Original Article

Comparison of Chromogenic (HiCrome Urinary Tract Infection Agar) Medium with Cysteine Lactose Electrolyte Deficient Agar in a Resource-Limited Setting

Abstract

Background and Objectives: Urinary tract infections (UTI) are one of the most frequent infections encountered in hospital settings as well as in community, making urine the most cultured specimens in laboratories across the world. Urine samples occupy most of the time and manpower in the form of resources in the microbiology laboratories. The microbiological performance of HiCrome UTI agar was compared with cysteine lactose electrolyte deficient (CLED) agar for isolation and presumptive identification of bacteria from urine culture with ease of reporting with less human resource and reduction in the cost. **Materials and Methods:** The study was conducted in a total of 208 collected midstream catch urine samples from patients attending the Department of Microbiology, Khartoum Teaching Hospital Central Laboratories. Urine samples received in the bacteriology laboratory were inoculated on CLED agar and for HiCrome UTI agar simultaneously and incubated overnight. Isolates were identified by the colony's color for HiCrome UTI agar and by standard microbiological techniques for CLED agar. **Results:** Out of 208 urine samples tested, significant growth was obtained in 94 (45.2%) plates of CLED agar and 84 (40.4%) of HiCrome UTI; moreover, 15 (7.2%) and 28 (13.5%) plates showed mixed with no growth observed in 99 (47.6%) and 96 (46.1%) on CLED agar and HiCrome UTI agar, respectively. The rate of presumptive identification was found significantly higher on HiCrome UTI agar (94%) than CLED agar (84%) ($P < 0.05$) as the primary urine culture medium. out of 43 (100%) polymicrobial growths 28 (65.1%) were demonstrated distinctly on HiCrome UTI agar and only 15 (34.9%) were obtained from CLED agar. **Conclusions:** HiCrome UTI agar was found to be more useful as a primary urine culture medium in both higher rate of isolation and presumptive identification of uropathogens in comparison to conventional media. Its inherent characteristics in demonstrating polymicrobial growth and ease of rapid identification by distinct colony color are unique.

Keywords: *Cysteine lactose electrolyte deficient agar, HiCrome urinary tract infection agar, presumptive identification, rate of isolation, urinary tract infection, urine culture, uropathogens*

Mohamed Khalid

Department of Laboratory Medicine, Kamal Medical Polyclinics, Riyadh, Saudi Arabia

Background

Urinary tract infections (UTIs) are the most common infectious diseases,[1] and it is a commonly encountered disease by clinicians in developing countries with an estimated annual global incidence of at least 250 million.[2] They affect a substantial proportion of otherwise healthy women, are common in males and women with structural or functional abnormalities of the genitourinary tract, and frequently are the cause of both community and hospital-acquired infections.[3,4] The most common route of infection is by the ascension of bacteria to colonize perianal and then ascent to introitus vagina. Furthermore, colonization can spread to the periurethral area, urethra, and bladder,[5] from these sites; the infections may ascend into the ureters (ureteritis) and subsequently involve the kidney (pyelonephritis). Females are more prone to infection of the urinary tract than males; in both males and females, UTI may be asymptomatic, acute, or chronic. Asymptomatic infection can be diagnosed by culture. Acute UTI is more frequently seen in females of all ages; these patients are usually treated on an outpatient basis and are rarely admitted to the hospital. In contrast, chronic UTI in both males and females of all ages is usually associated with an underlying disease (e.g., pyelonephritis, prostatic disease, or congenital anomaly of the genitourinary tract) and these patients

How to cite this article: Khalid M. Comparison of chromogenic (HiCrome urinary tract infection agar) medium with cysteine lactose electrolyte deficient agar in a resource-limited setting. Int J App Basic Med Res 2021;11:9-13.

