Iranian Journal of Microbiology
Volume 14 Number 6 (December 2022) 825-831

ORIGINAL ARTICLE

Evaluation of chromogenic agar medium, can it be a suitable alternative to conventional culture system for identification of uropathogens?

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Received: July 2022**, Accepted:** November 2022

ABSTRACT

Background and Objectives: Urinary tract infections (UTI) account for major proportion of outpatient load and hospital admission globally. In most of the clinical microbiology laboratories MacConkey agar (MAC) and Cystine lactose electrolyte-deficient (CLED) agar are being used for identification of uropathogens. The main objective of the present study was to evaluate the usefulness of HiCrome™ UTI by comparing isolation rate and presumptive identification of uropathogens against CLED and MAC agar.
Materials and Methods: This study was conducted over a period of three months on 672 non-duplicate midstream and/or catheter-catch urine samples. All samples were inoculated on to HiCrome™ UTI, CLED agar and MacConkey agar.
Results: Among the 672 samples received for culture, 113 (16.8%) showed significant growth. Among the 672 samples, 95 (14.1%) showed growth of a single organism while 18 (2.7%) showed polymicrobial growth. The rate of isolation and presumptive identification of the isolates and polymicrobial growth was found significantly higher on HiCrome™ UTI Agar.
Conclusion: HiCrome™ UTI Agar has the potential to streamline processing of samples for urine culture in a way that will reduce the workload for technicians, reduce turnaround time which in turn will benefit the laboratory ultimately leading to better patient care.

Keywords: Urinary tract infection; Laboratory; Polymicrobial; Isolation; Identification

INTRODUCTION

Urinary tract infection (UTI) is ideally characterized by the presence of more than 10^5 colony forming units (cfu) per ml in a midstream sample of urine (1). UTI is a very common infectious disease with an estimated annual global incidence of around 250 million (2, 3). UTIs account for major proportion of outpatient load and hospital admission globally, making urine the most commonly received sample for culture in the laboratory and thus accounting for a significant proportion of laboratory workload (4-6). UTIs can affect healthy women, are common in individuals of either sex with structural or functional abnormalities of the genitourinary tract, and can cause both community acquired and nosocomial infections (7, 8). Urinary tract infections can be asymptomatic, acute or chronic. Asymptomatic infection is usually diagnosed by culture, while acute infections are managed on an outpatient basis. Chronic UTIs

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are usually associated with underlying abnormalities and frequently require hospitalization (9).

The primary responsibility of a clinical microbiology laboratory is the accurate identification of pathogens in the shortest possible turnaround time. Approximately only 20-30% of the urine samples results in significant growth, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. *Enterococci* spp., and *Staphylococcus saprophyticus* being the predominant causative agents (10, 11). Culture media used for urine culture should ideally enhance the growth of all possible uropathogens while inhibiting the growth of contaminants. In most of the clinical microbiology laboratories, Blood agar (BA), MacConkey agar (MAC) and Cystine lactose electrolyte-deficient (CLED) agar are used. CLED agar and MAC agar can differentiate between lactose fermenters and non fermententing Gram-negative bacteria but further identification requires subculture and biochemical tests which subsequently increases cost and turnaround time. None of these media used either alone or in combination will support the growth of all uropathogens (12). Mixed cultures may not be detected in CLED agar or MAC agar due to the absence of differential genus-specific indicator property.

The issues with media used for urine culture have been addressed to certain extent by the introduction of chromogenic media which allows the presumptive identification of pathogenic organisms on the basis of colony morphology and distinctive colour patterns (13-15). Chromogenic substrates incorporated into the media are broken down by enzymes resulting in a distinct colour to the bacterial colonies, which results in easier identification. Chromogenic media also allows easier identification of polymicrobial infection (16, 17). A number of chromogenic media are available commercially for the detection of uropathogens such as Cps Id2 Agar, Chromagar Orientation, Uriselect, HiCrome™ UTI Agar, Rainbow UTI (18). Some of the advantages of these media include reduction in number of biochemical tests, reduction in work load, which has a direct impact on cost reduction (19).

The aim of the present study was to validate the usefulness of HiCrome™ UTI agar as a primary urine culture medium by comparing its isolation rate and presumptive identification of uropathogens against CLED and MAC in a tertiary care hospital in South India.

MATERIALS AND METHODS

This was a prospective study conducted over a period of three months from April 2021 to June 2021 in a tertiary care hospital in South India. The study included 672 non-duplicate midstream and/or catheter-catch urine samples from clinically suspected UTI patients on an outpatient basis or among hospitalized patients. Patients were advised to collect mid stream urine samples into a sterile wide mouth container with all aseptic measures. Samples from catheterized patients were taken with all aseptic measures. Samples were transported to the laboratory without delay. The study was approved by the Institutional Ethical Committee in the tertiary care Hospital.

