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UTILITY OF CHROMOGENIC AGAR FOR ISOLATION AND PRESUMPTIVE IDENTIFICATION OF UROPATHOGENS

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Abstract:

Urinary tract infections (UTIs) are highly prevalent and pose a significant health burden across all age groups. Accurate and timely diagnosis of UTIs is crucial for effective management and prevention of complications. This case study aims to evaluate the utility of chromogenic agar as a primary culture tool for the isolation and presumptive identification of uropathogens in UTI diagnosis. Conventional diagnostic protocols for UTIs often rely on time-consuming cultural characteristics and biochemical tests, leading to limitations in sensitivity and specificity. Chromogenic media, pioneered by Dr. Alain Rambach, have emerged as a promising alternative by incorporating specific chromogenic substrates that produce distinguishable colors in the presence of bacterial enzymes. This facilitates the identification of uropathogens without the need for further biochemical testing. By examining the existing evidence and insights from clinical practice, this case study aims to provide a comprehensive understanding of the utility of chromogenic agar in clinical microbiology laboratories for UTI diagnosis. The findings will contribute to enhancing diagnostic strategies and optimizing treatment decisions for patients with UTIs, potentially leading to improved patient outcomes and reduced healthcare costs.

Keywords: Urinary tract infections, Chromogenic agar, Uropathogens, Diagnosis, Conventional media

INTRODUCTION

Urinary tract infection is one of the most prevalent diseases affecting people from all age groups including pediatric and geriatric patients. Around 10% of postmenopausal women report having had a UTI in the previous year, and between 50% and 60% of adult women will experience at least one UTI in their lifetime (Ignacio Alós, 2005; Tan & Chlebicki, 2016). The current classification of urinary tract infections (UTIs) by the European Association of Urology and the European Section of Infection in Urology is a useful working instrument for daily patient assessment and clinical research. This new classification is based on clinical presentation, risk factors, and a severity scale. Symptomatic UTIs are categorized as cystitis, pyelonephritis, and urosepsis, with the urosepsis syndrome being the most severe form and pyelonephritis being more severe than cystitis (Smelov et al., 2016). Urinary tract infection (UTI) is one of the most common infections in clinical practice worldwide in both healthcare and community settings causing significant morbidity and mortality. It is one of the major conditions at the community level treated empirically and regarded as a potential cause of the emergence of antimicrobial resistance (AMR). The majority of cases of uncomplicated UTI are caused by uropathogenic *E. coli* (UPEC) and *Klebsiella spp*. and are acquired in the community, accounting for roughly 75–95% of all cases (Mohapatra et al., 2022a). The most frequently observed uropathogens were as follows: *E. coli*, followed by *K. pneumoniae*, *Proteus spp*, *Acinetobacter spp*, *Enterococcus spp*, and other pathogens such as *K. oxytoca, Enterobacter cloacae, Pseudomonas aeruginosa, Serratia marcescens, Providencia rettgeri, Citrobacter fruendii, and Citrobacter koseri*, which collectively made up 4% of cases (Behzadi et al., 2010)(Mohapatra et al., 2022b).

A quantitative Urine culture on standard agar media is required for the etiological diagnosis of UTI, as only 20 to 30% of urine samples yield significant growth with predominant causative agents. The media used for urine culture should be capable of supporting the growth of all urinary pathogens while inhibiting potential contaminants. Traditionally, most laboratories, particularly those in developing countries, have utilized a combination of conventional media such as Blood agar (BA) and MacConkey agar (MAC) for an extended period. Cystine lactose electrolyte-deficient (CLED) agar was later introduced, but none of these media, either individually or in combination, can adequately facilitate the growth and identification of potential uropathogens (Fung et al., 1982; Salvatore et al., 2011).

Conventional diagnostic protocols for urine sample processing require significant modifications as they solely rely on cultural characteristics and time-consuming analysis, which leads to limitations in terms of sensitivity and specificity.

Building upon the initial breakthrough by Dr. Alain Rambach, chromogenic media emerged as a highly influential microbiological method for identifying a diverse range of imperceptible microorganisms (American Society for Microbiology, 2021). The utilization of chromogenic media has been rapidly growing, positioning it as an increasingly versatile primary culture tool that offers enhanced capabilities in isolating, presumptively identifying, and differentiating bacterial species from clinical specimens. These media incorporate chromogenic substrates that, when acted upon by specific bacterial enzymes, produce unique and distinguishable colors within the growing bacterial colonies, facilitating their identification (Fallon, 2003). This innovative approach not only supports the growth of all uropathogens but also enables easier diagnosis of mixed infections, streamlining the diagnostic process. By harnessing the abilities of chromogenic media, it is possible to obtain more accurate and efficient results in clinical microbiology laboratories.