Submitted: 09-Sep-2020
Revised: 15-Jun-2020
Accepted: 28-Oct-2020
Published: 26-Jan-2021

Address for correspondence:
Dr. Mohamed Khalid,
Kamal Medical Polyclinics,
Riyadh, Zip Code 11461,
Saudi Arabia.
E-mail: maak16@hotmail.com

Access this article online

Website:
www.ijabmr.org

DOI:
10.4103/ijabmr.IJABMR_306_19

Quick Response Code:

are most often hospitalized. Asymptomatic, acute, and chronic UTI are three distinct entities and the laboratory results often require different interpretations.[6]

In most of the clinical laboratories, the use of cysteine lactose electrolyte deficient (CLED) agar is a better option for the detection of uropathogens.[7] The differentiation of lactose fermenter and nonfermenter is possible on CLED agar, but further species identification necessitates subculture or different biochemical tests with consequent longer reporting time and cost. Moreover, it limited capacities in maximizing the growth of possible pathogens rendered it unsuitable as an ideal primary isolation medium. Due to the absence of differential genus-specific indicator property in the CLED agar, there is no guarantee that mixed cultures are always detected. To overcome these limitations and difficulties, several chromogenic media have been available for some years.[8] Chromogenic media are nonselective media, which contain essential ingredients to support the growth of all common bacterial uropathogens and provide their presumptive identification.[9] Chromogenic media contain tryptophan deaminase which facilitates inhibition of swarming and detection of *Proteus* spp. The comparison of CLED agar with the chromogenic medium for the detection of mixed cultures showed a highly significant improvement in detection using the chromogenic medium. It can be seen that the chromogenic medium gave significantly fewer samples with no growth, indicating that the chromogenic medium supported the growth of more urinary isolates than CLED agar.[10] Chromogenic media have shown advantages, which include 20% reduction in time for identification, reduction in workload, easier recognition of mixed growth, and reduction in the number of biochemical tests performed for identification of bacteria, which ultimately results in cost reduction.[9]

Materials and Methods

Place of work

This cross-sectional study was carried out over a period of 4 months, from December 2012 to March 2013. It was conducted in the Department of Microbiology, Khartoum Teaching Hospital Central Laboratories.

Collection of urine

Patients were advised to pass urine, discarding the first part of the stream and collecting clean-catch "midstream" urine, in a graduated sterile wide-mouthed container covering around — volumes.

Transportation and processing of urine

Urine samples were transported to the laboratory without delay. About 2 ml of homogenized urine was centrifuged at 3000 r.p.m. for 4 min.

Preparation of media

HiCrome UTI agar and CLED agar were obtained as a dehydrated powder from HiMedia Laboratories Pvt. Ltd. Mumbai. All culture Petri plates were prepared in house by following the manufacturer's instructions and recommendations. Prepared plates were stored at 2°C–8°C for a month.

Urine culture

Culturing of uncentrifuged urine specimens was done using the standard loop technique. About 0.01 ml of urine was surface streaked by a calibrated loop holding 0.01 ml of urine on HiCrome UTI agar and CLED agar aseptically. The plates were incubated at 37°C aerobically and after overnight incubation; they were checked for significant bacteriuria as under by enumeration of colonies. If growth was seen, colonies were counted and recorded as colony-forming units (CFU)/ml by multiplying count by 100. Criteria for significant bacteriuria included (i) CFU more than or equal to 10^5 bacteria/ml of noncoliforms/ml or >102 CFU of coliforms/ml in a symptomatic woman, (ii) presence of >103 CFU bacteria/ml in a symptomatic man, and (iii) growth of two different organisms of possible uropathogens at a concentration >104 CFU/ml were considered for significant bacteriuria. Presumptive identification of bacterial growth was done on HiCrome UTI agar according to colony morphology and color as depicted by the manufacturer. Colonies on the CLED agar were also identified following colony characteristics against each of the uropathogens. The final identification of the isolates was done using standard identification protocol such as Gram's staining, motility test, catalase test, coagulase test, oxidase test, and other criteria described by Cheesbrough[11] and Yvette *et al.,*[12] as appropriate for the isolates.

Results

Out of 208 urine samples tested, significant growth of a single organism was obtained in 94 (45.2%) plates of CLED agar and 84 (40.4%) of HiCrome UTI; moreover, 15 (7.2%) plates of CLED agar showed mixed growth, whereas 28 (13.5%) obtained by HiCrome UTI agar. In contrast, no growth was observed in 99 (47.6%) of CLED agar plates and 96 (46.1%) of HiCrome UTI agar plates [Table 1].

