

Original Article

Detection of Uropathogens by Using Chromogenic Media (HiCrome UTI agar), CLED agar and other Conventional Media

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Abstract

This study was undertaken to find media better than routinely used media in isolation of uropathogens. Three hundred urine samples having pus cells ≥ 5 /HPF were enrolled for the study. Comparison of isolation and identification of uropathogens among HiCrome UTI Agar media, 5% Sheep Blood agar & MacConkey agar and CLED agar media were done. Among them 95(31.67%) samples showed single growth, 6 (2%) showed mixed growth and 199 (66.67%) showed no growth. Rate of presumptive identification of organisms in primary culture plate were high in HiCrome UTI agar media. For *Escherichia coli*, it was 94.20% whereas in CLED agar it was 79.71% and by Blood agar and MacConkey agar media in combination it was 82.61%. All the *Enterococcus spp.* were identified in HiCrome UTI agar media, 33.33% in CLED agar media but none in Blood agar and MacConkey agar media. Among the mixed growth, 100% organisms were identified on HiCrome UTI Agar media due to distinct colour produced by the different organisms, whereas in one (16.67%) sample (mixed *Esch.coli* and *Pseudomonas spp.*) organisms were identified on other three media.

Key words: UTI, Uropathogen, HiCrome UTI Agar media

Introduction

Urinary tract infection (UTI) is defined by the presence of more than 10^5 organisms per ml in a midstream sample of urine¹. It is estimated that about 35% of healthy women suffer symptoms of urinary tract infection (UTI) at some time in their life. Urinary tract infection is caused mainly by normal bowel flora-principally *Escherichia coli*, responsible for $\geq 75\%$ of cases². Other Gram-negative Enterobacteriaceae and Gram positive *Enterococcus faecalis* and *Staphylococcus saprophyticus* are responsible for

remainder of most commonly acquired UTI. More than 95% of urinary tract infections are caused by a single bacterial species^{3,4}. Urine samples are among the most numerous specimens sent for microbiology studies. This heavy workload demands a cost effective method for the diagnosis of urinary tract infections⁵. The aim of the microbiology laboratory is to reduce morbidity through accurate and timely diagnosis with appropriate antimicrobial sensitivity testing⁶.

Blood agar is considered to be the optimal medium for the isolation of organisms from clinical samples including urine⁷. Since 1905 the most widely used medium in the clinical laboratory for the isolation and differentiation of coliform organisms and enteric pathogens has been the MacConkey's agar media⁸. It differentiates Gram-negative bacteria but does not support the growth of all organisms involved in UTI, therefore conventionally Blood agar and MacConkey agar medium are used together for the isolation of urinary pathogens^{7,9}. CLED (Cystine Lysine Electrolyte Deficient) agar, introduced later, has proven to be useful as primary medium and helped to reduce the plate burden and workload. It has advantage of supporting the growth of certain *Staphylococci*, *Sterptococci* & *Candida*. Though MacConkey agar and CLED agar media distinguishes between lactose fermenting and lactose nonfermenting colonies, further identification of different genus of lactose fermenters

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Principle of chromogenic agar media is based on the fact that, bacteria have many enzymes for their physiological function that help them to utilize substrates. In such media, chromogenic substrates are specifically broken down by the enzyme present in the particular bacteria thereby imparting a distinct colour to the growing bacterial colony that can be visually observed, Chromogenic media 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 therapy¹².

Materials and methods

This study was carried out in the department of Microbiology of Dhaka Medical College during the period of January 2006 to December 2006. It was a cross sectional study. Clinically diagnosed UTI cases from out patient department and in-patient department of Dhaka Medical College Hospital, Dhaka, irrespective of age and sex were examined for presence of pus cells in urine. Among them, 300 cases having pus cells ≥ 5 /HPF in the deposits of centrifuged urine were included in this study. A detail medical history of the patient was taken and the data were recorded in a preformed data collection sheet.

With all aseptic measures clean-catch mid stream technique was employed to collect urine sample. All the urine samples were inoculated aseptically on HiCrome UTI agar, CLED agar, Blood agar and MacConkey agar media with a calibrated loop and were incubated aerobically at 37°C for 18-20 hours. Growth of 100 colonies indicate significant growth of organisms which equals to 10⁵ colony forming units (CFU) of bacteria /ml of urine.

