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Original Research Paper

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EVALUATION OF CHROMOGENIC AGAR AND CLED AGAR FOR DIAGNOSIS OF UROPATHOGENS IN A TERTIARY CARE HOSPITAL

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ABSTRACT The increase in resistance of uropathogens to antimicrobial agents demands rapid identification of the pathogen. Chromogenic media are increasingly being used as versatile tool in early identification of bacterial isolates from clinical specimens. The chromogenic media offers simultaneous presumptive identification of gram-positive and gram-negative bacteria and yeasts on a single medium by means of distinct colony colours It is an easy to use primary screening medium that considerably reduces the daily workload and thus minimizes or limits the use of identification tests.

KEYWORDS : Chromogenic agar, uropathogens, CLED agar

INTRODUCTION

Urinary tract infection (UTI) is the most common type ofinfection and continues to be a major health problem. Also, there isincrease in resistance of microorganisms to antimicrobialagents,especially in hospitalized patients, demands rapididentification of the pathogens.[1]

For many yearsCystine lactose electrolyte-deficient agar,Blood agar and MacConkey agar have been used for the detection ofurinary tract pathogens, as well as for the differentiationof a few of them. [2] These current detection methods aretime-consuming, taking 48 hours for reporting of uropathogens and antimicrobial sensitivity. [1] The aim of the microbiology laboratory is to reduce morbiditythrough accurate identification of pathogens withappropriate antimicrobial sensitivity testing in short turnaround time. [3]

In the last few years several chromogenic media have beendeveloped and commercialized, allowing for more specificdirect differentiation of microorganisms on primary plates.The chromogenic agar offers simultaneous presumptiveidentification of gram-positive and gram-negative bacteriaand yeasts on a single medium by means of distinct colonycolours produced by reactions of genus- or species-specificenzymes with a suitable chromogenic substrate. [2]

A rapid and definitive urine culture method capable of detectingbacteria and its antibiotic susceptibility would beenormously beneficial in ensuring timely treatment. However, the rapid methods are yet to replace standard bacterial culture method. [2]

The present study was undertaken to evaluate and compare HiCrome UTI agar for its utility as a primary isolation and identification medium for urinary microbial isolatesin comparison to CLED agar.

MATERIALS AND METHODS

This prospective study included 455 , clean catch midstreamurine samples obtainedfrom patients attending the out patient departments or admitted in various in patient units(wards & ICUs) of Chattrapati Shivaji Subharti Hospital ,Meerut , over a period of 6 months (January 2019 to June 2019)

Culture media :

The culture plates (CLED and UTI chrom agar) wereprepared in-house using commercially availabledehydrated media (Hi Media Laboratories, Mumbai,India) according to themanufacturer's recommendations and dispensed intopetridishes and stored at 2-8°C tilluse.

Quality control:

Each batch of media wastested for sterility, biochemicals and chromogenic reactionswith American Type Culture Collection (ATCC) strains.

ATCC Control strains:

*Staphylococcus aureus*ATCC 25923,*Enterococcus faecalis*ATCC 29212, *Escherichia coli* ATCC25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa*ATCC 27853, *Klebsiellapneumoniae*13883, and *Candida albicans*ATCC 10231 were used for quality control. [4]

Inoculation of media and incubation :

All urine samples were inoculated simultaneously onboth media with a calibrated loop (holding 10μ l ofsample) using Mayo's technique without flaming the loop in between for isolation. The cultured plates were incubated at 37°Covernight. A presumptive identificationof the isolates was attempted based on the colonycolours on the UTI chromagar plates. The isolatedmicroorganisms were identified upto the species level asper the standard protocols. [5]

Antimicrobial sensitivity test :

Antimicrobial sensitivity testing was done by Kirby bauer disk diffusionmethod as per CLSI recommendations. [4]

Study Population:

All urine samples collected from patients with clinical symptoms of UTI.

INCLUSION CRITERIA:

- Patients ≥ 18 years of age , either admitted in ChattrapatiShivajiSubharti Hospital or visit the outpatient department(OPD) of the Hospital with clinical suspicion of UTI(Fever >38oC, Dysuria, Frequency, Suprapubic tenderness
- Presence of single type of bacterial morphology in gram stained smear and pus cells all genders

EXCLUSION CRITERIA:

- Patients < 18 years of age.
- Patients with history of antibiotic therapy.
- Patients on Foley's Catheter
- Pregnant woman
- Presence of epithelial cells and two or more types of bacterial morphology in gram stained smear

Ethics

The approval from the Institutional Ethics and Research Committee was obtained before conducting the study.

RESULTS

Out of 455 urine samples,growth was observed in 119/455(26.15%) and 112/455(24.61%)samples on UTI CHROM agar and CLED agar respectively. While remaining were found to be sterile.

Bacterial growth was observed in 111/455 (24.39%) and 104/455(22.86%) cases on UTI CHROM agar and CLED agar respectively. Growth of Candida species was observed in 8/455(1.76%) in each on UTI CHROM agar and CLED agar. (Table 1)

The predominant urinary pathogen isolated was *E.Coli*,50/119 (42.01%). All isolates grew onUTI chromogenic agar and CLED agar as well .This was followed by *Klebsiella pneumoniae*22/119(18.48%), again all grew on UTI chromogenic andCLEDagar.

