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RESEARCH ARTICLE

COMPARISON OF CHROMOGENIC MEDIA AND COVENTIONAL MEDIA IN PRIMARY ISOLATION AND IDENTIFICATION OF URINARY TRACT PATHOGENS

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ABSTRACT

Introduction: Urinary tract infections (UTIs) are prevalent worldwide and are considered to be the most common type of bacterial infection in humans.The present study was undertaken to evaluate the use of chromogenic media as primary media for isolation of uropathogens and to compare it with the convention methods of isolation and identification of bacteria.

Materials and Methods: A total of 200 samples were tested and inoculated on Blood agar(BA) and MacConkey agar(MA)and HiCrome UTI Agar media, Isolates were identified based on colour on HiCrome UTI Agar media and by standard identification protocol on MA and BA.

Results: Out of 200 urine sample tested, 59 (29.5%) yielded significant growth of single organism and 5(2.5%) yielded polymicrobial growth of 2 or 3 organisms.Rate of presumptive identification of organisms in primary culture plate was high in HiCrome UTI agar media. For *Escherichia coli,* it was 94.73%) whereas and by Blood agar and MacConkey agar media in combination it was 76.31%.

Conclusion: The result of this study showed that HiCrome UTI Agar media can replace use of conventional media in clinical laboratory as primary isolation media for urine culture particularly with common uropathogens like E.coli as it is user-friendly ,facilitates early reporting, saves time as well as cost and less labour intensive.

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INTRODUCTION

Urinary tract infections (UTIs) are prevalent worldwide and are considered to be the most common type of bacterial infection in humans. In the community, the majority of UTIs are uncomplicated but are often recurrent. The incidence of these infections is high, with an estimated 80-90% of women experiencing at least one episode during their lifetime. Its importance in the hospital setting is also profound, where they are responsible for 40-60% of all nosocomial infections. (Elly Sekikawa *et al*., 2011) The etiology of nosocomial UTIs are often more diverse than uncomplicated community-acquired infections and can be frequently polymicrobial. (Ronald, 2003) Conventionally, Blood agar (BA), MacConkey agar (MA) and cystine lactose electrolyte deficient medium (CLED) are used as primary media for the processing of urine samples by culture. Over the last few years, several chromogenic media have been developed and commercialized, allowing more specific and direct differentiation of microorganisms on the primary plates. They not only minimize the need for further identification tests but also reduce the time required to report the results to the clinician to facilitate early initiation of antibiotic therapy. (Saba Qaiser *et al*., 2011) Previous studies comparing chromogenic media with traditional ones have shown advantages which include 20% reduction in time for identification, reduction in workload, easier recognition of mixed growth and reduction in number of biochemical test performed for identification of bacteria which ultimately results in cost reduction. (Lakshmi *et al*., 2004) Most of these studies have been conducted in developed countries; with only one study assessing the feasibility of chromogenic media in a resource limited setting.

In developing countries the apparent higher cost of chromogenic media is a major hindrance for their routine use and their utility has never been explored. (Saba Qaiser *et al*., 2011) The present study was undertaken to evaluate the use of chromogenic media as primary media for isolation of uropathogens and to compare it with the conventional methods of isolation and identification.

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MATERIALS AND METHODS

Source of data

The present study included urine samples from 200 clinically suspected cases of UTI attending Victoria and Vani Vilas hospitals, Bengaluru (both outpatient and inpatients) between August 2014 and September 2014. Urine samples yielding non fermenters and yeasts were excluded from the study. Urine samples were processed in Department of Microbiology, BMCRI. Approximately 20 ml of clean-catch mid-stream urine sample was collected aseptically in a sterile wide mouth container. (Forbes *et al*., 1998) The samples were inoculated in Blood agar (BA), MacConkey agar (MA), and HiCrome UTI agar media using calibrated loop (holding 10µl of sample) and incubated overnight as standard protocol. Samples showing significant bacterial growth ($>10^5$ /ml) were further processed.

A presumptive identification of the isolates was made on the colony colour on the HiCrome UTI agar based on manufacturer's instructions (Himedia); the isolated pathogens on BA and MA agar plates was identified by standard identification protocol such as Gram's staining and standard biochemical tests. (Collee and Marr, 1996; Garcia *et al*., 1998) Instituitional ethical clearance was obtained for the present study.