Criteria for Significant Bacteriuria^{4,13}.

- 1) Presence of $\geq 10^5$ CFU of non-coliforms/ml or $\geq 10^2$ CFU of coliforms/ml in a symptomatic woman.
- 2) Presence of $\geq 10^3$ CFU bacteria /ml in a symptomatic man.
- 3) Growth of two different organisms of possible uropathogens at a concentration $> 10^4$ CFU/ ml.

A presumptive identification of the isolated organisms were made on the morphology and colour of colonies on HiCrome UTI Agar media, CLED agar, Blood agar and MacConkey agar plates and was confirmed by standard identification protocol such as Gram's staining, motility test, oxidase test, catalase test and other relevant biochemical tests⁷.

Results

Among 300 cases, most of them (31.33%) were in the age group of 21-30 years. Of them, 95 (31.67%) samples showed growth of single organism, 6 (2%) showed mixed growth and 199 (66.33%) samples yielded no growth. Total 12 (6x2) organisms were isolated from 6 mixed growth (Fig.1).

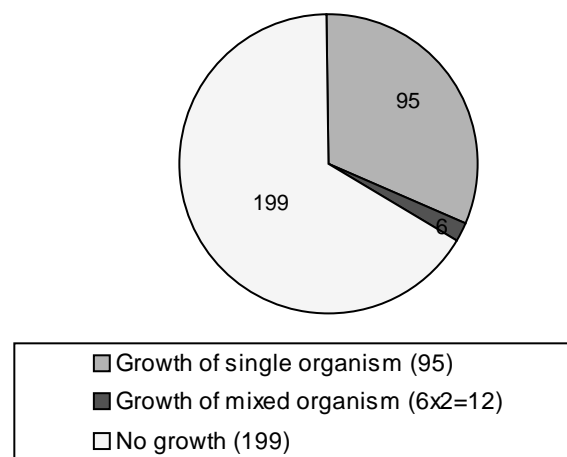


Figure 1: Pie diagram shows the results of urine culture

Total 107 strains were isolated, of which *Esch.coli* (64.49%) was the most common aetiologic agents followed by *Klebsiella spp.*(11.21%) (Table I).

Table I: Isolation of different organisms among culture positive cases (n= 107) *

Organism	Single	Mixed	Total
<i>Escherichia coli</i>	64	5	69 (64.49)
<i>Klebsiella spp.</i>	11	1	12 (11.21)
<i>Pseudomonas spp.</i>	8	2	10 (9.35)
<i>Enterococcus spp.</i>	3	3	6 (5.61)
<i>Proteus spp.</i>	4	0	4 (3.74)
<i>Staph.saprophyticus</i>	3	0	3 (2.80)
<i>Enterobacter spp.</i>	2	1	3 (2.80)
Total	95	12	107 (100)

Figures in parentheses indicate percentage.

*{95 from single growth+ 12(6x2) from mixed growth}

was detected by CLED agar and Blood agar & MacConkey agar media respectively (Table III).

Table III: Detection of mixed growth in different media. (n= 6)

Type of mixed growth	Organisms detected	HiCrome UTIagar media	CLED agar media	Blood agar & MacConkey agar media
<i>Esch. coli+ Enterococcus spp.</i>	3	3(100.00)	0(00)	0 (00)
<i>Esch. coli+ Pseudomonas spp.</i>	1	1(100.00)	1(100.0)	1(100.00)
<i>Enterobacter+Pseudomonas</i>	1	1(100.00)	0(00)	0(00)
<i>Esch.coli + Klebsiella spp.</i>	1	1(100.00)	0(00)	0(00)
Total	6	6(100.00)	1(16.67)	1(16.67)

Figures in parentheses indicate percentage.