Both CLED agar and MAC agar were obtained as dehydrated powder from Himedia laboratories (Hi-Media Laboratories, Mumbai, India). Both media were prepared following manufacturer's instructions. HiCrome™ UTI Agar Plate (MP1353, HiMedia Laboratories, Mumbai, India) is a readymade chromogenic medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections. It gives the following colours to bacterial colonies (Table 1 and Fig. 1). All urine samples were inoculated aseptically on to HiCrome™ UTI agar, CLED agar and MacConkey agar media by a calibrated loop holding 0.01 ml of urine. The plates were incubated at 37°C aerobically and after overnight incubation they were checked for significant bacteriuria.

Criteria for significant bacteriuria (20) includes

1. Urinary catheter $\geq 10^4$ cfu/ml
2. Midstream $\geq 10^5$ cfu/ml
3. Samples which grow 10,000-50 000 cfu/ml will be included if there is a high clinical suspicion of UTI (fever + pyuria or bacteriuria or patients with renal disease).

Presumptive identification of bacterial isolates. Presumptive identification on CLED agar and MacConkey agar was based on colony morphology while presumptive identification on HiCrome™ UTI agar was based on colour and colony morphology (Table 1 and Fig.1). The final identification of the isolates was done using standard biochemical tests such as Gram staining, tests for motility, catalase, coagulase, oxidase, sugar fermentation and other relevant biochemical tests as appropriate for the isolates. Consumption of media and reagents were recorded to analyze cost.

Table 1. Colour of different bacteria on HiCrome UTI Agar

Bacteria	Colour
E. coli	Pink
Enterococci/Klebsiella spp.	Blue
Proteus spp.	Brown
Pseudomonas spp.	Colourless
Staphylococcus aureus	Golden yellow
Staphylococcus saprophyticus	White colonies

Fig. 1. Colonies of *E. coli, Klebsiella* and *Enterococci* on HiCrome UTI Agar
1. Pink colonies of *E. coli*
2. Blue colonies of *Enterococci*
3. Blue Mucoid Colonies of *Klebsiella*

Statistical analysis. The data was entered in MS excel and analysed using JASP V.18. Proportions were calculated and expressed. The yield of outcomes of HiCrome™ UTI agar over MAC Agar and CLED Agar was compared using Chi squared test recommended by Campbell and Richardson (21). A p value less than 0.05 was considered as statistically significant.

RESULTS

Out of the 672 samples received for culture, 113 (16.8%) showed significant growth and the remaining 559 (83.2%) samples showed no growth or insignificant growth (20). Among the 672 samples, 95 (14.1%) showed growth of a single organism while 18 (2.7%) showed polymicrobial growth. The 113 culture positive samples yielded a total of 131 organisms which included 95 monomicrobial growths and 18 (18 samples yielded 2 bacteria each ie 18 ×2 =36 bacterial growths) polymicrobial growths. Isolates from urine culture is shown in Table 2.

Klebsiella spp., *E. coli*, *Enterococcus* spp. and *Pseudomonas* spp. were the major uropathogens in the study. Comparison of the rate of isolation of uropathogens in the three media used in the study is shown in Table 3. Hicrome™ UTI gar showed 100% isolation of all the microorganisms in the study. The rate of presumptive identification of microorganism by colony colour and morphology on all three media is shown in Table 4. The rate of isolation and presumptive identification of the isolates was found significantly higher on HiCromeTM UTI compared to either CLED agar or MAC agar as primary urine culture medium ($P < 0.05$). The Rate of isolation of polymicrobial growth on different media is shown in Table 5. All 18 (100%) polymicrobial growths were correctly identified only on HiCrome™ UTI agar medium. The difference in

Table 2. Various types of Organism isolated from Urine Culture

Organism	Number (%)
Escherichia coli	38 (29)
Klebsiella spp.	43 (32.8)
Pseudomonas spp.	12 (9.2)
Enterococcus spp.	19 (14.5)
Enterobacter spp.	5 (3.8)
Citrobacter spp.	5 (3.8)
Acinetobacter spp.	2 (1.5)
Burkholderia spp.	1 (0.7)
Group B streptococci	1 (0.7)
Candida	5 (3.8)
Total	131 (100)

Table 3. Rate of isolation of uropathogens in MAC agar, CLED Agar, HiCrome UTI Agar

Bacteria (Number)	MAC Agar N (%)	CLED Agar N (%)	HiCrome UTI Agar N (%)
Escherichia coli (38)	36 (94.7)	38 (100)	38 (100)
Klebsiella spp. (43)	43 (100)	43 (100)	43 (100)
Pseudomonas spp. (12)	9 (75)	8 (66.7)	12 (100)
Enterococcus spp. (19)	17 (89.5)	14 (73.7)	19 (100)
Enterobacter spp. (5)	5 (100)	5 (100)	5 (100)
Citrobacter spp. (5)	5 (100)	5 (100)	5 (100)
Acinetobacter spp. (2)	2 (100)	2 (100)	2 (100)
Burkholderia spp. (1)	1 (100)	1 (100)	1 (100)
Group B streptococci (1)	1 (100)	1 (100)	1 (100)
Candida spp. (5)	5 (100)	5 (100)	5 (100)
Total	124 (94.7)	122 (93.1)	131 (100)

isolation of polymicrobial growth between HiCrome™ UTI agar and either CLED agar or MAC agar was found to be statistically significant ($P < 0.05$).