In the chromogenic media, *E. coli*, the most prevalent uropathogen, manifests as pink-red colonies due to its production of β-galactosidase. This distinctive coloration allows for the definitive identification of *E. coli* without the need for further biochemical tests. On the other hand, strains that produce β-glucosidase, such as *Enterococci* and the *Klebsiella-Enterobacter-Serratia* group, form blue colonies as a result of the hydrolysis of a glucoside, which serves as a chromogenic substrate incorporated in the medium. Furthermore, the medium also contains tryptophan to detect members of the *Proteus* group. These bacteria generate a diffuse brown coloration due to the production of tryptophan deaminase (Chang et al., 2008).

In contrast, *Pseudomonas spp*. produce colorless colonies, while *Staph.saprophyticus* forms white colonies on the chromogenic media (Gaillot et al., 2000a). This streamlined approach eliminates the need for additional biochemical tests and if needed can be done from the chrome agar plates which enables more efficient and accurate identification in clinical microbiology laboratories.

This case study aims to explore the utility of chromogenic agar for the isolation and presumptive identification of uropathogens in the context of diagnosing UTIs. By utilizing this innovative approach, Laboratory professionals can streamline the diagnostic process, improve the accuracy of identification, and enhance the efficiency of clinical microbiology laboratories. The potential benefits of chromogenic agar in isolating and presumptively identifying uropathogens hold significant promise for optimizing UTI diagnosis and subsequent treatment decisions.

OBJECTIVES

The objective of this case study was to evaluate the utility of chromogenic agar for the isolation and presumptive identification of uropathogens in the diagnosis of urinary tract infections (UTIs). By assessing the effectiveness of chromogenic media in comparison to traditional diagnostic protocols, this study aims to determine the advantages of using chromogenic agar for improving the accuracy, efficiency, and specificity of UTI diagnosis. The objective is to provide evidence-based insights into the potential benefits of incorporating chromogenic agar as a primary culture tool in clinical microbiology laboratories, ultimately optimizing the management of UTIs and informing treatment decisions.

METHODOLOGY

Selection of ATCC Strains:

A panel of well-characterized reference strains from the American Type Culture Collection (ATCC) was selected. These strains represent commonly encountered uropathogens, including uropathogenic *E. coli, Klebsiella spp*., and other relevant species.

The selection of strains was based on their clinical relevance and prevalence in urinary tract infections.

(*E. coli ATCC 25922, K. pneumoniae ATCC 13883, P. aeruginosa ATCC 27853, P. mirabilis ATCC 4630*

S. aureus ATCC 25923, E. faecalis ATCC 19433)

Preparation of Chromogenic Agar and Conventional Media:

HicromeUTI agar

Choice of media and Preparation of HicromeUTI agar was prepared with the readymade dehydrated powder from HiMedia laboratories. Culture plates were prepared in the lab by following the manufacturer's instructions and recommendations.

Suspend 56.8 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates with final pH (25°C) of 6.8±0.2. Conventional media, including Blood agar (BA), MacConkey agar (MAC), Chocolate agar (CA), and Cystine–lactose–electrolyte-deficient (CLED) agar were prepared following standard laboratory protocols (Hicrome, n.d.).

Standardization of Inoculum:

Each ATCC strain was subcultured to obtain pure cultures. The subcultured isolates were standardized to a 0.5 McFarland turbidity standard to ensure consistent inoculum density.

Comparative Culturing:

For each ATCC strain, a streak plate technique was employed to inoculate both chromogenic agar and conventional media simultaneously.

Incubation and Colony Observation:

The inoculated plates were incubated at an appropriate temperature, typically 37°C, for 18-24 hours. Following incubation, the plates were examined for colony growth and coloration. Colonies on chromogenic agar were assessed for the characteristic colors associated with specific uropathogens, such as pink-red for *E. coli,* blue for *Enterococci* and *Klebsiella-Enterobacter-Serratia* group, brown for the *Proteus* group, colorless for *Pseudomonas* spp., and white for *Staph. saprophyticus*.