Table II: Presumptive identification of organisms from different primary culture plate. (n= 107)

Name of organism presumptively identified	In HiCrome UTI agar media	In CLED agar media	In Blood agar and MacConkey agar
<i>Esch.coli</i> (n= 69)	65 (94.20)	55 (79.71)	57 (82.61)
<i>Klebsiella spp.</i> (n=12)	12 (100.00)	12 (100.00)	12(100.00)
<i>Pseudomonas spp.</i> (n=10)	10 (100.00)	7 (70.00)	10(100.00)
<i>Enterococci spp.</i> (n=6)	6 (100.00)	2 (33.33)	0(00)
<i>Proteus spp.</i> (n=4)	4(100.00)	4(100.00)	4(100.00)
<i>Staph.saprophyticus.</i> (n=3)	2(66.67)	0(00)	0(00)
<i>Enterobacter spp.</i> (n=3)	2(66.67)	0(00)	0(00)
Total	101(94.39)	80 (74.77)	83 (77.57)

Highest presumptive identification rate (94.39%) was found in primary culture plates of HiCrome UTI Agar media followed by 77.57% in combination of Blood agar and MacConkey agar media. Lowest rate (74.77%) was found in CLED agar media (Table II).

Out of 6 mixed growth, 100% were detected by HiCrome UTI Agar media, whereas only 1 (16.67%)

Discussion

A total 300 samples of urine from clinically diagnosed UTI cases having pus cell ≥ 5 /HPF were examined. Of them, 95 (31.67%) samples showed single growth, 6 (2%) showed mixed growth and 199 (66.33%) samples yielded no growth. From 101 (33.67%) culture positive

samples, 107 strains of organisms were isolated, of which 95 strains were from 95 samples of single growth and 12 (6x2) strains were from 6 samples of mixed growth. Findings of the present study were similar with a study done in Bangladesh which showed 38.5% single growth and 4% mixed growth¹⁴. In contrast to the findings of the present study a higher rate was reported from UK (54.2% single growth and 21.6% mixed growth)¹⁵. This might be due to the fact that urine samples having pus cell > 200/cmm were included in that study. A lower bacterial isolation rate were reported from Israel (19.55% single growth and 1.66% mixed growth)¹¹, India (20% single growth and 4% mixed growth)¹² and California (24.5% single growth and 17.5% mixed growth)¹⁶. Such lower isolation rate in their study were probably due to fact that all urine samples were cultured irrespective of pus cell count, while in the present study urine samples having pus cells ≥ 5 /HPF were included.

In the present study, from 101 culture positive cases a total 107 strains were isolated. Of which, 69 (64.49%) were *Esch.coli* followed by 12 (11.21%) *Klebsiella* spp, Various studies in Bangladesh noted that *Esch.coli* is the predominant organism^{17,18}. In the present study, 94.39% of isolated organisms were presumptively identified by the primary culture plate of HiCrome UTI agar media, 74.77% by CLED agar media and 77.57% by combined Blood agar and MacConkey agar media. The isolation rate of uropathogens in chromogenic agar media of the present study is consistent with reports of studies published elsewhere^{5,14}.

In the present study out of 69 *Esch.coli*, 94.20% were identified in HiCrome UTI agar, whereas 79.71% were in CLED agar media and 82.61% in MacConkey & Blood agar media. Similarly another study in India reported 90% *Esch. coli* in chromogenic media (Uricrom II)¹³. *Escherichia coli* produce the enzyme Beta-glucuronidase that attack Beta-glucuronide chromogenic substrate and grow as distinct pink coloured colonies. In our study, among 6 *Enterococcus* spp., 100% were identified on HiCrome UTI agar media because of small blue coloured colonies. On CLED agar media only 33.33% *Enterococci* were identified. *Enterococci* produce Beta-glucosidase, that attacks Beta-glucoside chromogenic substrate, generate distinct blue colour colonies. Therefore, all *Enterococci* were presumptively identified. Almost similar findings were reported by investigators from different countries^{12,14}. Lower rate of identification were observed by other investigators^{9,11}. These variations might be due to different types of chromogenic media.

Out of 101 culture positive samples, 06 (2%) samples showed mixed growth. Among the mixed growth, 100% organisms were identified on HiCrome UTI Agar media due to distinct colour produced by the different organisms, whereas only one (16.67%) sample (mixed *Esch.coli* and *Pseudomonas* spp.) organisms were identified on CLED agar and Blood agar & MacConkey agar media. Similar results were observed by investigators in different countries^{12,14,15}.

The overall findings of this study suggests that though expensive, chromogenic media like HiCrome UTI Agar media, offer an excellent and time saving method for the reliable identification of most of the uropathogens and differentiation of mixed bacterial cultures in primary culture plate, and thus reduces laboratory workload (i.e. plate burden and sheep house). Moreover HiCrome UTI Agar media have an advantage over conventional media for identification of *Enterococcus* spp.

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