugars such as lactose, urea, glucose, maltose, and others are used, which are incorporated into an agar for a final culture medium (Iseri, Nilsson, van Belkum, van der Wijngaart, & Özenci, 2024; Khutade, Shah, Patil, & Patel).

Bacteria can be identified through morphological characteristics (size, shape, arrangement, and structure) and molecular, cellular, metabolic, serological, physiological, taxonomic, and pathogenic actions in the organism. The ability to metabolize specific sugars, using a pH indicator in the composition of the culture medium, aids in the colour that each bacterium produces when it comes into contact with fermentation substrates, is analyzed under a microscope, and can generate identification. The Bactray system is a rapid identification technique composed of three series of biochemical tests (Bactray I, II, and III) for a total of 30 substrates (Liu et al., 2024; Varney, Mannix-Fisher, Thomas, & McLean, 2024).

This system is specific for identifying oxidase-negative Gram-negative bacilli, which ferment or do not ferment glucose, and oxidase-positive Gram-negative bacilli, which do not ferment glucose. Bactray I and II are used to identify oxidase negative, while Bactray III is used for oxidase positive. Each set includes a 10-compartment disposable polystyrene holder for performing biochemical tests. Another rapid technique is the chromogenic medium, which, through specific colours released by the colonies through substrates metabolized by enzymes produced by microorganisms, allows the presumptive identification of the appropriate therapeutic species for antimicrobial-affected patients (Alonso-Tarrés et al., 2024; Asmare et al., 2024; Behere & Haldar, 2024).

Table 1: Common Bacteria Causing Urinary Tract Infections

Pathogen	Classification	Common Site of Infection	Reference
Escherichia coli	Gram-negative	Urethra, bladder, ureters, kidneys	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024
Klebsiella pneumoniae	Gram-negative	Urethra, bladder, ureters, kidneys	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024
Enterococcus faecalis	Gram-positive	Urethra, bladder, ureters, kidneys	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024

Table 2: Sources and Characteristics of Common UTI Pathogens

Pathogen	Source	Characteristics	Reference
Escherichia coli	Gastrointestinal tract	Common in urinary infections	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024
Klebsiella pneumoniae	Gastrointestinal tract	Infects patients with weakened immune systems	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024
Enterococcus faecalis	Gastrointestinal tract	Migrates into the urinary tract to cause infections	Benjumea, Navarro, & Alonso-Tarrés, 2024; Koca, Gur, & Cekin, 2024

Table 3: Identification Techniques for UTI Pathogens

Technique	Description	Reference
Manual Methods	Involves substrates, biochemical tests, pH	Iseri, Nilsson, van Belkum, van der Wijngaart, & Özenci, 2024; Khutade,

	indicators, and sugars	Shah, Patil, & Patel
Morphological Characteristics	Size, shape, arrangement, structure	Liu et al., 2024; Varney, Mannix-Fisher, Thomas, & McLean, 2024
Molecular and Metabolic Actions	Specific sugars, pH indicators, fermentation substrates	Liu et al., 2024; Varney, Mannix-Fisher, Thomas, & McLean, 2024
Battery System	Three series of biochemical tests for Gram-negative bacilli	Liu et al., 2024; Varney, Mannix-Fisher, Thomas, & McLean, 2024
Chromogenic Medium	Colour changes for rapid bacterial identification	Alonso-Tarrés et al., 2024; Asmare et al., 2024; Behere & Haldar, 2024

Table 4: Rapid Identification Techniques

Technique	Description	Reference
Battery System	Identifies oxidase-negative and oxidase-positive Gram-negative bacilli	Liu et al., 2024; Varney, Mannix-Fisher, Thomas, & McLean, 2024
Chromogenic Medium	Allows presumptive identification through colour changes of colonies	Alonso-Tarrés et al., 2024; Asmare et al., 2024; Behere & Haldar, 2024

Table 5: Research Findings on Chromogenic Culture Medium

Research Finding	Description	Reference
Development	Created in three Petri dishes for Gram-positive and Gram-negative bacteria	Garcia, Martins, Porto, Nobrega, & Dos Santos, 2024; Nwankwo, Ezebialu, Ezeadila, & Ikechukwu, 2024
Diagnostic Simplification	Identifies bacteria via colour change, eliminating the need for biochemical testing	Garcia, Martins, Porto, Nobrega, & Dos Santos, 2024; Nwankwo, Ezebialu, Ezeadila, & Ikechukwu, 2024

In the research, a chromogenic culture medium was created in three Petri dishes, intended for the growth of specific Gram-positive and Gram-negative bacteria that mostly cause urinary infections through different colours compared to traditional media. Simplify diagnosis and treatment by identifying bacteria via colour change, eliminating the need for biochemical testing, and making the process more efficient and specific (Garcia, Martins, Porto, Nobrega, & Dos Santos, 2024; Nwankwo, Ezebialu, Ezeadila, & Ikechukwu, 2024).