Out of 208 urine culture, It was observed that HiCrome UTI agar supported 134 bacterial growths, while 126 growths were observed in CLED agar. HiCrome UTI agar was supported 59/134 (44.0%) of Escherichia coli isolates compared to 57/126 (45.2%) in CLED agar. The difference between the isolation of common uropathogens on HiCrome UTI agar and CLED agar is significant $P < 0.05$ [Table 2].

For the presumptive identification of bacterial species by colony characteristics on primary culture plates, of

131 bacterial isolates, 123 (94%) were successfully presumptively identified from HiCrome UTI agar and 110 (84%) isolates were identified from CLED agar. Of importance, 100% of *Pseudomonas* spp. isolated from HiCrome UTI agar plates matched with the CLED agar isolates. The rate of presumptive identification of the isolates was found significantly higher on HiCrome UTI agar than CLED agar as primary urine culture medium $P < 0.05$ [Table 3].

Out of 43 polymicrobial growths reported from HiCrome UTI agar and CLED agar, 28 (65.1%) were detected by HiCrome UTI agar, whereas only 15 (34.9%) were detected by CLED agar. The difference in detection of polymicrobial growth between HiCrome UTI agar and CLED agar is significant $P < 0.05$ [Table 4].

Table 1: Results obtained on cysteine lactose electrolyte deficient agar and HiCrome urinary tract infection agar after 24 h incubation

Growth	CLED agar, *n* (%)	HiCrome UTI agar, *n* (%)
Single bacterial growth	94 (45.2)	84 (40.4)
Mixed bacterial growth	15 (7.2)	28 (13.5)
No growth	99 (47.6)	96 (46.1)
Total	208 (100)	208 (100)

UTI: Urinary tract infection; CLED: Cysteine lactose electrolyte deficient

Table 2: Comparison of isolation rate of uropathogens recovered HiCrome urinary tract infection agar and cysteine lactose electrolyte deficient agar

Name of bacteria	HiCrome UTI agar isolates, *n* (%)	CLED agar isolates, *n* (%)
E. coli	59 (44.0%)	57 (45.2%)
Klebsiella spp.	30 (22.4%)	22 (17.5%)
Pseudomonas spp.	7 (5.2%)	11 (8.7%)
Staphylococcus spp.	8 (6.0%)	12 (9.5%)
Proteus spp.	6 (4.5%)	4 (3.2%)
Enterococcus spp.	17 (12.7%)	11 (8.7%)
Citrobacter spp.	0 (0.0%)	2 (1.6%)
Candida spp.	7 (5.2%)	7 (5.6%)
Total	134 (100%)	126 (100%)

UTI: Urinary tract infection; CLED: Cysteine lactose electrolyte deficient; *E. coli: Escherichia coli*

Discussion

The present study was formulated to evaluate HiCrome UTI agar for its usefulness as a primary isolation medium for uropathogens from clinical specimens. Two hundred and eight urine samples were tested by parallel inoculation on HiCrome UTI agar and CLED agar.

The present study shows in agreement with Hengstler *et al.*;[13] The total number of organisms recovered was higher in HiCrome UTI agar than on CLED agar. as HiCrome UTI agar was superior to CLED agar in detecting Enterococci, this may due to hidden of Enterococci by colonies of Gram-negative bacteria.

The rate of isolation and majority of organisms isolated by the present study were *E. coli, Klebsiella* spp., *Enterococci faecalis,* and *Staphylococcus* spp. This finding is as similar as finding reported by Fatema *et al.*[14]

The identification of urinary pathogens and detection of mixed cultures on CLED agar as well as other traditional media is time-consuming and requires extensive experience in clinical microbiology; this finding is accepted by Raafat *et al.*[15] Several researchers have previously demonstrated the equal or superior performance of various chromogenic media over traditional media for the identification of urinary tract pathogens.[9,15-17]

Among 43 cases of mixed growth of two organisms, HiCrome UTI agar was able detect 28 (65.1%) in contrast, CLED agar detect 15 (34.9%), this finding similar to that reported by Biji et al.,[18] and. Kaskar *et al.*[19]

CLED agar has several advantages, such as the inhibition of *Proteus* swarming, but it has only minimal differential potencies because it differentiates only lactose fermenter from lactose nonfermenter bacteria.[16] In addition, it does not recover all of the organisms involved in UTIs as conducted by Mohan *et al.*[20] This study has shown with the agreement of Mohan *et al.*,[20] and Sharmin *et al.*[21] The substantially higher number of strains can be recovered using HiCrome UTI agar for the isolation of urinary tract

