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Original Article

Microbiology Section

Utility of Chromogenic Media against Conventional Culture Techniques for Isolation of Bacterial Uropathogens in Resource Poor Settings

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ABSTRACT

Introduction: 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.

Aim: The study was planned to evaluate chromogenic media against conventional techniques in terms of correct identification and colony count with ease of reporting with less human resource and reduction in the cost.

Materials and Methods: This Cross-sectional Prospective Analytical study was carried out from 1st July 2018 to 31st August 2018 at Raipur Institute of Medical Sciences, Raipur after due approval from Institutional Ethics Committee for waiver of consent. Urine samples received in bacteriology laboratory were inoculated on CLED agar and chromogenic media simultaneously and incubated overnight. Isolates were identified by colony's colour for Hichrome UTI agar and by standard microbiological techniques for CLED agar. The results were then maintained in Microsoft Excel and were analysed using test of proportion and significance.

Results: A total of 35/77 samples were culture positive with 46 clinical isolates. *E.coli*, 20/46 (43.47%) and *Klebsiella pneumoniae* 8/46 (17.39%) followed by *S. aureus* and *Candida albicans* with 6/46(13.04%) each were predominant isolates. HiChrome UTI agar was found to be highly significant with p-value of <0.001 with a sensitivity and specificity of 88.57% and 92.85% respectively with an average time of 20 hours for identification when compared with conventional media.

Conclusion: Chromogenic media is highly sensitive and specific for detection of urinary pathogens and will help to reduce time, cost and workload in resource poor settings.

Keywords: CLED agar, HiChrome UTI agar, Urinary tract infection

INTRODUCTION

UTI are one of the most frequent infection encountered in hospital settings as well as in community, making urine one of the most cultured specimens in laboratories across the world [1]. UTI constitutes the third most common infection found in India affecting people of all age group and of every socioeconomic strata. It is common in the females due to short urethra and about 60% women are reported to have experienced the UTI once in their lifetime. The increasing risk factor in females is due to short urethra, absence of prostatic secretions, pregnancy and easy contamination of urinary tract with faecal flora. A 90% of pregnant women develop urethral dilation and are susceptible to UTI [2].

The Urine culture occupies most of the time of the clinical microbiology laboratory which commonly employs routine media like Blood Agar (BA), MacConkeys Agar (MA) or Cysteine Lactose Electrolyte Deficient agar (CLED) agar for isolation, presumptive identification and further confirmation of identification using battery of biochemical tests or specific tests for the organisms requiring almost 48 hour for identification and AST reporting. Accurate identification of Uro-pathogens in shortest possible time is the primary responsibility of a clinical microbiologist.

Various chromogenic medias that can identify the bacterial uropathogens in 24 hours time with accuracy and ease have been developed to achieve the same [3].

Bacteria possess various enzymes for utilisation of various substrates for their physiological function. In Chromogenic Medias, chromogenic substrates are specifically broken down by the enzyme possessed by the particular bacteria thus imparting a distinct colour to the isolated bacterial colony which can be visualised and identified. Thus, Chromogenic media does not require various identification tests for confirmation of isolate and can reduce the time required for accurate reporting and can help physicians in not only minimising the need for further identification tests but also reduce the time and cost required to report the results to the clinician to facilitate early initiation of antibiotic therapy. This is of particular importance in resource poor setting, lacking the medias, battery of biochemical tests and the laboratory technicians as human resource [3].

Thus, the present study was planned primarily to establish the utility of Chromogenic media in isolation of pathogens in urine against conventional techniques in terms of correct identification and colony count which will help reduce the cost in resource poor settings.

MATERIALS AND METHODS

This Cross-sectional Prospective Analytical study was carried out in the Department of Microbiology, at Raipur Institute of Medical Sciences, Raipur from 1st July 2018 to 31st August 2018. Clean catched mid-stream urine sample or aseptically collected samples from catheterized patients received in bacteriology laboratory from patients of all ages and gender attending the OPD and IPD of the tertiary care hospital were processed after due approval from Institutional Ethics Committee (IEC) with a waiver of consent. (Approval Letter no. RIMS/ADMIN/DIS/05/040518 dated 04.05.2018 from IEC RIMS, Raipur Registration No. ECR/969/Int./CG/2017) All the repetitive samples from the same patient and fungal as well as mycobacterial isolates were excluded.

