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Original Article

Comparative Study of Hicrome Agar Medium with Conventional Culture System for the Isolation of Uropathogens

S Gul Nahar[1], M. Bulbul Hasan[2], Mst. Rokeya Khatun[3], M. Nawshad Ali[4]

Abstract

Objective: The present study was done to compare the performance of chromogenic agar medium and conventional culture media for the isolation and presumptive identification of uropathogen.

Methodology: A total 300 sample were collected from Rajshahi Medical College Hospital, Bangladesh during January to June, 2008. Urine samples of the suspected UTI cases, showing pus cells >5/HPF on microscopic examination were included for urine culture simultaneously onto 2 conventional media (Blood agar and MacConkey agar) and chromogenic agar medium (HiCrome UTI agar medium).

Results: Culture yielded 139 (46.33%) bacterial growth among them, 133 (44.33%) showed single organism and remaining 06 (2.00%) showed mixed growth of two organisms in different combinations. It is evident from the present study that both HiCrome UTI agar and Blood agar (BA) media supported growth of all 145 bacteria, while MacConkey (MAC) agar yielded 133(91.72%) bacterial growths. The rate of presumptive identification of the isolates was found significantly higher (97.24%) on HiCrome UTI agar when compared with the MacConkey agar (80.68%) and Blood agar (27.58%) media. Out of 91 *E. coli* isolated, 88(96.70%) could be identified differentially on HiCrome UTI agar medium in contrast to 85(93.40%) on MacConkey agar and only 06(06.59%) on Blood agar. Again, all 06 (100%) of the isolate-pairs of mixed growth were identified distinctly on HiCrome UTI agar, whereas both Blood agar and MacConkey agar media could revealed only 01(16.66%) of the polymicrobial growth.

Conclusion: HiCrome UTI agar medium has been documented for its very high yielding rate, rapid presumptive identification of both single and polymicrobial growths with greater precision and avoidance of biochemical tests for further identification of uropathogens. Thus it can be recommended as primary urine culture medium to be used by the clinical microbiology laboratories.

Key Words: Urinary tract infections, chromogenic agar medium, HiCrome UTI agar, conventional media.

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Introduction

Urinary tract infections (UTI) are important clinical problems and account for significant morbidity. UTI refer to invasion of urinary tract including urinary bladder, prostate, collecting system or kidney by different microorganisms[1]. Predominant causative agents of UTI are *E. coli*,

[1] Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.
[2] Associate Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi.
[3] Assistant Professor, Department of Gynae and Obstetrics, Rajshahi Medical College, Rajshahi.
[4] Associate Professor, Department of Pediatric Surgery, Rajshahi Medical College, Rajshahi.

Klebsiella spp., *Pseudomonas* spp., *Proteus* spp. *and Enterococci* spp., *Staph. saprophyticus*[2,3].

Urinary tract infections result from the interaction between uropathogens and the host. The microorganisms may have particular uropathogenic properties, explaining the occurrence of infection in an otherwise normal urinary tract. On the other hand, non-uropathogenic strains can induce acute infection in the presence of urological abnormalities or when the host's defense mechanisms are impaired[4].

Most urinary tract infections irrespective of age are the result of enteric bacteria, especially *Escherichia coli*. It colonizes the perineum and then ascends the urethra to multiply and infect the bladder, kidney, and adjacent structures. The most common site of infection is the urinary bladder.

The media chosen must be able to support the growth of all urinary pathogens, inhibit possible contaminants and distinguish between pathogenic lactose and non-lactose fermenters. Routinely used conventional media like Blood agar (BA), MacConkey agar (MAC) and Cystine lactose electrolyte-deficient (CLED) agar have some drawbacks as all uropathogens cannot be cultured and differentiated in a single medium. Being an enriched media, BA can support the growth of all uropathogens but regarding identification of bacteria, its performance is very poor. Again, differentiation of lactose fermenter from non-lactose fermenter is possible on MAC and CLED agar media, but further species differentiation demands different biochemical reactions. Although CLED agar is preferable to MAC agar media for its ability to inhibit the swarming growth of *Proteus* spp. and to support the growth of certain species of *Staphylococci, Strptococci* and *Candida* that fail to grow on MacConkey agar media. But CLED agar provides poor growth of some gram positive bacteria as for example, alpha-haemolytic *Streptococci*, Group B-*Streptococci* and some strains of coagulase negative *staphylococci* and it does not have the differential capacity to distinguish mixed growth. On CLED agar medium the presence of *Enterococci* is frequently masked by larger colonies of gram negative bacteria[5,6]. Therefore it cannot be used alone as a primary isolation medium[7].

