Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublisher.com/sjams/

OPEN ACCESS

Microbiology

An assessment of Chromogenic Agar Medium and Conventional Culture System for the Isolation of Uropathogens

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DOI: 10.36347/sjams.2020.v08i01.022 | **Received:** 06.01.2020 | **Accepted:** 13.01.2020 | **Published:** 17.01.2020

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Abstract **Original Research Article**

Introduction: Urinary tract infections (UTI) account for significant health burden among all age groups. Isolation and identification of the uropathogens by bacterial culture and selection of appropriate antimicrobial drugs through susceptibility testing is the mainstay in management of UTI cases. ***Material & Methods:*** This was a cross-sectional study which was carried out in the Department of Microbiology, Rajshahi Medical College (RMC) to evaluate the performance of a Chromogenic agar medium (commercially named as HiCrome UTI agar) and conventional culture system like Blood agar (BA) and MacConkey (MAC) agar for isolation and presumptive identification of the uropathogens. ***Results:*** Slightly higher bacterial growth was noted among female (29.33%) than male (17.00%) patients as a whole and 15-45 years was the leading age group with higher number of culture positive cases. Out of 300 urine samples cultured, a total of 139 (46.33%) yielded bacterial growth and 161 (53.67%) were negative for bacterial growth. Bacterial isolates included E. coli 91(62.75%), Klebsiella spp. 18(12.41%), Enterococcus spp. 16(11.03%), Pseudomonas spp. 09(06.28%), Staph. saprophyticus 05(3.44%), Enterobactor spp. 04(2.75%) and Proteus spp. 02(1.37%). It is evident from the present study that both HiCrome UTI agar and BA media supported growth of all 145 bacteria, while 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. In antimicrobial susceptibility testing, majority of the isolates showed very high (78% -100%) sensitivity to Imipenem. Ceftazidime and Cefuroxime were also found efficacious against E. coli and Staph. saprophyticus, while Klebsella spp., Enterococcus spp., Pseudomonas spp., Enterobacter spp. and Proteus spp. showed variable sensitivity to these drugs. Further, most of the isolates showed moderate to poor sensitivity to Ciprofloxacin, Nalidixic acid, Cephalexin, Amoxicillin and Cotrimoxazole. (Minimize that red mark) ***Conclusion:*** HiCrome UTI agar can be recommended as primary urine culture medium to be used by the clinical microbiology laboratories.

Keywords: Urinary Tract Infections, Chromogenic Agar Medium, Conventional Culture System, HiCrome UTI Agar, Bacterial Growth.

INTRODUCTION

Urinary tract infections (UTIs) is the most common infections both in community and hospital settings. They represent 10–20% of all infections treated in primary care and 30–40% of infections treated in hospitals. UTI cause considerable morbidity in women and account for 1-3 % of all consultations in general practice [1]. About 150 million people become infected with UTI each year worldwide [2]. Interestingly, UTI account for approximately 23% of all hospital acquired infections [3]. Clinical microbiology laboratory in the management of UTI reduces the morbidity and mortality through accurate and timely identification of the etiological agent with selection of appropriate antimicrobials through in-vitro sensitivity testing. Most urinary tract infections irrespective of age are the result of enteric bacteria, especially Escherichia coli. More than 90% of community acquired infections are due to E. coli [4]. It colonizes the perineum and then ascends the urethra to multiply and infect the bladder, kidney, and adjacent structures. