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**Research Article**

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# *Evaluation of Chromogenic Agar Media for Isolation, Identification and Direct Antibiotic Susceptibility Testing of Uropathogens*

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## ABSTRACT

*Urinary tract infections (UTIs) are one of the most commonly occurring infections in outpatient settings and hospital admissions globally. No single medium is capable of supporting the growth and identification of all uropathogens. Recently, in order to improve isolation and presumptive identification in urine samples, chromogenic agar media are being used more and more frequently as versatile primary culture tools. Direct susceptibility testing by disc diffusion on clinical samples offers a rapid and inexpensive means of obtaining information to guide antimicrobial therapy. The objective was to evaluate and compare the effectiveness of conventional culture systems and chromogenic agar medium for the isolation, identification, and direct testing of uropathogen's antibiotic susceptibility. The study comprised 155 clinically suspected UTI patients. Uncentrifuged urine samples underwent wet mount analysis and centrifuged samples for Gram staining before the culture. All urine samples that were received in the lab underwent Direct Susceptibility Testing (DST) right away. A total of 155 urine samples were cultured on all three media (HiChrome UTI Agar, Sheep Blood Agar, and MacConkey Agar). 58 (37.32%) showed significant bacterial growth, while 97 (62.58%) showed no growth. A single organism was isolated in 54 (36% of the samples), and two (1.42%) samples yielded mixed growths of two species. We conclude that the Hichrome agar medium can be a desirable, simple primary isolation and identification medium that significantly lessens the daily workload associated with urine culture in microbiology labs.*

**Key words:** *Urinary tract infections, Hi chrome agar, Sheep blood agar, Direct susceptibility test, Polymicrobial, Conventional culture*

## INTRODUCTION

Urinary tract infections (UTIs) are one of the most frequent clinical presentations encountered in a hospital setup. In the need to confirm the diagnosis and manage the patient, a microbiological diagnosis is a must. This directly results in a significant amount of workload in the microbiology laboratory for their etiological diagnosis and antimicrobial susceptibility report [1]. Empirical therapy is usually started after taking samples depending on the patient's presentation and local epidemiology. Conventional methods of diagnosis require at least 24 hours for a negative culture and up to three days for susceptibility data if positive cultures are discovered.

Rapid pathogen identification is, therefore, necessary to decrease the turnaround time. This will also help in preventing misuse and overuse of antimicrobials and thus affect the rise in antimicrobial agent-resistant microorganisms, particularly in hospitalized patients [2]. Early knowledge using Chromogenic media permits the selection of the appropriate antibiotic prior to the findings of conventional susceptibility tests [3]. Most laboratories have long used Blood Agar (BA) and MacConkey Agar (MAC) in combination, particularly in developing nations. Later, cytosine lactose electrolyte-deficient (CLED) agar was added, but neither of these

media used singly or in combination can enable direct identification of potential uropathogens [1]. These media require subculture or alternative biochemical tests for further identification of the isolate, which results in a longer reporting time and higher cost [4]. Chromogenic agar (CA) was developed to solve the problem of urine culture [2]. It is increasingly being employed as a flexible primary culture method for better bacterial species separation, presumptive identification, and isolation from clinical specimens. Much chromogenic media for urinary pathogens have been manufactured and introduced to the market recently, enabling more precise direct distinction of microorganisms on basic plates. By using unique colony colors produced by interactions of genus- or species-specific enzymes with a suitable chromogenic substrate, chromogenic agar provides simultaneous presumptive identification of gram-positive and gram-negative bacteria and yeasts on a single medium [2]. The optimum media for urine culture should be able to maintain the growth of all urinary pathogens and inhibit potential commensals. These media contain chromogenic substrates that, when broken down by bacterial enzymes, give the developing bacterial colonies a recognizably visual color that can be used to identify them. The chromogenic medium might help less experienced bench technicians evaluate urine culture plates more consistently and with increased sensitivity [2]. Antimicrobial susceptibility testing (AST) using Muller Hinton (MHA)agar takes on average 18 to 24 hours. Direct inoculation of urine for the AST has been reported to be of value in decreasing the turnaround time by reporting reliable results on day two of sample submission [2, 5, 6]. Therefore, in the interest of patient care, direct antimicrobial susceptibility testing (DST) of urine samples by chromogenic media was attempted in this study. The objective was to evaluate and compare the effectiveness of conventional culture systems and chromogenic agar medium for the isolation, identification, and direct testing of uropathogens' antibiotic susceptibility.