The problem of urine culture can be addressed by using chromogenic agar (CA) medium. CA medium is increasingly being used as a versatile tool in early differentiation and identification of bacterial isolates from clinical specimens[8]. This single medium supports not only the growth of all uropathogens but mixed infection can also be diagnosed more easily[9]. Since Chromogenic agar medium facilitates direct identification of the organism on the basis of distinct colour production and characteristic colonial morphology thus it reduces the burden of biochemical characterization of the bacterial species in most instances.

The principle of chromogenic agar medium is based on the fact that bacteria posses many enzymes for their physiological function that help them to utilize substrates. In such media specific chromogenic substrates are broken down by the enzymes liberated by the particular bacteria thereby imparting a distinct colour to the growing bacterial colonies that can be visually observed[10,11,12].

Over the last few years, several chromogenic urine culture media have been developed and commercialized, allowing more specific and direct differentiation of microorganism on the primary plate itself[7,12,13,14]. HiCrome UTI agar is such a chromogenic medium designed to isolate and identify all uropathogens. *E. coli* appears as pink-red colonies because of β-galactosidase production, thus allows a definite identification of *E. coli* without the need for further biochemical tests. Strain that produces β-glucosidase, such as *Enterococci* and the *Klebsiella-Enterobacter-Serratia* group, form colonies that generate a blue coloration as a result of hydrolysis of glucoside chromogenic substrate. Tryptophan is also present in the medium to detect members of the *Proteus* group, which generates a diffuse brown coloration as a result of tryptophan deaminase production. *Pseudomonas* spp. produce colourless colony whereas *Staph. saprophyticus* white coloured. An important aspect of HiCrome UTI agar is that it allows an easy differentiation of various species from mixed cultures due to specific colony colour[14,15].

Certain identification tests like the catalase, oxidase and indole test can be done directly from the colonies on HiCrome UTI agar. Another advantage of this HiCrome UTI agar is that it can be used to perform antibiotic sensitivity testing without subculture onto another basic medium[7,12,14]

Thus HiCrome UTI agar is advocated as an attractive and easy to use primary screening medium that hopefully will considerably reduce the daily workload and limits the use of further identification tests[15,16].

Methodology

Patients

The study included 300 clinically suspected patients of UTI of different age and sex attending either at the outpatient department (OPD) or admitted in the Rajshahi Medical College Hospital from July, 2007 to June, 2008.

Clinically suspected patients of UTI having pus cells >5/ HPF detected on microscopy of centrifuged deposit of urine were included as study cases [17].

Culture of urine

Urine samples for culture were selected on the basis of presence of pus cells >5/ HPF on microscopy[17]. Inoculation was done aseptically with urine samples using a calibrated wire loop of 28G with an internal diameter of 3.26 mm holding 0.004 ml of urine into all three selected media plate[18]. The plates were incubated at 37°C aerobically and after overnight incubation, they were checked for bacterial growth. Colony count was done with bacterial growth to calculate the number of colony forming unit **(**CFU**)** or viable bacteria per ml of urine[18].

Presumptive identification

Bacterial growth was identified presumptively on HiCrome UTI agar, MAC agar and Blood agar, following colony characteristics against each of the uropathogens (Table-I).