Major errors in using Hi crome UTI agar. HiCrome™ UTI agar can reliably identify *E. coli, Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *S. aureus* and *S. saprophyticus.* Misidentification or inability to identify any of the above mentioned isolates was regarded as a Major Error in the study. There were two major errors in our study which included wrong identification of isolates of *E. coli* and *Enterococcus* as *Klebsiella* spp. and *Staphylococcus aureus* respectively. Inability to identify *Citrobacter, Burkholderia, Acinetobacter*, Group B *Streptococcus* and *Enterobacter* were not included as major errors as this media can only reliably detect the major uropathogens.

Table 4. Rate of presumptive identification on different media

Bacteria (Number)	MAC Agar N (%)	CLED Agar N (%)	HiCrome UTI Agar N (%)
Escherichia coli (38)	27 (71.1)	31 (81.6)	37 (97.4)
Klebsiella spp. (43)	38 (88.4)	37 (86)	43 (100)
Pseudomonas spp. (12)	7 (58.3)	6 (50)	12 (100)
Enterococcus spp. (19)	17 (89.5)	14 (73.7)	18 (94.7)
Enterobacter spp. (5)	0 (0)	0 (0)	3 (60)
Citrobacter spp. (5)	3 (60)	3 (60)	0 (0)
Acinetobacter spp. (2)	2 (100)	2 (100)	0 (0)
Burkholderia spp. (1)	0 (0)	0 (0)	0 (0)
Group B streptococci. (1)	1 (100)	1 (100)	0 (0)
Candida spp. (5)	5 (100)	5 (100)	5 (100)
Total (131)	100 (76.3)	99 (75.6)	118 (90.1)

Table 5. Rate of isolation of Polymicrobial growth on different media

Organism Pair (Number)	MAC Agar N (%)	CLED Agar N (%)	HiCrome UTI Agar N (%)
E. coli + *Klebsiella* (5)	2 (40)	1 (20)	5 (100)
Enterococcus + *Pseudomonas* (4)	2 (50)	1 (25)	4 (100)
E. coli + *Enterococcus* (4)	0 (0)	0 (0)	4 (100)
Klebsiella + *Pseudomonas* (3)	1 (33.3)	1 (33.3)	3 (100)
Enterococcus + *Klebsiella* (1)	1 (100)	1 (100)	1 (100)
E. coli + *Pseudomonas* (1)	0 (0)	0 (0)	1 (100)
Total (18)	6 (33.3)	4 (22.2)	18 (100)

DISCUSSION

The present study was formulated to evaluate the usefulness of HiCrome™ UTI agar as a primary isolation medium. Microbial identification can be a very time consuming process when traditional culture media like Blood agar, MacConkey agar or CLED agar are used (22). In order to overcome the disadvantages of traditional culture media, a range of chromogenic agar media have become available. Chromogenic agars not only support the growth of uropathogens but also allow easier identification of polymicrobial growths. The first report of a chromogenic medium for diagnosis of urinary tract infections was the use of CPS ID2 media in 1995 (14, 23).

In our study *Klebsiella* spp., *E. coli*, *Enterococcus* spp. and *Pseudomonas* spp. were the major uropathogens. Low rate of polymicrobial growth in our study was concordant with other studies but the rate of monomicrobial growth was lower when compared to other studies (24). High rates of both monomicrobial and polymicrobial growth has been reported (25). Our study showed 100% rate of isolation of bacteria including polymicrobial growth while using HiCrome™ UTI agar. Rate of presumptive identification while using HiCrome™ UTI agar was also very

Table 6. Sensitivity of different chrom agar media

Authors	Chrom Agar Medium Used	Sensitivity (%)
Payne et al. (17)	1.CPS (CPS4) agar	1. 93.1
	2. UriSelect™ 4 (URS4) agar	2. 93
Meddeb et al. (18)	1. ChromID CPS	1.98.7
	3. Uri select 4	2.97.7
Khalid et al. (27)	Hicrome UTI agar	94 (RPI)
Akter et al. (26)	Hicrome UTI agar	97.49 (RPI)
Present study	Hicrome UTI agar	90.1% (RPI)

RPI- Rate of presumptive Identification

high (90.1%) when compared to other media. Our findings were concordant with the study conducted by Akter et al. which also showed 100% isolation rate of bacteria including polymicrobial growth while using Hicrome™ UTI agar (26). Akter et al. also reported a very high presumptive identification rate (97.49%) while using Hicrome™ UTI agar. Another study conducted by Khalid et al. also showed 100% isolation rate of bacteria and presumptive identification rate of 94% while using HiCrome™ UTI agar but the rate of isolation of polymicrobial growth was lower (65.1%) (27). High identification rate for polymicrobial growth while using HiCrome™ UTI agar was also reported by other studies (26, 27).