Table 3: Comparison of rate of presumptive identification on HiCrome urinary tract infection agar than cysteine lactose electrolyte deficient agar as primary culture plate

Bacteria (*n*)	HiCrome UTI agar		CLED agar	
	Positive, *n* (%)	Negative, *n* (%)	Positive, *n* (%)	Negative, *n* (%)
E. coli (59)	57 (97)	2 (3)	55 (93)	4 (7)
Klebsiella spp. (30)	29 (97)	1 (3)	22 (73)	8 (27)
Pseudomonas spp. (7)	7 (100)	0	7 (100)	0
Staphylococcus spp. (12)	8 (67)	4 (33)	11 (92)	1 (8)
Proteus spp. (12)	6 (100)	0 (0)	4 (67%)	2 (33%)
E. faecalis (17)	16 (94)	1 (6)	11 (65)	6 (35)
Total (131)	123 (94)	8 (6)	110 (84)	21 (16%)

UTI: Urinary tract infection; CLED: Cysteine lactose electrolyte deficient; *E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis*

Table 4: Comparison of rate of isolation of polymicrobial growth on culture media

Polymicrobial growth (*n*)	Detected by	
	HiCrome UTI agar, *n* (%)	CLED agar, *n* (%)
E. coli and *Enterococci* spp. (8)	7 (87.5)	1 (12.5)
E. coli and *Citrobacter* spp. (1)	0	1 (100.0)
Enterococci spp. and *Klebsiella* spp. (3)	2 (66.7)	1 (33.3)
Proteus spp. and *Pseudomonas* spp. (3)	2 (66.7)	1 (33.3)
E. coli and *Klebsiella* spp. (18)	14 (77.8)	4 (22.2)
E. coli and *Pseudomonas* spp. (3)	0	3 (100.0)
E. coli and *Proteus* spp. (2)	1 (50.0)	1 (50.0)
K.oxytoca and *Pseudomonas* spp. (1)	0	1 (100.0)
E. coli and *Candida* spp. (2)	1 (50.0)	1 (50.0)
Pseudomonas spp. and *Candida* spp. (2)	1 (50.0)	1 (50.0)
Total (43)	28 (65.1)	15 (34.9)

UTI: Urinary tract infection; CLED: Cysteine lactose electrolyte deficient; *E. coli: Escherichia coli*

pathogens. Although CLED agar remains an excellent medium for the isolation of single pathogens, CLED agar lacks differential capacity to distinguish between some mixtures of species, in contrast HiCrome UTI agar offer a far superior means of differentiating polymicrobial cultures from pure cultures, thus enabling microbiologists to assess more accurately the clinical relevance of urine culture results. Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics and turnaround time for urine culture.[9,19]

Conclusions

Although chromogenic media on its own are still expensive at the moment, considering the overall costs incurred for the use of multiple media and/or different biochemical tests necessary to identify the organism in the conventional urine culture system, it seems to be cost-effective. The overall findings of this study suggest that the HiCrome UTI agar can be used as a single medium for the isolation and presumptive identification of uropathogens as conventional isolation and identification requires a great deal of experience when using traditional media. HiCrome UTI agar offers an excellent and time-saving method for the reliable identification with lesser training of most of the uropathogens and differentiation of mixed bacterial cultures in primary culture plate compared to the conventional culture system. Although expensive, the use of HiCrome UTI agar may improve the quality of urine culture by contributing to a uniform interpretation of uropathogens by the different personnel engaged in this task at the laboratory.