Table I: Colony characteristics of uropathogens on three urine culture media

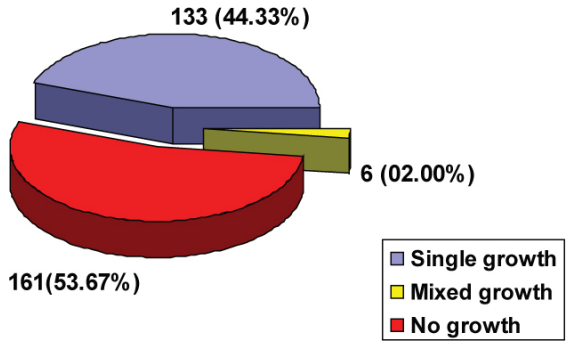
Organisms	HiCrome UTI agar	Blood agar	MacConkey agar
E. coli	Pink to Red colour colony due to β-D-galactosidase	Large, grey coloured colonies, Some strains haemolytic	Smooth, Pink colonies, lactose fermenting, Some strains are late or non-lactose fermenting
Klebsiella spp.	Blue colour due to β-glucosidase.Large, mucoid colony	Yellow to cream or occasionally white, large colonies, Some strains beta-haemolytic	Mucoid, Pink colonies, lactose fermenting
Enterococcus spp.	Blue colour due to β –glucosidase, Small colony	Non-haemolytic colonies	some strains lactose fermenter/non-lactose fermenter, Pale/Pink coloured colonies
Pseudomonas spp.	Colourless : not explained by the manufacturer	Large flat, spreading greenish-blue coloured colonies	Pale coloured colonies, non-lactose fermenting, grape like odour
Staph. Aureus	Golden Yellow: not explained by the manufacturer	Yellow to cream or occasionally white, large colonies Some strains beta-haemolytic	Smaller colonies and some strains lactose fermenting
Proteus spp.	Light brown colour due to tryptophan deaminase	Characteristic fishy odour, and swarming growth	Pale (non-lactose fermenting) colonies
Enterobacter spp.	Blue colour due to β –glucosidase.Mucoid colony	Produces large and mucoid colonies	Produces large, mucoid, pink coloured colonies, lactose fermenting
Staph. saprophyticus	White, small colony: not explained by the manufacturer	Produce white or yellow and non-haemolytic colonies.	Growth does not occur

The isolates were further identified using standard identification protocol such as Gram's staining, motility test, catalase test, coagulase test, oxidase test and other relevant biochemical tests as appropriate for the isolates[18].

Results

A total of 300 patients of different age and sex suffering from UTI were included in this study. Out of 300 samples of urine cultured, a total of 139(46.33%) samples yielded significant bacterial growth with colony count $>10^5$ CFU/ml and 161(53.67%) samples yielded no growth. Culture-positive cases included 133(44.33%) growth of single organism and 06(02.00%) mixed growth of two organisms each (Figure-I).

Figure I: Patterns of bacterial growth from urine culture (N=300)



Patterns of bacterial isolates from urine culture include: 139 culture positive samples of urine yielded 145 bacterial isolates including both single (133) and polymicrobial growths (06) of two bacteria each. Out of 145 isolates, *E. coli* was the leading bacteria 91(62.75%) followed by *Klebseilla* spp. 18(12.41%), *Enterococcus* spp. 16(11.03%), *Pseudomonas* spp. 09(06.28%), *Staph. saprophyticus* 05(03.44%), *Enterobacter* spp. 04(02.75%) and *Proteus* spp. 02(01.37%). **(Table –II)**

Table II: Pattern of bacteria isolated from urine culture (N=145)

Bacteria	Number	Percentage
E. coli	91	62.75
Klebsiella spp.	18	12.41
Enterococcus spp.	16	11.03
Pseudomonas spp.	09	06.28
Staph. saprophyticus	05	03.44
Enterobacter spp.	04	02.75
Proteus spp.	02	01.37
Total	145	100

Results of three culture media for the rate of isolation of uropathogens showed that both chromogenic agar media and Blood agar media supported 100% bacterial growth, while MAC agar yielded 133(91.72%) bacterial growth out of 145 total isolates (Table –III).

Table III: Comparison of three culture media for the rate of isolation of uropathogens (N=145)

Bacteria	Total isolates	HiCrome UTI agar	MAC agar	Blood agar
E. coli	91	91(100)	91(100)	91(100)
Klebsiella spp.	18	18(100)	18(100)	18(100)
Enterococcus spp.	16	16(100)	09(56.25)	16(100)
Pseudomonas spp.	09	09(100)	09(100)	09(100)
Staph. Saprophyticus	05	05(100)	00	05(100)
Enterobacter spp.	04	04(100)	04(100)	04(100)
Proteus spp.	02	02(100)	02(100)	02(100)
Total	145	145(100)	133(91.72)	145(100)

Regarding presumptive identification of bacterial isolates by defined colony characteristics on primary culture plates, it was found that out of total 145 bacterial isolates, 141(97.24%) could be differentially identified on chromogenic agar media and 117(80.68%) on MAC agar media whereas only 40(27.58%) on BA. The rate of presumptive identification of the isolates was found significantly higher on chromogenic agar media in comparison to MAC agar and BA (Table -IV).