## MATERIALS AND METHODS

*Study population*

The study comprised 155 clinically suspected acute UTI patients of all ages and sexes who either visited the outpatient department (OPD) or were hospitalized at the ESIC Medical College Hospital. This research was conducted at the ESIC Medical College in Faridabad, Haryana, in the Department of Microbiology. It was conducted between January 2018 and April 2018.

*Sample processing*

Midstream urine samples from patients were taken. Uncentrifuged urine samples underwent wet mount analysis and centrifuged samples for Gram staining before the culture [2]. Culture samples were kept in the refrigerator from the time of collection until they were processed in the microbiology lab.

Common surface streak procedure was used to perform the cultures on MAC (HI Media), 5% sheep blood agar (HI Media), and HiChrome agar (HiMedia, code no. M1353), and then incubated at 37°C overnight using a semiquantitative technique [7, 8]. Microbiological diagnosis of UTI was made as per standard definitions [9, 10]. The colonies on HiChrome agar were interpreted as per the manufacturer's interpretation. Identification of organisms was done by VITEK 2 and AST by disk diffusion method.

*Direct susceptibility testing (DST) on chromogenic agar media*

All urine samples that were received in the lab underwent direct susceptibility testing right away. An unadjusted, well-mixed urine sample was used to test for confluence on a HiChrome agar plate using a sterile cotton swab. The extra fluid was then expelled. Using sterile forceps, commercial antimicrobial discs were firmly placed on the agar surface after being distributed via a multidisc dispenser. Following incubation at 35°C for 16 to 18 hours, plates were read the following day [11]. According to the Clinical and Laboratory Standards Institute (CLSI), zone diameter was measured and interpreted. In accordance with CLSI standards, the antimicrobial susceptibilities of isolates from pure cultures were evaluated for comparison using a conventional disc diffusion method [12].

*Statistical analysis*

The standard culture method is used as the gold standard when calculating the prediction values for HiCrome UTI agar. For categorical data like the type of bacteria, frequencies with percentages were calculated. Using the computer program Microsoft Excel Professional Plus 2016, calculations were performed.

## RESULTS AND DISCUSSION

A total of 155 urine samples were cultured on all three media (HiChrome UTI Agar, Sheep Blood Agar, and MacConkey Agar). Among the samples tested, 58 (37.32%) showed significant bacterial growth, while 97 (62.58%) showed no growth. A single organism was isolated in 54 (36%) of the samples, and 02 (1.42%) samples yielded mixed growths of two species. **Table 1** displays the bacterial isolate patterns from urine culture. *Escherichia coli* accounted for the majority of the 58 isolates (39), followed by *Enterobacter aerogenes* (6), and *Klebsiella pneumoniae* (04) **(Table 1)**. Chromogenic Agar (CA) supported the growth of all isolates grown on Blood Agar (BA), whereas Mac Conkey supported only 93% of growth. Two polymicrobial growths were distinguishable on Cain comparison to BA and MaC. The DST result was in perfect agreement with traditional AST except for minor variations in zone sizes **(Table 2, Figures 1 and 2)**.