Colonies on conventional media were evaluated based on their growth characteristics, colony morphology, staining, and specific biochemical tests (IMVIC – Indole, Methyl red, Voges proskouer, Citrate)

OBSERVATIONS

Cultural characteristics of ATCC strains cultured on HicromeUTI agar is given in Table 1.0

Organism	Cultural characteristics observed
E coli ATCC 25922	Small purple pink
K. pneumoniae ATCC 13883	Large metallic blue mucoid colony
P. aeruginosa ATCC 27853	Transparent yellow to green colony with serrated edge
P. mirabilis ATCC 4630	Diffusible pale brown
S. aureus ATCC 25923	Small colorless to a golden yellow colony
E. faecalis ATCC 19433	Pinpoint blue or turquoise (blue to green)

Table 1.0

The expected cultural characteristics of the ATCC strains on HiCrome UTI agar according to the information provided by the manufacturer are shown in Table 1.1.

Organism	Color of the colony (According to manufacturer)
Escherichia coli ATCC 25922	Pink-purple
Klebsiella pneumoniae ATCC 13883	Blue to purple, mucoid
Pseudomonas aeruginosa ATCC 27853	Colourless (greenish pigment may be observed)
Proteus mirabilis ATCC 12453	Light brown
Staphylococcus aureus subsp. aureus ATCC 25923	Golden yellow
Enterococcus faecalis ATCC 29212	Blue, small

Table 1.1

Hi chrome UTI agar

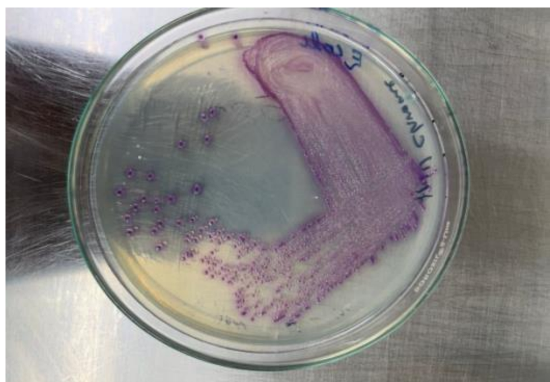


Figure 1.0 *E coli* ATCC 25922

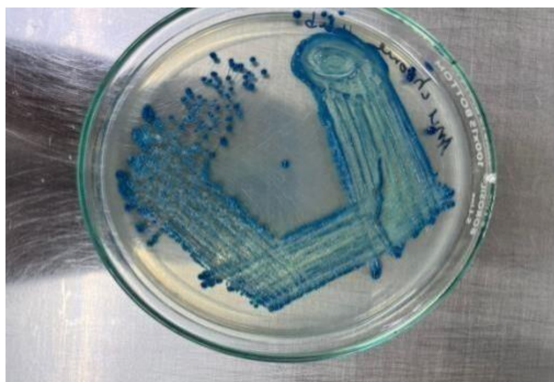


Figure 1.1 *K. pneumoniae* ATCC 13883

Figure 1.2 *E. faecalis* ATCC 19433

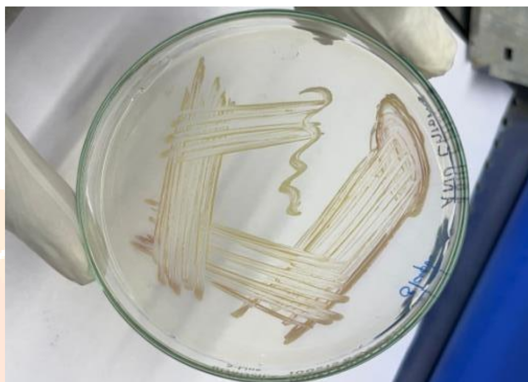


Figure 1.3 *S. aureus* ATCC 25923

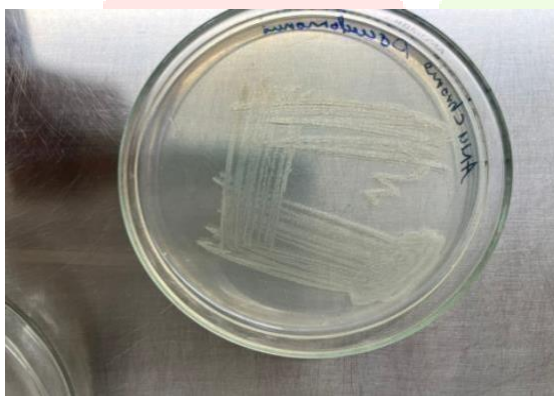


Figure 1.4 *P. aeruginosa* ATCC 27853

Conventional culture media

Figure 1.5- *K. pneumoniae* ATCC 13883 (Blood agar)