MATERIALS AND METHODS:

INOCULUM PREPARATION:

Strains 1, 3, and 4 of the freeze-dried bacteria were inoculated into BHI and brought into the greenhouse for 24 hours at 35+/-2°C until the point of growth and development. On MacConkey Agar and Cled Agar media, growth bacteria were grown using a 10 ul loop to increase the bacterial colonies used in the research. Where were these lands incubated in the oven for another 24 hours at 35+/-2°C? These bacteria were subjected to the Gram staining methodology, and the microscopic analysis confirmed that the specific genera of the strains used in the research are indeed those pre-established as Gram-negative and Gram-positive (Cherkaoui, Renzi, & Schrenzel, 2024; Yang et al., 2024).

With the confirmation of the bacterial strains, injection into the media in triple plates was started to carry out the research. Nutrient Agar was used as a base, together with pH indicators and sugars: lactose, Ueria, maltose, and glucose, which make up the chromogenic medium (Leroux, Montvernay, Davenas, LeBihan, & Fulchiron, 2024).

PREPARATION OF THE CHROMOGENIC GROWING MEDIA:

200 ml of deionized water and 5.6 g of Nutrient Agar powder were used as a base solution. The three pH indicators (Phenol Red Broth Base = 3.2 g, Violet Crystal = 0.010 g, and Thymol Blue = 0.010 g). Then he added the sugars: 2.6 g of lactose, 5 ml of 40% urea, 5 g of maltose, and 5 g of glucose, and distributed the contents into three Petri dishes. Two plates for the Negative Control and another for the Positive Control were separated and incubated in an insulated and closed greenhouse for 24 hours, which favours an ideal temperature for the growth of the desired bacterial colonies. Gram-negative bacteria, such as K. pneumoniae and E. coli, and the gram-positive bacterium E. faecalis were seeded into three different types of medium that make up the triple plate (Table 1) (El-Shabrawy et al., 2024; Jihad & Salih, 2024; Na et al., 2024).

	SUGARS	pH INDICATOR	BACTERIA
Medium 1	Urea 40% + Lactose	Phenol Red Broth Base	E. coli
Medium 2	Lactose	Crystal Violet	Klebsiella pneumonia
Medium 3	Maltose + Glucose	Thymol Blue	Enterococcus Faecalis

Table 1: Seeding of gram-negative and gram-positive bacteria used during research in three different types of media composed of sugars and a pH indicator that constitute the triple plate.

Incubate in an oven at 35+/-2°C for a minimum of 24 hours to observe the growth of the bacterial colonies, to simultaneously carry out identification by colour change without the aid of biochemical tests (Damola, Adeoshun, Bakarey, & Udeh, 2024).

RESULTS AND DISCUSSION:

MEIOCOM LACTOSE EUREIA:

The E. coli bacterium, through the metabolism of enzymes, ferments the sugars Lactose and Urea 40%, which make up the sown tri-plate medium, reacts with the pH indicator PhenolRedBrothBase, developing colourless colonies with a dry appearance after 24 hours (Image 1) (Kritikos, Prod'hom, Jacot, Croxatto, & Greub, 2024).

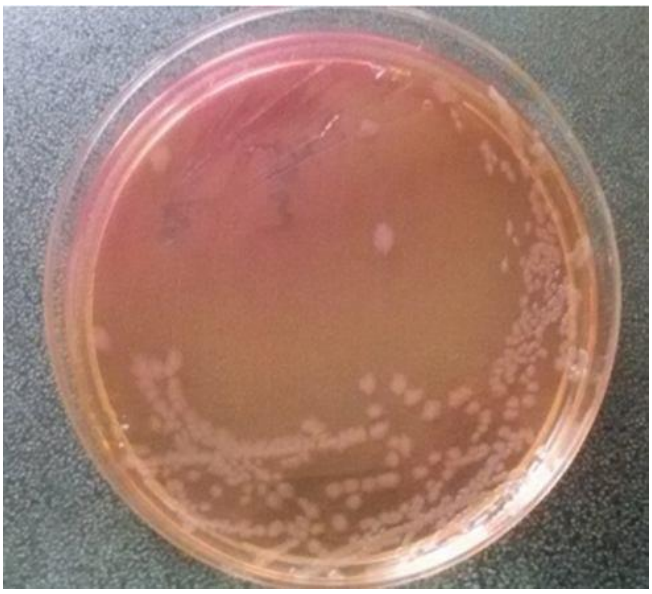

Image 1: Triple plate with first medium for E. cholina with fermentation of lactose and 40% urea, which becomes colourless after 24 hours.