**Table 1.** Distribution of bacteria among positive urine culture (n=58)

| Organisms | Sheep Blood Agar | MacConkey Agar | HiChrome UTI Agar |
|---|---|---|---|
| Escherichia coli | 39 | 39 | 39 |
| Enterobacter aerogenes | 6 | 4 | 6 |
| Klebsiella pneumoniae | 4 | 4 | 4 |
| Pseudomonas aeruginosa | 4 | 4 | 4 |
| Staphylococcus aureus | 2 | 1 | 2 |
| Proteus vulgaris | 2 | 2 | 2 |
| Staphylococcus haemolyticus | 1 | 00 | 1 |

**Table 2.** Antibiotic susceptibility of Gram-positive isolates (n=3)

| Antibiotic disc | No. of isolates | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | Direct Sensitivity on CA | | | Conventional AST | | | |
| | S | I | R | S | I | R | |
| Ampicillin | 0 | - | 3 | 0 | - | 3 | >0.5 |
| Ciprofloxacin | 1 | - | 2 | 1 | - | 2 | >0.5 |
| Gentamicin | 1 | - | 2 | 1 | - | 2 | >0.5 |
| Nitrofurantoin | 3 | - | 0 | 3 | - | 0 | >0.5 |
| Erythromycin | 1 | - | 2 | 1 | - | 2 | >0.5 |
| Vancomycin | 2 | - | 1 | 2 | - | 1 | >0.5 |
| Teicoplanin | 2 | - | 1 | 2 | - | 1 | >0.5 |
| Cefoxitin | 1 | - | 2 | 1 | - | 2 | >0.5 |

**Table 3.** Antibiotic susceptibility of Gram-negative isolates (n=55)

| Antibiotic disc | No. of isolates | | | | | | P value |
|---|---|---|---|---|---|---|---|
| | Direct Sensitivity | | | Conventional AST | | | |
| | S | I | R | S | I | R | |
| Nalidixic Acid | 16 | 2 | 37 | 16 | 1 | 38 | >0.5 |
| Norfloxacin | 21 | 1 | 33 | 22 | 1 | 32 | >0.5 |
| Ciprofloxacin | 20 | 1 | 34 | 22 | 0 | 33 | >0.5 |
| Cotrimoxazole | 19 | 1 | 35 | 34 | 5 | 16 | >0.5 |
| Gentamicin | 35 | 6 | 14 | 44 | 2 | 9 | >0.5 |
| Amikacin | 45 | 2 | 8 | 37 | 47 | 14 | >0.5 |
| Nitrofurantoin | 35 | 5 | 15 | 22 | 2 | 31 | >0.5 |
| Cefixime | 23 | 0 | 32 | 29 | 3 | 22 | >0.5 |
| Amoxy-clav | 28 | 3 | 23 | 17 | 2 | 36 | >0.5 |