Figure 1.6- *E coli* ATCC 25922(MacConkey agar)

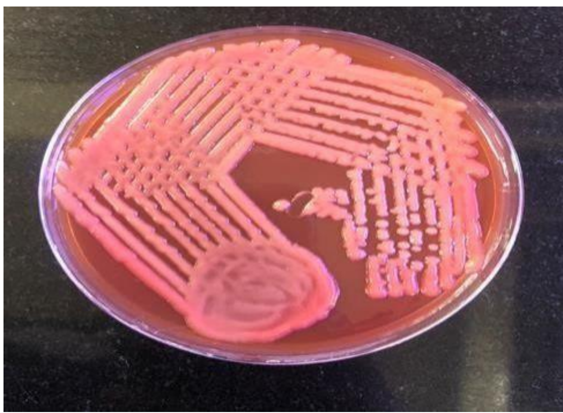


Figure 1.7 -*K. pneumoniae* ATCC 13883(MacConkey agar)

Bio-chemical tests for identification from conventional culture media(IMVIC- Indole, Methyl red, Voges Proskauer, Citrate)

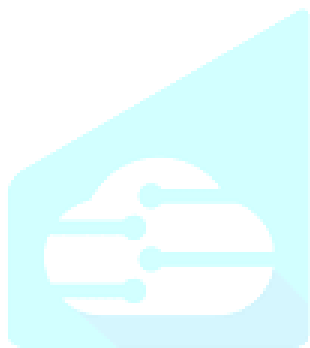


Fig 1.8 Biochemical reaction showing *E.coli*Indole –positive
Mannitol motility test-Motile, fermentativeCitrate- negative TSI-A/A

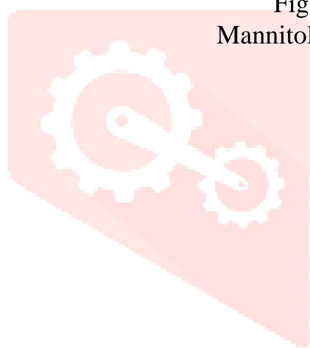


Fig 1.9: Biochemical reaction of *Klebsiella pneumonia* Indole – negative
Mannitol motility test – Non-motile, fermentingCitrate –positive TSI-A/A