MEIOCOM LACTOSE:

The K. pneumoniae bacterium, through the metabolism of enzymes, ferments the lactose which constitutes the seeded tri-plate medium, reacts with the Crystal Violet pH indicator, developing violet/purple coloured colonies with a mucoid appearance, after 24 hours (Image 2) (Tilahun et al., 2024).

Image 2: Triple plaque with K. pneumonia in the second half, after 24 hours. Verification of lactose fermentation and purple/purple colour.

MEIOCOMMALTOSE GLUCOSE:

The bacterium E. faecalis, through enzymatic metabolism, ferments the sugars Maltose and Glucose, which make up the three-plate medium, which was sown, reacting as a pH Indicator. Growing colonies of Thymol Blue with orange colour after 24 hours (Image 3) (Molina et al., 2024).

Image 3: Tri-plate like E. faecalis in the middle third, after 24 hours, Colour control turns orange.

During testing, K. pneumoniae was found to exhibit two types of staining in two different media. In the medium composed of Nutrient Agar, lactose sugar, and Violet Crystal pH indicator, the bacterial colonies had a purple colour, as shown above, and were established for use in the triple plate. In the medium composed of the same base, sugars such as Lactose and Urea at 40%, and the Phenol Red Broth Base indicator in a Petri dish, K. pneumoniae colonies showed a pink colour after 24 hours (Image 4) (Alkan et al., 2024; Gonçalves et al., 2024).

Image 4: Plate with K. pneumonia in the first medium type with lactose fermentation and 40% urea. Transformation of the body into mucoid after 24 hours.

Being an identification method by colour change through chromogenic culture media, it makes the generation of culture media very similar compared to the study carried out in the Cendilab laboratory, located in Curitiba. Using the CPSID3 chromogenic medium to identify bacteria only through the colour of the medium, the results obtained in the research are innovative; thanks to the colours obtained, it is possible to analyze and carry out bacterial identification (Gulia, Shrivastava, & Sharma; Sachu, Sunny, Mathew, Kumar, & David, 2024).

Chromogenic Agarcoliform agar-CCA is another ready-to-use agar medium composition used for the bacterial identification and differentiation of E. coli and coliform bacteria through different substrates incorporating the medium, causing the specific enzymes of the bacteria to form coloured complexes. We can analyze the colour change reactions in the chromogenic medium, and it is very easy to identify the microorganism. This study aims to facilitate the commercialization of a chromogenic culture medium in hospital and outpatient laboratories (Gomez et al., 2024; Laanait, Elmoussaoui, Lahmini, & Bourrous, 2024).

The results obtained are associated with the specific staining of the bacteria identified with the function of this medium, validating the usefulness of the chromogenic medium for bacterial identification. Based on the results obtained from the indicators and sugars in a specific medium, compared to conventional methods of bacteria identification, the effectiveness of this new identification method can be confirmed within 24 hours. The goal is to speed up the diagnosis of patients with urinary infections and reduce the time needed to identify the bacteria.

However, it is important to highlight that the research was unable to test new pH indicators, which could be explored in future studies (Aththanayake, Samarasinghe, Priyadarshana, Weerakkody, & Weerasinghe, 2024; Qader, 2024; Saleh, Dheyab, Hadi, Hasan, & Jasim, 2024).

CONCLUSIONS:

The identification of bacterial pathogens and the evaluation of their susceptibility provide important information for the rational use of antimicrobials and the reduction of the mortality rate. Taking into account the results obtained, it was possible to demonstrate the objectivity of the method in chromogenic culture media for the growth of Gram-negative and Gram-positive bacteria in 24 hours, considering that fermentation of sugars occurred in a triple plate, allowing identification of bacteria by staining.

As this is a new form of identification carried out using a methodology currently used in some laboratories, chromogenic culture media with new indicators and different sugars composing a new triple plate base can still be very practical because it follows the core aspect turn analysis method, which facilitates and simplifies simultaneous analysis in a triple plate, providing faster and more accurate results in the detection of pathogens and indicators, with high selectivity, specificity, and efficiency, facilitating and simplifying routines, thanks to principles based on chromogenic substrates specific for each target microorganism. However, it is worth mentioning that the development and commercialization of this chromogenic culture medium involves high costs.

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