Preperation of Chromogenic Media

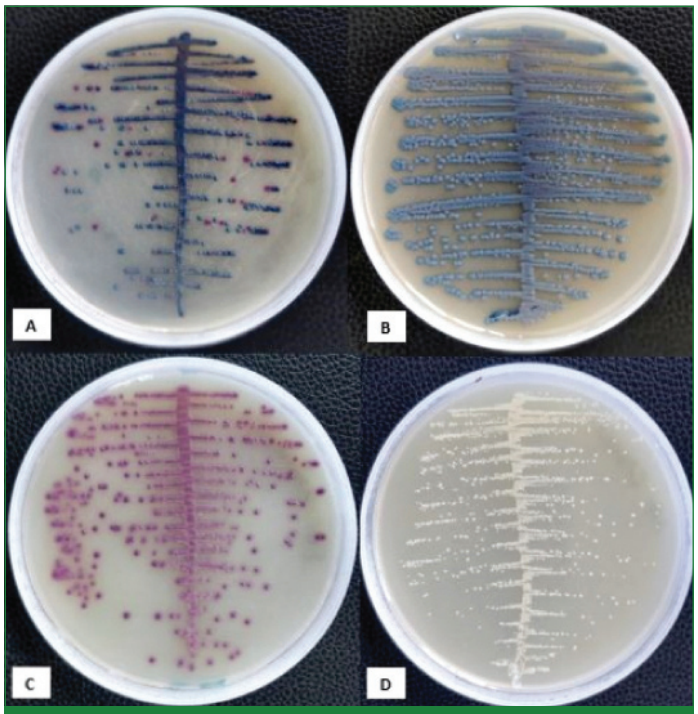
The HiChrome UTI agar from Hi-Media India (M-135 R) was procured. The dehydrated powder 56.8 gm was mixed with 1000 mL distilled water which was heated to dissolve the medium completely which then was sterilized by autoclaving at 15 Lbs pressure (121°C) for 15 minutes, cooled to 50°C and poured into sterile Petri plates [4].

Inoculation of Sample

The urine samples received in bacteriology laboratory was inoculated on the HiChrome agar as well as CLED using a calibrated loop to know the colony forming units per mL of urine and to obtain the isolated colonies for identification. Both the medias were incubated at 37°C for 18 to 24 hours [5].

Reading of Plates

After incubation, the HiChrome agar plates were read as per the instructions of the manufacturer where different isolate produce colonies of the specific colour [Table/Fig-1a-d]. The colony count was done manually using the colony counter.



[Table/Fig-1]: Showing growth on HiChrome UTI Agar: A) Mixed growth of *E. coli* and *Klebsiella pneumoniae* on Chrome agar; B) Growth of *Klebsiella pneumoniae* on Chrome agar; C) Growth of *E. coli* on Chrome agar; D) Growth of *S. aureus* on Chrome agar.

The CLED agar plates were also read for colony count. The isolates were identified on the basis of colony characteristics with standard microbiological identification techniques followed by AST [5].

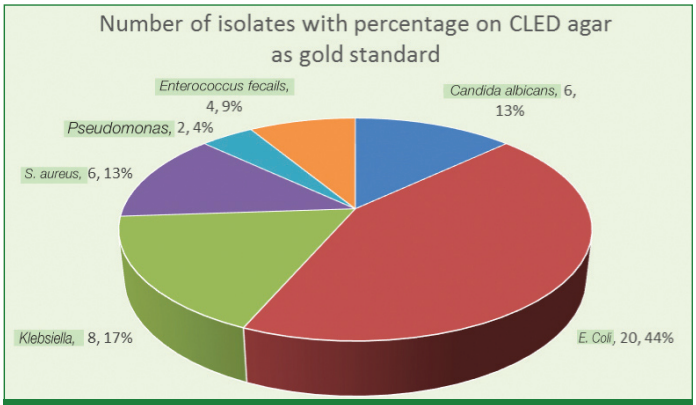
The identification as per the HiChrome agar and the colony count was compared for utility of the Chromogenic media [4,5].

All data was maintained in Microsoft office Excel. All the tabulated data was then analysed using Excel and appropriate Statistical tools like tests of proportion and test of significance like Pearson's Chi-Square Test.

RESULTS

A total of 77 urine specimens received in Bacteriology laboratory included 37 males and 40 females, with 22 (29%) sample coming from OPD while 55 (71%) samples coming from IPD. Majority of the samples were received from casualty, General Medicine and Obstetrics and Gynecology department with 18/77 (23.37%), 17/77 (22.07%) and 14/77 (18.18%) respectively followed closely by paediatrics and ICU with 9/77 (11.68%) each and Surgery 8/77 (10.38). One sample each was obtained from Ophthalmology and Surgical ICU.