Ethical clearance

Study was conducted after approval from the Institutional Ethics Committee.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Behzadi P, Behzadi E, Pawlak-Adamska EA. Urinary tract infections (UTIs) or genital tract infections (GTIs)? It's the diagnostics that count. GMS Hyg Infect Control 2019;14:Doc14.
2. Worku S, Derbie A, Sinishaw MA, Adem Y, Biadglegne F. Prevalence of bacteriuria and antimicrobial susceptibility patterns among diabetic and nondiabetic patients attending at Debre Tabor Hospital, Northwest Ethiopia. Int J Microbiol 2017;5809494. [doi: 10.1155/2017/5809494].
3. Nicolle LE. Urinary tract infections. In: Engleberg C, editor. Schaechter's Mechanisms of Microbial Disease. Philadelphia: Lippincott Williams & Wilkins; 2012.
4. Anjum M, Moorani KN, Sameen I, Mustufa MA, Kulsoom S. Functional and structural abnormalities of the kidney and urinary tract in severely malnourished children - A hospital based study. Pak J Med Sci 2016;32:1135-40.
5. Davis NF, Flood HD. The pathogenesis of urinary tract infections. In Clinical management of complicated urinary tract infection 2011;978-953. [doi: 10.5772/22308].
6. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC, Heuck CC. Basic Laboratory Procedures in Clinical Bacteriology. World Health Organization; 2003.
7. Chaturvedi A, Garg R, Singh VA. Evaluating the correlation of different culture media for the identification and isolation of common gram positive uropathogens and yeast causing UTI. Int J Adv Microbiol Health Res 2018;2:47-50.
8. Parveen R, Saha SK, Shamshuzzaman SM, Rashid AL, Chowdhury A, Muazzam N. Detection of uropathogens by using chromogenic media (Hicrome UTI agar), CLED agar and other conventional media. Faridpur Med Coll J 2011;6:46-50.
9. Akter L, Haque R, Salam MA. Comparative evaluation of chromogenic agar medium and conventional culture system for isolation and presumptive identification of uropathogens. Pak J Med Sci 2014;30:1033-8.
10. Fallon D, Andrews N, Frodsham D, Gee B, Howe S, Iliffe A, Nye KJ, and Warren RE. A comparison of the performance of cystine lactose electrolyte deficient (CLED) agar with Oxoid

chromogenic urinary tract infection (CUTI) medium for the isolation and presumptive identification of organisms from urine, J Clin Pathol 2002;55:524-9.

11. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed. Part 2. New York: Cambridge University Press; 2006.
12. McCarter YS, Burd EM, Hall GS, Zervos M, Sharp SE. Cumitech 2C: laboratory diagnosis of urinary tract infections. American Society for Microbiology, Washington, DC. 2009.
13. Hengstler KA, Hammann R, Fahr AM. Evaluation of BBL CHROMagar orientation medium for detection and presumptive identification of urinary tract pathogens. Journal of Clinical Microbiology. 1997 Nov 1;35:2773-7..
14. Fatema N, Alam A, Ahmed CO. Comparative study between chromogenic medium and conventional media for detection of uropathogens in urine. Jalalabad Med J 2009;6:5-10.
15. Raafat R, Dwedar R, El-Seidi E. Evaluation of the chromogenic medium (CPS) in the isolation and identification of urinary tract pathogens. Egypt J Med Microbiol 2015;38:1-6.
16. Rajaratnam A, Baby NM, Kuruvilla TS, Machado S. Diagnosis of asymptomatic bacteriuria and associated risk factors among pregnant women in Mangalore, Karnataka, India. J Clin Diagn Res 2014;8:OC23-5.
17. Bajoria A, Kaore M, Kaur NS, Gautam KS. Utility of chromogenic media against conventional culture techniques for isolation of bacterial uropathogens in resource poor settings. Natl J Laboratory Med 2019;8:MO05-8.
18. Biji SM, Sasikumari O, Bai JR. A study on evaluation of different culture media for the isolation of routine urinary pathogens. Int J Curr Microbiol App Sci 2017;6:195-201.
19. Kaskar SN, Tendolkar MR, Vaidya SP, Angadi SA, Dalal AR, Karr S, Koppikar GV. Comparative evaluation of performance and cost effectiveness of HiCrome UTI agar in detection of urinary tract pathogens. IJAR 2018;4:358-66.
20. Mohan B, Gupta P, Appannanavar SB, Singh G, Taneja N. Evaluation of new chromogenic media for identification of uropathogens from complicated urinary tract infections in a tertiary healthcare setting. Indian J Med Microbiol 2015;33:183-4.
21. Sharmin S, Alamgir F, Begum F, Jaigirdar MQ. Use of chromogenic agar media for identification of uropathogen. Bangladesh J Med Microbiol 2010;4:18-23.