Bacterial isolation rates while using Hi Crome™ UTI agar, CLED agar and MAC agar were 100%, 93.1% and 94.7% respectively. Both CLED and MAC agar have minimal ability to differentiate between bacterial species. It can only differentiate between lactose fermenters and non-fermenters (28). CLED agar does not isolate all the uropathogens as reported by Mohan et al. (29). The slightly lower bacterial isolation rate in MAC agar can be explained by the fact that it does not support the growth of all organisms involved in UTI like *Enterococcus* spp. Higher presumptive identification rate (90.1%) while using HiCrome™ UTI agar was possible due to the ease in identification by seeing the specific colour produced by each bacteria on this medium. This differential colour production is one of the main reasons why HiCrome™ UTI agar is being increasingly recognized as a primary isolation medium. In our study, MAC agar and CLED agar produced lower presumptive identification rates of 76.3% and 75.6% respectively probably due to failure in identifying few non lactose fermenting variety of *E. coli* and few non mucoid type of *Klebsiella*. Chromogenic media gave the added advantage of identifying these organisms. HiCrome™ UTI agar also offers the benefit of limiting the spread of organisms such as *Proteus* spp., *Klebsiella* spp. and *E. coli* mucoid strains thus increasing the ability of the medium to detect pathogens when polymicrobial growth is present (30). In this study the rates of isolation of polymicrobial growth while using HiCrome™ UTI agar, CLED agar and MAC agar were 100%, 22.2% and 33.3% respectively. MAC and CLED agar were unable to differentiate between the colonies. Improved detection in polymicrobial infection can lead to judicious use of antibiotics.

Ever since the introduction of chromogenic media, a large number of other media has since been commercialized and evaluated with clinical samples, including CHROM agar Orientation, UriSelectmedium, Rainbow Agar UTI medium, Chromogenic UTI medium, USA agar, Urichrom agar and Hicrom™ UTI Agar (30). Sensitivities of these different media according to different studies are shown in Table 6. There were two major errors in our study which included misidentification of one isolate each of *E. coli* and *Enterococcus* spp. HiCrome™ UTI agar also failed to presumptively identify all isolates of *Citrobacter, Burkholderia, Acinetobacter* and Group B *Streptococci*. It also failed to identify two isolates of *Enterobacter* spp. Their final identification required additional biochemical reactions (31). Inability to identify *Citrobacter, Burkholderia, Acinetobacter,* Group B *Streptococcus* and *Enterobacter* were not included as major errors as this media does not reliably detect them.

In our Laboratory both CLED agar and MAC agar are used for urine culture. Two samples are inoculated per agar plate. Several Biochemical reactions like indole, citrate, triple sugar iron are required. Overall cost of media and reagents used for culture of a single sample by conventional culture comes to around 21 Indian Rupees. HiCrome™ UTI Agar obviates the need for biochemical reactions. During the study three samples were inoculated per HiCrome™ UTI Agar plate as shown in Fig. 1. Cost required for culture of a single sample on HiCrome™ UTI agar comes to around 24 Indian Rupees. Due to the colour produced by each bacteria, identification was very easy in Hicrome™ UTI agar as very little technical expertise was required and led to reduction in turnaround time. Since biochemical reactions are not required if HiCrome™ UTI agar is used as a primary isolation medium, workload will be significantly reduced. Eventhough culture by Hicrome™ UTI Agar is slightly more expensive than conventional culture, the reduction in workload and turnaround time associated with Hicrome™ UTI agar makes it very beneficial for the laboratory.

While Hicrome TM UTI agar was found to very reliable in identifying major uropathogens like *E. coli* and *Klebsiella*, the inability to identify organisms like *Burkholderia, Acinetobacter* is a potential draw back. This was the limitation of this study.

CONCLUSION

Hicrome™ UTI agar had a superior isolation and presumptive identification rate when compared to CLED agar and MAC agar especially useful in identifying polymicrobial infection. With Hicrome™ UTI agar there is reduction in turnaround time and workload. Although the media is a bit expensive, it can be considered cost-effective as it obviates the need for additional media and reagents that are required in conventional culture system.

ACKNOWLEDGEMENTS

Authors would like to thank all the staff in Department of Microbiology, Believers Church Medical College, Thiruvalla for all their support throughout the study.

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