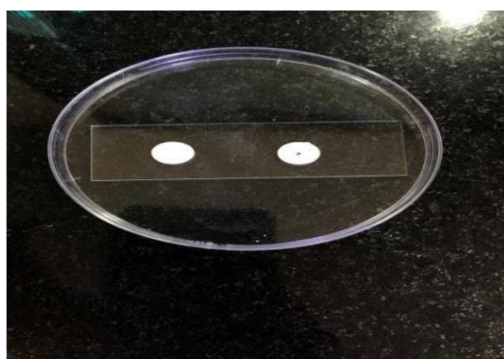


Figure 2. Oxidase test Left –Negative, Right- Positive

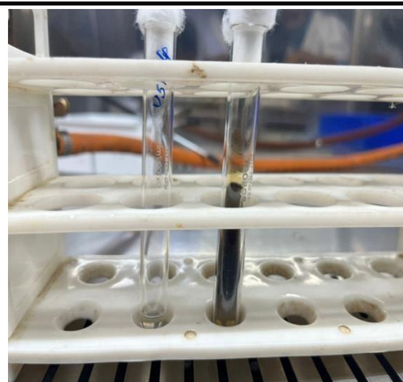


Fig 2.1: Catalase test Left –Negative, Right -Positive

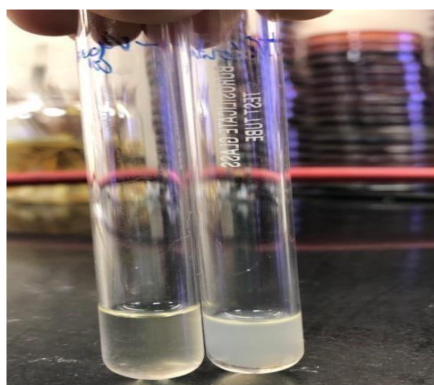


Fig 2.2: Tube coagulase test Left Negative, Right- Positive

Fig 2.3: Bile esculin test Left Negative, Right Positive

RESULTS

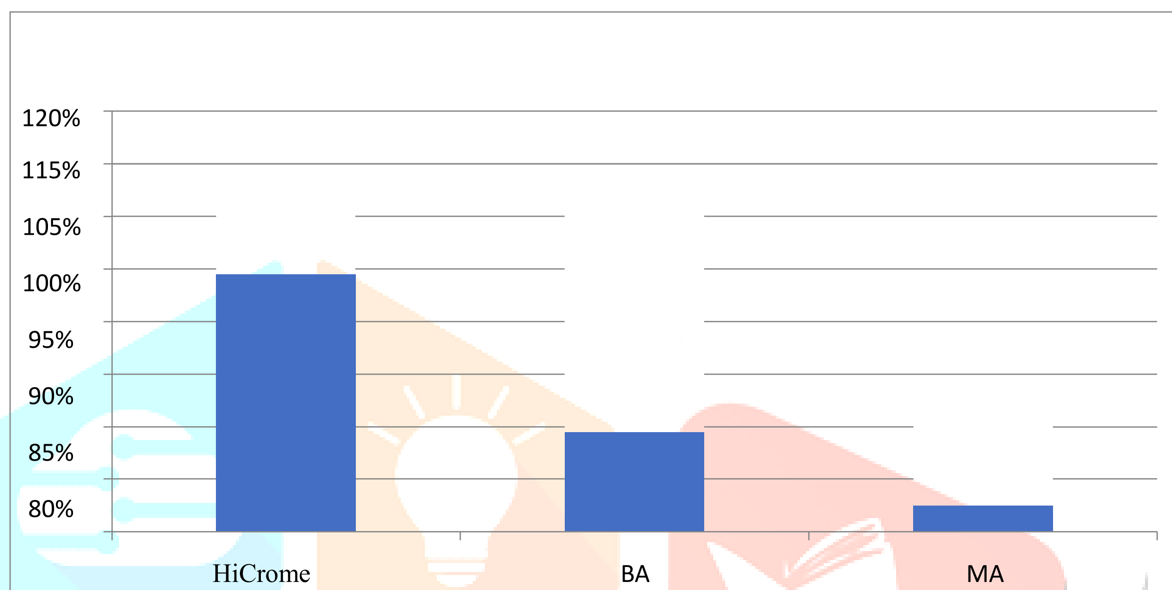
In this study, we conducted an analysis of the isolation and identification of 6 ATCC strains using both conventional agar and HiCrome UTI agar. Our findings revealed that HiCrome UTI agar demonstrated superior performance in terms of isolation and identification compared to conventional agar media.

Table 1.3: Comparison of three culture media for the rate of isolation.

ATCC STRAIN INOCULATED	TOTAL NUMBER OF PLATES STREAKED	HICROME UTI AGAR		BLOOD AGAR		MACCONKEY AGAR	
		N	%	N	%	N	%
Escherichia coli ATCC 25922	5 PLATES EACH	5	100%	5	100%	5	100%
Klebsiella pneumoniae ATCC 13883	5 PLATES EACH	5	100%	3	60%	3	60%
Pseudomonas aeruginosa ATCC 27853	5 PLATES EACH	5	100%	2	20%	2	60%
Proteus mirabilis ATCC 12453	5 PLATES EACH	5	100%	5	100%	5	100%
Staphylococcus aureus subsp. aureus ATCC 25923	5 PLATES EACH	5	100%	5	100%	5	100%
Enterococcus faecalis ATCC 29212	5 PLATES EACH	5	100%	4	80%	5	100%
TOTAL	**30**	**30**	**100%**	**25**	**83.3%**	**24**	**80%**

Table 1.3 provides the results of the inoculation of ATCC strains on different agar plates, including HiCrome UTI agar, Blood agar, and MacConkey agar. Each ATCC strain was streaked on 5 plates of each agar type, and the percentages indicate the rate of presumptive identification for each strain on each agar. Overall, the HiCrome UTI agar demonstrated the highest rate of presumptive identification, with a 100% identification rate for all ATCC strains tested. It was followed by Blood agar, which had a presumptive identification rate of 83.3%, and MacConkey agar with a rate of 80%.

For specific strains, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 12453, and Staphylococcus aureus subsp. aureus ATCC 25923 showed a 100% presumptive identification rate on all agar types. Klebsiella pneumoniae ATCC 13883 had a 60% presumptive identification rate on MacConkey agar, while Pseudomonas aeruginosa ATCC 27853 had a 60% presumptive identification rate on MacConkey agar.