Table IV: Comparison of rate of presumptive identification on different media

Bacterial strains	HiCrome UTI agar	MAC agar	Blood agar
E. coli (n=91)	88 (96.70)	85 (93.40)	06(06.59)
Klebsiella spp. (n=18)	18 (100)	15 (83.33)	14(77.77)
Enterococcus spp. (n=16)	16 (100)	05 (31.25)	10(62.50)
Pseudomonas spp. (n=9)	08 (88.88)	07 (77.77)	03(33.33)
Staph. saphrophyticus (n=5)	05 (100)	00	05 (100)
Enterobacter spp. (n=4)	04 (100)	03 (75.00)	00
Proteus spp. (n=2)	02 (100)	02 (100)	02 (100)
Total (N=145)	14 1 (97.24)	117(80.68)	40(27.58)

Rate of matching with the standard colour for bacterial isolates on HiCrome UTI agar medium is shown in Table-V It is evident that the colony colour of all isolates of *Klebsiella* spp., *Enterococcus* spp., *Staph. saprophyticus*, *Enterobacter* spp. and *Proteus* spp. matched 100% with the standard colony colour on HiCrome UTI agar medium. While, out of 91 isolates of *E. coli*, 03(03.30%) did not match with the standard colony colour. The mismatch in colony colour was also noted in 01 (11.12%) case of *Pseudomonas* spp.

Table-VI shows the rate of presumptive identification of polymicrobial growth in different culture media used. It was found that all 06(100%) polymicrobial growths were distinctly identified only in HiCrome UTI agar medium, while except in a single case consisting of *E. coli* and *Proteus* spp., all other mixed bacterial growths could not be identified on both MAC and Blood agar media.

Table V: Rate of matching of bacterial isolates on HiCrome UTI agar medium with the standard colony colour

Organisms	Matched	Not matched
E. coli (n=91)	88 (96.70)	03 (03.30)
Klebsiella spp. (n=18)	18 (100)	00
Enterococcus spp. (n=16)	16 (100)	00
Pseudomonas spp. (n=9)	08 (88.88)	01(11.12)
Staph. saphrophyticus (n=5)	05 (100)	00
Enterobacter spp. (n=4)	04 (100)	00
Proteus spp. (n=2)	02(100)	00
Total(N=145)	14 1 (97.24)	04 (02.76)

Figures in the parenthesis indicate percentage

Table VI: Comparison of HiCrome UTI agar medium with conventional culture system for detection of polymicrobial growth

Organisms in polymicrobial growth	HiCrome UTI agar	Blood agar	MAC agar	Total
E. coli & *Enterococci*	02 (100)	Nil	Nil	02
E. coli & *Klebsiella*	02(100)	Nil	Nil	02
E. coli & *Proteus*	01(100)	01(100)	01(100)	01
E. coli & *S. saprophyticus*	01(100)	Nil	Nil	01
Total	06(100)	01(16.66)	01(16.66)	06

Figure in the parenthesis indicate percentage

Discussion

Urinary tract infections continue to be an important clinical problem with significant morbidity. UTI accounts for approximately 23% of all hospital acquired infections[19].

For the isolation and identification of uropathogens, routinely used culture media like MacConkey agar, Blood agar, and Cystine Lactose Electrolyte Deficient (CLED) agar media are used together conventionally for long time. Each of these media has several limitations of their own for identification of the common uropathogens.

In order to overcome disadvantages of traditional urine culture media, a range of chromogenic media has become commercially available in recent

years. Chromogenic agar medium supports not only the growth of all uropathogens but mixed infections can also be diagnosed properly. Since Chromogenic agar medium facilitates prompt isolation and presumptive identification of the organisms on the basis of distinct colour production and colonial morphology, thus it reduces the burden of biochemical characterization of the bacterial species in most instances.

Three hundred urine samples were tested by parallel inoculation on chromogenic (HiCrome UTI agar), Blood agar and MacConkey agar media based on samples having microscopic detection of pus cells >5/HPF in centrifuged deposit of urine.

Out of 300 urine samples, a total of 139(46.33%) samples yielded bacterial growths and 161(53.67%) had no growth. Culture-positive cases included 133(44.33%) with significant growth of single organism and remaining 06(02.00%) yielded mixed growth of two organisms (Figure-I).

The growths of all 145(100%) isolates were supported by HiCrome UTI agar and Blood agar whereas MacConkey agar yielded 133(91.72%) bacterial growths (Table-III). Blood agar is an enriched medium and HiCrome UTI agar contains all essential nutrients to support the growth of possible uropathogens that is why all isolates have grown on to these two media. On the other hand, the slightly lower yielding rate on MAC agar can be explained by its limitations of not supporting all organisms involved in UTI like *Staph. saprophyticus* and *Enterococcus* spp., *because* it is a selective medium to support the growth of members of Enterobacteriaceae .