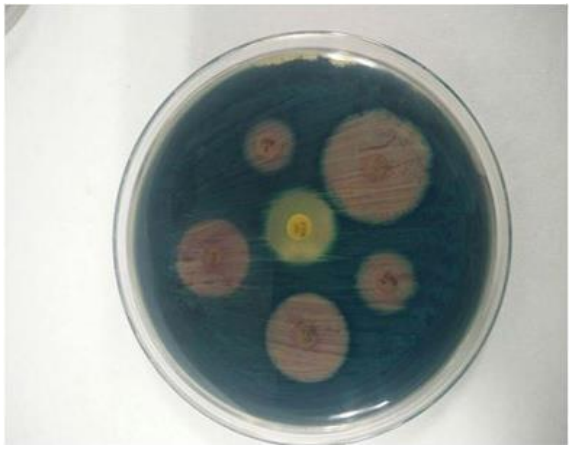

**Figure 1.** DST on Hi Chrome agar showing *E.coli*

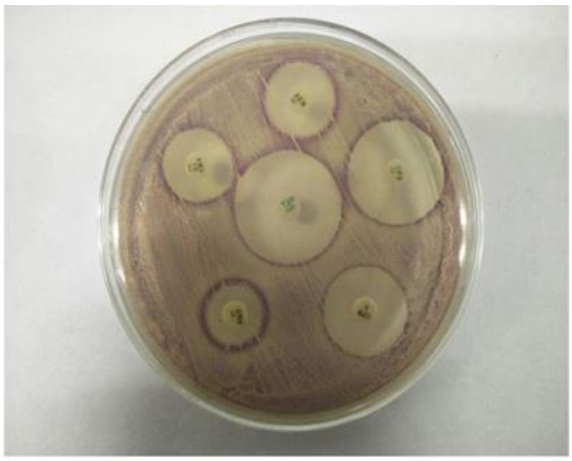

**Figure 2.** DST on Hi Chrome agar with polymicrobial growth *(Klebsiella & E.coli)*

UTIs are a common presentation requiring antimicrobial therapy. The etiology is mostly gram-negative bacteria, especially in community settings. To provide optimal therapy, time is of the essence. Considering alternative culture media like chromogenic agar can decrease the turn-around time by a significant amount without compromising the sensitivity and specificity. This will prevent morbidity and even mortality in some UTI cases [2]. A total of 155 urine samples were examined using parallel inoculations on chromogenic (HiCrome UTI agar), blood, and MacConkey agar media. A total of 58 (37.42%) of the 155 urine samples examined produced bacterial growth, whereas 97 (62.58%) showed no growth at all. This outcome is consistent with other related investigations conducted by Gul Nahar *et al*. [13] and Sharmin [14], who discovered bacterial growth to be around 46.33% and 42.5%, respectively. 54 (36%) of the culture-positive cases showed significant development of only one organism, whereas the remaining 2 (1.42%) showed mixed growth in two organisms. Studies by Gul Nahar *et al*. [13] revealed 44.33% single growth and 2.00% polymicrobial growth. Similar results were reported from two other studies carried out by Sharmin *et al.* [14] and Fatema *et al.* [15]. A study by Perry *et al*. [16] revealed 54.20% single growth and 21% polymicrobial growth, indicating that the rate of polymicrobial growth may fluctuate depending on multiple factors like patient age, sex, and geographical locations. According to a study done in India, where samples were randomly cultured without first checking for pus cells under a microscope, the frequency of bacteriological culture positivity in urine was 19.79%, with 95.12% antimicrobial and 04.87% polymicrobial growths [17]. In the present study *E. coli* was the most common 39(67.24%), followed by *Enterobacter aeruginosa* 06(10.34%), *Klebsiella pneumoniae* 04(6.89%), *Pseudomonas aeruginosa* 04(06.89%), *Staphylococcus aureus* 02(03.44%), *Proteus vulgaris* 02(03.44). Other researchers [13, 14] also noted the same trend with gram-negative being the predominant agent. Similar investigations carried out in Israel and the USA also revealed a high prevalence of *E. coli* isolation from urine cultures [18]. Hi, Chrome agar growth was in perfect agreement with Sheep Blood Agar. Since there is no statistically significant difference, it may be concluded that UTI chromogenic agar is equally effective at isolating bacteria from the urine of UTI patients. Furthermore, the differentiation of species was possible in Chromogenic media, thus giving it an advantage over

the conventional media. The cost-effectiveness of such specialized media may be justified in the case of predominantly monomicrobial infections, as usually reported for UTIs.

The conventional antibiotic sensitivity test and the direct antibiotic sensitivity on chromogenic media both yielded similar results ($p > 0.05$). Selective use of DST and careful interpretation will affect efficient rapid and cost-effective methods for AST determination of UTI cases. Concern regarding the accuracy especially with mixed culture for AST on direct samples using non-standardized methodology should always be evaluated with utmost care. Despite the above practical results using such methods may offer better services in the interest of patient care [19]. The direct antibiotic sensitivity test benefits from the fact that urine is typically sterile and is primarily monobacterial during a UTI. Except for a little change in the zone diameter, the direct antibiotic sensitivity test provided results that were identical to those of the conventional antibiotic sensitivity test. Direct antibiotic sensitivity testing has been the subject of numerous investigations, all of which have shown it to be more effective than traditional antibiotic sensitivity testing [2, 13, 14]. The standardization of urochrome for AST is a potential issue as the CLSI zone sizes are interpreted with Muller Hinton Agar (MHA). This should always be kept in mind, and the sent data should be carefully interpreted. The patient benefit generated from such a test can justify the use of Hi-Chrome UTI media for DST. The proven advantage of color-changing media can assist the laboratory in providing faster results.