Graph 1.0: Overall Differential isolation rate of media

In summary, HiCrome UTI agar consistently demonstrated the highest rate of presumptive identification across all tested ATCC strains, followed by Blood agar and MacConkey agar. These results suggest that HiCrome UTI agar may be a reliable choice for the isolation and presumptive identification of uropathogens.

DISCUSSION

The utility of chromogenic agar for the isolation and presumptive identification of uropathogens in the diagnosis of urinary tract infections (UTIs) holds great promise in improving the accuracy, efficiency, and specificity of UTI diagnosis. One of the key advantages of chromogenic agar is its ability to streamline the diagnostic process.

The rate of isolation and pattern of major uropathogens observed in this study are consistent with findings from previous studies conducted using both chromogenic and conventional media.

A similar study yielded a total of 199 bacterial isolates from positive plates, with a prevalence rate of 42.67%. Among these, 179 (40.40%) were unimicrobial growths, while 10 (2.26%) were polymicrobial growths. Both HiCrome UTI agar and Blood agar media supported 100% of the growth, while MacConkey agar demonstrated growth in 75.88% of the cases. The rate of presumptive identification was significantly higher on HiCrome UTI agar (97.49%) compared to MacConkey agar (67.34%) as the primary urine culture medium (Akter et al., 1969).

The leading uropathogen isolated was *E. coli*, accounting for 59.30% of the samples, with a high presumptive identification rate of 95.76% on HiCrome UTI agar. In contrast, the presumptive identification rates on MacConkey agar and Blood agar were 93.22% and 5.93%, respectively. Interestingly, all 10 cases of polymicrobial growth were distinctly demonstrated on HiCrome UTI agar, while only one case each was identified on Blood agar and MacConkey agar. The slightly lower isolation rate on MAC agar can be explained by its limitations of not supporting all organisms involved in UTIs like Staph.saprophyticus and Enterococcus *spp*. because it is a selective medium for members of Enterobacteriaceae (Akter et al., 1969; Gaillot et al., 2000b).

Clinical trials revealed that approximately 70% of the urinary tract infections (UTIs) analyzed were caused by gram-negative pathogens, while gram-positive pathogens accounted for 26% of the cases. Fungal infections represented the remaining 4% of the total UTIs. These results are consistent with recent reports. Among the gram-negative isolates, Escherichia coli (E. coli) emerged as the

predominant species, responsible for 65% of the infections (Emori & Gaynes, 1993). Notably, all these E. coli isolates exhibited distinctive reddish colonies on CHROME agar enabling easy differentiation. Given that E. coli is a significant causative agent of UTIs, particularly in nosocomial settings, the use of CHROME agar appears highly appropriate as a selective medium for the direct isolation of urine samples (Edberg & Kontnick, 1986).

It is important to consider the implications of these results in the context of UTI management. The high proportion of gram-negative pathogens underscores the ongoing clinical challenge posed by these organisms, especially in healthcare-associated infections. Rapid identification and appropriate treatment of these pathogens are crucial in order to effectively manage UTIs and prevent the development of complications.

Future studies should aim to evaluate the performance and cost-effectiveness of CHROM agar Orientation compared to other existing methods for the detection and differentiation of uropathogens. Additionally, further investigation is needed to assess the utility of CHROM agar in different healthcare settings, including both community and hospital-based laboratories.

CONCLUSION

The study investigated the effectiveness of chromogenic agar in diagnosing urinary tract infections (UTIs) compared to conventional media. The researchers used HiCrome UTI agar as primary culture tools to isolate and presumptively identify uropathogens. The results showed that chromogenic media outperformed traditional media in terms of both growth rates and identification accuracy.

In conclusion, this study highlights the potential of HiCrome UTI agar as a valuable tool for optimizing UTI management. Incorporating these media into clinical microbiology laboratories can lead to improved accuracy in identifying uropathogens, ultimately informing appropriate treatment decisions for UTIs.

Future studies should prioritize the evaluation of Hicrome in terms of its performance and cost-effectiveness compared to conventional methods for detecting and differentiating uropathogens. It is essential to assess the utility of CHROMagar in various healthcare settings, encompassing both community and hospital-based laboratories. Given the potential benefits of CHROMagar, such as the reduction in costs and human labor associated with the preparation and culturing process compared to conventional media, it holds promise as a superior alternative for UTI diagnosis. Further research will provide valuable insights into the practical implementation of CHROMagar and its impact on optimizing UTI management and treatment decisions.

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