Out of 145 isolates of uropathogens, 141(97.24%) had been presumptively identified on HiCrome UTI agar by matching with standard colours as stated and supplied by the manufacturer. In contrast, 117(80.68%) isolates were identified on MAC agar and only 40 (27.58%) on Blood agar media (Table-IV). The rate of presumptive identification of the uropathogens was found significantly higher on HiCrome UTI agar in comparison to Blood agar and MAC agar. This high rate of identification could be correlated with the ease of distinct colour production by each of the bacterial isolate on Chromogenic agar medium. Moreover, HiCrome UTI agar offered the advantage of limiting the spread of some isolates such as *Proteus* spp., *Klebsiella* spp. and *E. coli* mucoid strains thus increased the ability of the medium to detect urinary tract pathogens when mixed organisms were present[15].

Regarding the pattern of bacterial isolates causing UTI, *E. coli* was the highest 91(62.75%), followed by *Klebsiella* spp. 18(12.41%), *Enterococcus* spp. 16(11.03%), *Pseudomonas* spp. 09(06.28%), *Staph. saprophyticus* 05(03.44%), *Enterobactor* spp. 04(02.75%) and *Proteus* spp. 02(01.37%) shown in (Table-II).

As far as the rate of presumptive identification of individual bacterial isolates on different culture plates is concerned, it has been seen that except *E. coli* (96.70%) and *Pseudomonas* spp. (88.88%), all bacteria could be differentially identified onto the HiCrome UTI agar medium. While, MAC agar provided 93.40% identification for *E. coli*, followed by 83.33% for *Klebsiella* spp., 31.25% for *Enterococcus* spp., 77.77% for *Pseudomonas* spp., 75% for *Enterobacter* spp., 100% for *Proteus* spp. and 00% for *Staph. saprophyticus*. Blood agar also showed variable rate of identification ranging from 00 to 77.77% for all bacterial isolates except *Proteus* spp. and *Staph. saprophyticus*, which were identified 100% on BA plates (Table-V).

The reason behind the highest rate of identification of *E. coli*, *Klebsiella* spp., *Enterococci* spp. and *Enterobacter* spp. on HiCrome UTI agar medium in comparison to other two conventional media used is that each of these organisms produces characteristic, distinct and easily perceivable colour on HiCrome UTI agar medium. In fact, this differential colour production by individual bacterial isolates is among the exciting features of chromogenic agar for which it has been advocated to be used as primary culture medium. The chromogenic media also provided added advantage on identification of a few non-lactose variety of *E. coli*, which might be the reason of decreased rate of identification on MAC agar.

However, HiCrome UTI agar failed to produce expected colony colours for 03(03.33%) of the *E. coli* and 01(11.12%) *Pseudomonas* spp. This failure can be correlated with inadequate production of enzymes at that point of time or absence of enzymes in those strains.

The HiCrome UTI agar also reigned over the conventional media by providing specific identifying characteristics of the organisms isolated in mixed growth. As it was found in this study, all 06(100%) of the isolate-pairs of mixed growth (*E. coli* and *Enterococci* spp., *E. coli* and *Klebsiella* spp., *E. coli* and *Proteus* spp., *E. coli* and *Staph. saprophyticus)* were all identified distinctly on HiCrome UTI agar, whereas only 01(16.66%) in combination of *E. coli* and *Proteus* spp. was identified on both BA and MAC agar media (Table-VI). The rate of identification of mixed culture on the BA and MAC agar was poor due to difficulty in differentiating the colonies. Now it is obvious from the results of the present and similar studies that chromogenic media can replace CLED and MAC agar in order to identify uropathogens more efficiently by its characteristic colony colour for each of the organism in both community acquired and nosocomial UTI cases. Moreover, chromogenic media also provide an added advantage of requiring less time in mastering the skill in the identification of the uropathogens. But considerable efforts are required to acquire the similar desired level of competence in identification using conventional media. Though chromogenic media on its own are still prohibitively expensive at the moment but it can cut down the total cost of urine culture by replacing the use of multiple conventional media. Additionally, expense will come down as use of chromogenic media will make many routinely used identifying biochemical tests redundant. This will be an attractive easy to use primary screening medium that considerably reduce the daily workload and thus minimize the use of troublesome biochemical tests[8].

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All correspondence to:
Sabera Gul Nahar
Assistant Professor
Department of Microbiology
Rajshahi Medical College, Rajshahi, Bangladesh
E-mail: sabera170764@yahoo.com