RESULTS

Out of total 200 patients, 98 (49%) were male and 102(51%) were female. Among the 200 study population, 70% cases were in more than 18 years age group. Out of 200 urine sample tested, 59 (29.5%) yielded significant growth of single organism and 5(2.5%) yielded polymicrobial growth of 2 or 3 organisms. Total 11 organisms were isolated from 5 polymicrobial growth. Of which E.coli(n=5) was isolated from all 5 cases, Klebsiella (n=3) was isolated from 3 cases and enterococci (n=3) was isolated from 3 cases.No growth was observed in total 136(68%) cases. (Table 1)

Table 1. Details of organisms isolated

Organisms	Single	Polymicrobial	Total
E.coli	33	05	38(54.28%)
Klebsiella sp	12	03	15(21.42%)
Proteus	02	00	02(2.85%)
Enterococcus spp	10	03	13(18.57%)
Staph.aureus	02	00	02(2.85%)
Total	59	11	70(100%)

Of the 5 samples with polymicrobial growth,80% was detected by HiCrome UTI agar, where as only 20% was detected by BA/MA agar. (Table 2) Total of 70 strains were isolated of which E.coli(54.28%) was the most common etiologic agent followed by Klebsiella spp. Presumptive identification rate was found to be highest in Hi Crome UTIagar (91.42%) followed by conventional media BA/MA(64.28%). (Table 2)

Of the 5 samples with polymicrobial growth, 80% was detected by HiCrome UTI agar, whereas only 20% was detected by BA/MA agar. (Table 3)

Table 2. Detection of Polymicrobial growth in different Media (n=11)

Polymicrobial growth	Total no. of cases	Cases detected by HiChrom UTI agar	Cases detected by conventional media
Klebsiella spp & E.coli	02	02(100%)	00
E.coli & Enterococcus Spp	02	01(50%)	01(50%)
E.coli,Klebsiella spp & Enterococcus Spp	01	01(100%)	00
	05	04(80%)	01(20%)

Table 3. Presumptive identification of organisms in different culture media

Organism presumptively identified	In Hichrom UTI agar	In BA/MA
E.coli(n=38)	36(94.73%)	29(76.31%)
Klebsiella spp(n=15)	14(93.33%)	09(60%)
Proteus spp(n=2)	01(50%)	02(100%)
Enterococcus spp(n=13)	11(84.61%)	04(30%)
Staph.aureus(n=2)	01(50%)	02(100%)
	63(90%)	46(65.71%)

DISCUSSION

Quantitative culture of urine samples on solid media forms the mainstay of diagnosis of UTI. Conventional media used include CLED agar, BA and MA agar. Presumptive identification of urinary pathogens on conventional media is limited and requires expertise. The media which utilise various chromogenic substrates has made the process of presumptive identification and interpretion of urine cultures easier and faster. Previous researchers have demonstrated the equal or superior performance of various chromogenic media compared to conventional media for the isolation and identification of urinary tract pathogens. (D'Souza *et al*., 2004; Chang *et al*., 2008) In the present study, a total of 200 samples of urine from clinically diagnosed cases of UTI were included. 59(29.5%) showed single growth, 5(2.5%) showed polymicrobial growth and 136(68%) cases yielded no growth. Of total 64(32%) culture positive cases, 70 strains of organisms were isolated, 59 strains were monomicrobial and 11 strains were from 5 samples of polymicrobial growth. Out of total 38 *Esch.coli,* 36(94.73%) were identified in HiCrome UTI agar, wheras 29(76.31%) were identified in MacConkey &Blood agar media. Among the other gram negative pathogens 14(93.33%) of Klebsiella sp (n=15)15 were identified on HiCrome UTI agar, and 09(60%) were identified on BA/MA and of the Proteus sp, 50% were identified on HiCrome UTI agar and 100% were identified on BA/MA. In our study, among 13 *Enterococcus spp.*, 11 (84.61%) were identified on HiCrome UTI agar media because of small blue coloured colonies. On conventional media only 4(30.76%) *Enterococci* were identified. Findings of the present study are comparable with the results of study done by Parveen *et al*. (2011) Most of the organisms isolated in the study were correctly presumptively identified based on colour production as described by the manufacturers except Staph.aureus. The colour produced by S.aureus was also produced by Pseudomonas and yeasts. The findings were consistent with the findings of Sekikawa *et al*. (2011). The limitation of the study was the moderate number of

samples included in the study as only samples received during August and September 2014 were included in the study. Hence the present study could not provide insight into further differentiation of other Gram negative pathogens causing UTI like Enterobacter Spp and Citrobacter spp. Results of the present study showed that HiCrome UTI agar is a user friendly medium that supports the growth of uropathogens like E.coli, Klebsiella and Proteus with presumptive identification within 18 hrs. Additional workload of setting up biochemical reactions for identification can be avoided if chromogenic media is used. Identification of individual pathogen in polymicrobial growth was better appreciated in HiCrome UTI agar due to colour contrast than in conventional media. To conclude, the results of the present study suggests that though expensive, chromogenic media like HiCrome UTI Agar media, offer an excellent time saving method for the reliable direct identification of common urinary tract pathogens especially E.coli by colony colour .It was also observed that differentiation of mixed bacterial cultures in HiCrome UTI Agar media was better when compared to conventional media. HiCrome UTI Agar media can replace use of conventional media in clinical laboratory as primary isolation media for urine culture particularly with common uropathogens like E.coli as it is user-friendly, facilitates early reporting, saves time as well as cost.

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