The samples were cultured on CLED and HiChrome Agar and identified using standard microbiological techniques. The isolates by conventional culture methods were taken as a gold standard. A total of 35/77 (45.45%) samples were culture positive of which around 10 samples yielded a poly-microbial growth. Out of 35 positive samples, 16 belonged to males and 19 to females. The 35 culture positive samples grew a total of 46 strains [Table/Fig-2]. The predominant isolates were *E.coli*, 20/46 (43.47%) and *Klebsiella pneumoniae* 8/46 (17.39%) closely followed by *S.aureus* and *Candida albicans* with 6/46 (13.04%) each. A total of 4/46 (8.69%) isolates were of *Enterococcus fecalis* and 2/46 (4.34%) of *Pseudomonas aeruginosa*.



[Table/Fig-2]: Number of isolates with percentage on CLED agar as gold standard.

A total of 10 samples have shown the poly-microbial growth in CLED as well as Chrome agar of which 6/35 (17.14%) culture positive samples grew *E. coli* and *Klebsiella*. 2/35 (5.71%) samples grew *Enterococcus fecalis* along with *Candida albicans* whereas 1/35 (2.85%) sample each showed growth of *E. coli* and *Candida albicans* and *E. coli*, *Enterococcus fecalis* and candida species respectively.

Three samples have shown growth in Hichrome agar which CLED agar has missed. The additional pathogens identified on HiChrome agar were one strain of *S. aureus* and two strains of *Candida albicans*, with a significant bacterial count. Whereas four Samples have shown growth on CLED and not on HiChrome UTI agar, of which three strains were of *S.aureus* and one of *E.coli* but the bacterial count was only five to six colonies for each of the strain which was not significant. It is also important to note that isolates of *Candida albicans* have not shown any colour change on chromogenic media but white opaque colonies were seen which can be clearly differentiated.

Evaluation of HiChrome Agar Compared to CLED

Growth on HiChrome was then compared with the gold standard i.e., conventional CLED agar. [Table/Fig-3] which was found to be highly significant with p-value <0.001 with a sensitivity and specificity of 88.57 and 92.85% respectively.

	CLED Positive	CLED Negative	Total	Chi-Square Value and p-value	Significance
HiChrome UTI Agar Positive	31	3	34	Chi-Square- 51.33 p-value -<0.001	Highly Significant
HiChrome UTI Agar Negative	4	39	43		
Total	35	42	77		

[Table/Fig-3]: HiChrome agar compared with gold standard CLED agar using Pearson's Chi-Square test as test of significance.

Sensitivity	88.57%
Specificity	92.85%
Positive predictive value	91.17%
Negative predictive value	90.69%

The average time taken for identification on HiChrome Agar was 20 Hours whereas for CLED using all the required standard microbiological test was 36.26 hours. Most importantly the use of chromogenic media was hassle-free as it gave the direct identification of the clinical isolates similar to the gold standard media routinely used in the laboratory. The isolates identified on the chromogenic media were in 100% agreement with that of the CLED agar. In the present study, any reduction in the colony count could not be seen as well as compared to conventional media in chromogenic media used. In-fact three additional colonies could be identified on the Chrome agar which would have been missed on the conventional agar.

DISCUSSION

In the present study, the culture positivity rate was 35/77 (45.45%). Similar results were seen with other studies that include a study by Akter L et al., where he recorded the prevalence rate of UTI as 42.66% [6] and another study by Qaiser S et al., that recorded the prevalence rate as 69.32% [7]. In the present study, the urine samples were cultured irrespective of pus cell count as the utility of HiChrome agar was assessed, which may be the reason for lower percentage of growth in the study than that of their study results. Other studies by Parveen R et al., and Manjushree BS et al., recorded the prevalence rate of 33.66% and 34.84% respectively [8,9].

The prevalence rate in females and males was 19/35 (54.28%) and 16/35 (45.71%) respectively. The minor difference in culture positivity in different gender in the present study because of smaller sample size and also because the majority of samples were collected from IPD which rules out the greater prevalence rate in females generally reported in various studies. In a study carried out by Kolawole AS et al., (2009) in Nigeria, reported 120/180 (66.67%) were females against 60/180(33.33%) males [10]. Another study by Premanatham N et al., at Andhra Pradesh concluded that the rate of culture positivity in females was 131/143(91.6%) and in males was 23/54 (40.3%) [11]. The increased number of samples is contributed by females because of more prevalence of UTI amongst females. Different reasons like shorter urethra, absence of prostatic secretions, delay in micturition may contribute to this result [2].

Around 55/77 (71.42%) samples in the present study came from IPD and 22/77 (28.57%) from OPD. The majority of IPD patients in the present study are because in a rural based tertiary care hospital like ours people follow-up only after multiple opinions from quacks and pharmacists outside or when their condition deteriorates to an extent requiring hospitalisation.