## CONCLUSION

In our investigation, chromogenic culture media appear to be at par if not better than conventional culture media because they have a higher rate of target pathogen detection or a higher ability to distinguish between mixed cultures. We conclude that chromogenic UTI agar medium can be a desirable, simple primary isolation and identification medium that significantly lessens the daily workload associated with urine culture in a microbiology laboratory. In monomicrobial illnesses, especially in OPD settings with no complications and a high-risk factor, DST using HiCrome media helps with the concurrent confirmation of bacterial species. It may be suggested for use as the main urine culture medium by clinical microbiology laboratories.

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## REFERENCES

1. Bajoria A, Kaore NM, Kaur S, Gautam SK. Utility of chromogenic media against conventional culture techniques for isolation of bacterial uropathogens in resource poor settings. Natl J Lab Med. 2019;8(1):MO05-8.
2. Khalid M. Comparison of Chromogenic (HiCrome Urinary Tract Infection Agar) Medium with Cysteine Lactose Electrolyte Deficient Agar in a Resource-Limited Setting. Int J Appl Basic Med Res. 2021;11(1):9-13.
3. Sikora A, Zahra F. Nosocomial infections. InStatPearls [Internet] 2021 Feb 10. StatPearls Publishing.
4. Sekikawa E, Vidicki MB, Bilkey MK. Comparison of two chromogenic media and conventional media in the primary isolation and identification of urinary tract pathogens. N Z J Med Lab Sci. 2011;65(3):77-82.
5. Burg L, Crewe G, DiMeo J, Guo X, Li CG, Mayol M, et al. Rapid pathogen identification and phenotypic antimicrobial susceptibility directly from urine specimens. Sci Rep. 2022;12(1):18315.
6. Kapur S, Gupta S, Dv P, Pal A, Pant J, Jain R. Rapid Sensor Based Technology: A Novel Tool for Direct Antimicrobial Susceptibility Testing in Urinary Tract Infection. Transl Med Biotecnol. 2014;2:22-8.
7. Collee JG. Mackie & McCartney Practical Medical Microbiology. 14th Edition; 2016.
8. Hay AD, Birnie K, Busby J, Delaney B, Downing H, Dudley J, et al.. The Diagnosis of Urinary Tract infection in Young children (DUTY): a diagnostic prospective observational study to derive and validate a clinical algorithm for the diagnosis of urinary tract infection in children presenting to primary care with an acute illness. Health Technol Assess. 2016;20(51):1-294.

9. Tille P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences; 2015.
10. Versalovic J, editor. Manual of clinical microbiology. American Society for Microbiology Press; 2011.
11. Mohammad RN, Omer SA. Direct disk testing versus isolation and antimicrobial susceptibility testing of urine from urinary tract infection. Iran J Microbiol. 2018;10(1):37.
12. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
13. Nahar SG, Hasan MD B, Aktar R, Naznin M. An assessment of Chromogenic Agar Medium and Conventional Culture System for the Isolation of Uropathogens. Sch J App Med Sci. 2020;8(1):114-9.
14. Sharmin S. Use of chromogenic media (Urochrom II) for detection of Uropathogen. [MPhil (Microbiology) Thesis]. University of Dhaka. 2005:55-68.
15. 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.
16. Perry JD, Butterworth LA, Nicholson A, Appleby MR, Orr KE. Evaluation of a new chromogenic medium, Uriselect 4, for the isolation and identification of urinary tract pathogens. J Clin Pathol. 2003;56(7):528-31.
17. Lakshmi V, Satheeshkumar T, Kulkarni G. Utility of Urichrom II - a chromogenic medium for uropathogens. Indian J Med Microbiol. 2004;22(3):153-8.
18. Gomaa SSE, EL-Mashad NB, EL-Deeb DR, Essa SG. Microbial Spectrum and Antibiotics Susceptibility Profile of Nosocomial Urinary Tract Infections in Mansoura Oncology Center. Int J Curr Microbiol Appl Sci. 2020;9(12).
19. Chooramani G, Jain B, Chauhan PS. Prevalence and antimicrobial sensitivity pattern of bacteria causing urinary tract infection; study of a tertiary care hospital in North India. Clin Epidemiology Glob Health. 2020;8(3):890-3.