Other uropathogens isolated were *Staphyloccus aureus* (15.12%) all isolates grew on chromogenic agar while only 12.60% isolates grew on CLED agar, , *Enterococcus species*(5.04%) all isolates grew on chromogenic agar while only 1.68 % isolates grew on CLED agar.Other isolates were *Pseudomoas species,* (8.40%), *Non albicans candida species* (5.88%)*Klebsiella oxytoca*(3.36%), *Proteus mirabilis*(0.84%) *and Candida albicans*(0.84%).Similar isolation rate was observed for all these uropathogens in both UTI chromogenic agar and CLED agar. (Table 2)

Overall isolation of uropathogens on UTI CHROM agar was 100% while 94.12% on CLED agar. (Table 3)

RESULT

Table 1 Results of urine culture on UTI CHROM Agar and CLED agar (n=455)

Growth	UTI CHROM Agar	CLED Agar
Bacterial	111(24.39%)	104(22.86%)
Candida Species	8(1.76%)	8(1.76%)
No Growth	336(73.85%)	343(75.38%)
Total	455	455

Table no. 2 Distribution of uropathogens among positive urine culture specimens on UTI CHROM Agar and CLED agar

Microorganism	Total	UTI CHROM Agar	CLED Agar
Escherichia coli	50	50(42.01%)	50(42.01%)
Klebsiella pneumoniae	22	22(18.48%)	22(18.48%)
Staphylococcus aureus	18	18(15.12%)	15(12.60%)
Pseudomonas species	10	10(8.40%)	10(8.40%)
Non albicans candida	7	7(5.88%)	7(5.88%)
Enterococcus species	6	6(5.04%)	2(1.68%)
Klbesiella oxytoca	4	4(3.36%)	4(3.36%)
Proteus mirabilis	1	1(0.84%)	1(0.84%)
Candida albicans	1	1(0.84%)	1(0.84%)
Total	119	119	112

Table 3 Overall isolation of uropathogens on UTI CHROM agar and CLED agar

Total no of uropathogens isolated	Uropathogens isolated on UTI CHROM Agar	Uropathogens isolated on CLED Agar
119	**119/119(100%)**	**112/119(94.12%)**

DISCUSSION

Out of 455 urine samples,single type of growth was observed in 119/455(26.15%) samples. While remaining were found to be sterileOur finding correlates with Parveen *et. al.* [6] in which 31.67% samples showed single growth.alsoD'Souza HA et al from California reported single growth in 24.5% samples. [7]

The rate of isolation and pattern of major urinary pathogens oftheour study are in accordance with a few other studies carriedout on both chromogenic and conventional media. Accordingto present study, *E.coli*was the predominant causativeagent (42.01%), followed by *Klebsiellapneumoniae*. (18.48%),*Staphylococcus aureus*(15.12%). (Table 1)

Similarly Jadhav*et al*. also reported *E.coli* as the predominant causative agent (33.74%),followed by *Klebsiella pneumonia* (14.01 %), *Staphylococcus aureus(12.97%).* [1]

Similar results were found in a study from Hyderabad (India),most common organism was *Escherichiacoli* (47.5%) followed by *Klebsiella*spp (18.1%), [8] Parveen *et al* reported higher isolation rate of *E.coli*(64.49%) followed by *Klebsi ella*spp (11.21%). [6]

Presumptive identification of bacterial isolates in urine culture is time consuming and requires a great deal of experience and labour when using conventional media like CLED agar. HiCrome UTI agar medium was found to be much superior over conventional media for its higher rate of isolation and uniform interpretation for identification of uropathogens. As many of the extra tests for bacterial identification associated with conventional culture methods were no longer required, chromogenic medium substantially reduced the laboratory workload with high bench output. [9]

In our study, on Hicrome UTI agar approximately 100% ofthe isolates were identified while CLEDagar, presumptively identified only 94.12% (Table 3). Similarly R Perveen*et al*. concluded that 94.39% of isolateswere presumptively identified by HiCrome UTI agar media and 74.77% by CLED agar media [6] a study by Sharmin et al. [10] also concluded that the chromogenic medium considerably reduced workload and minimized the use of conventional identification tests.

A high percentage of bacterial species were possible to be identified on HiCrome UTI agar by matching with standard colours as compared to conventionalculture system. This high rate of identification could becorrelated with the ease of identification technique by seeing the distinct colony colour produced by each of the bacterial species on chromogenic agar medium.

The present study revealed that, 100% *E. coli* were isolated and presumptively identified on Hicrome UTI agar and CLED agar.

The present study also reveals that Hicrome UTI agar and CLED agar contributed 100% presumptively identification of *Klebsiella spp.* Our results are similar with the results reported by Akter L *et al* which stated 100% *Klebsiella* isolates were presumptively identified on Hicrome UTI agar. [9]

In our study, approximately all isolates of *Enterococcus spp.* were presumptively identified on Hicrome UTI agar by colour production while only 2 /6 isolates grew on CLED agar.

This could be due to the reason that in CLED medium the presence of *Enterococci* was frequently masked by larger colonies of Gram negative bacteria.

Our finding is consistent with study by Sharmin et al [10] , who also reported that100% of Enterococci were identified on chromogenic agarbut only 95% on CLED agar. According to R Parveen 100%were identified on HiCrome UTI agar media but on CLEDagar media only 33.33% *Enterococci* were identified. [6]

CONCLUSION

The authors conclude that though expensive, chromogenic media like HiCrome UTI Agar media, is an acceptable alternative to traditional media for the isolation of urinary pathogens. HiCrome UTI Agar may facilitate improved sensitivity of identification of some Gram positive cocci (e.g., enterococci) in mixed cultures with *Enterobacteriaceae*. Chromogenic media may also promote more rapid identification of the uropathogens and may provide clinicians with relevant information regarding their choice of empirical antimicrobial therapy for patients.

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