By conventional culture techniques, 46 clinical isolates were seen on CLED, of which the predominant isolates were of *E.coli*, 20/46 (43.47%) and *Klebsiella pneumoniae* 8/46 (17.39%) followed by *S.aureus* and *Candida albicans* with 6/46 (13.04%) each. A total of 4/46 (8.69%) isolates were of *Enterococcus fecalis* and 2/46 (4.34%) of *Pseudomonas aeruginosa*. This is in accordance with the study carried out by Manjusree BS et al., which recorded 45/123 (36.58%) *E coli* as the major organism followed by 26/123 (21.13%) *Klebsiella*, 15/123 (12.19%) *Candida*, 14/123 (11.38%) *Enterococcus spp*., 12/123 (9.75%) *Pseudomonas aeruginosa*, 5/123 (4.06%) each of *Staphylococcus aureus* and *Acinetobacter* and 1/123 (0.84%) *Serratia species* [9]. Another study carried out by Okonko IO et al., amongst the pregnant women also concluded that *E.coli* was the most frequent isolate [12].

There was 100% correlation between the growth and colony count observed on CLED agar as well as Chromogenic media with ease of identification because of specific colour production on it. According to the technical data it is said that the chromogenic media shows approximately 30% reduction in colony count which was not evident in the present study. This difference could have been produced because the study included the use of urine samples with clinical isolates whereas the technical data was recorded using the standard strains of the organism [4].

A total of 10/35 (28.57%) culture positive samples have shown poly-microbial growth and all the 10 poly-microbial growths were identified in both CLED and chrome agar making them equally sensitive for identification of bacterial uro-pathogen. This is in accordance with the study carried out by Qaiser S et al., which stated that the number of samples showing mixed growth was same for both the media but the mixed growth could easily be appreciated on chromogenic media because of variation in colour [7]. But the study carried out by Manjusree BS et al., compared various medias for growth and concluded that there is 60/60 (100%) identification by chromogenic agar in mixed growths whereas 36/60 (60%) identification by CLED, 35/60 (58%) by Mac-conkey agar and 30/60 (50%) by blood agar [9]. Parveen R et al., in their study showed 100% detection by HiCrome UTI Agar media, whereas only 1/6 (16.67%) was detected by CLED agar and Blood agar and Mac Conkey agar media respectively [8].

In the present study 3/77 (3.89%) samples (one strain of *S. aureus* and two strains of *Candida albicans*) had shown growth in Hichrome agar which were missed by CLED agar. This is in accordance with a study carried out by Qaiser S et al., where 7/174 (4.02%) samples showed growth on chromogenic media which was evident after prolonged incubation of 48 to 72 hours on CLED [7].

In the present study also showed that four Samples have shown growth on CLED and not on HiChrome UTI agar (three strains of *S.aureus* and one of *E.coli*) but the bacterial count were only five to six colonies for each of the strain which was not significant. This is in accordance with the study carried out by Paul S et al., where out of 100 samples 68 were found to be positive in conventional media and 64 were found to be positive in chromogenic media. They concluded that the difference was not significant with $p>0.05$ [13].

Comparison of chromogenic media with conventional CLED media reveals the sensitivity and specificity of chromogenic media was recorded as 88.57% and 92.85% respectively with positive predictive value and Negative predictive value of over 90% [Table/Fig-3].

In the present study, it was also seen that the results on chromogenic agar were evident within 20 hours whereas for CLED using the entire required standard microbiological tests it was an average of 36.26 hours. Similarly in a study by Paul S et al., it was concluded that the chromogenic agar could produce results for urine specimen within 24 hours while the conventional media requires 48 hours to give the positive results [13].

Hence, it can be concluded that the use of Hichrome media requires less time, it is user friendly and reduces the cost as also evident from the study by Qaiser S et al., [7] and decreases the workload in the resource poor settings which is in accordance with the study carried out by Manickam K et al., which concluded that by the use of chromogenic media the average no. of workload units (one workload unit equals one minute of hands on labor) per urine specimen were significantly reduced from 2.67 in 2006 over to 1.88 in 2011 [14].

The higher cost of chromogenic media as compared to the conventional media can be compensated by reduction in the additional cost for the biochemical tests required in case of other conventional media to identify the organism. This was also concluded by Qaiser S et al., that in their study the reagents required were less in case of chromogenic agar as compared to those used for identification of organism by using other conventional media [7].

LIMITATION

The study is having a limitation of small sample size being a short term studentship project to be completed within a small time frame but the utility of the chromogenic media in resource poor settings needs to be explored in large scale and multi-centric trials for correct identification using less human hours and cost benefit.

CONCLUSION

This study has established the utility of chromogenic media in isolation of pathogens in urine against conventional techniques in terms of earlier response, correct identification and correct colony count which has helped to reduce the cost in resource poor settings. It obviates the need of various biochemical tests for correct identification of the isolates thus reducing the overall cost of the investigation for the benefit of the patients, institute and community at large.

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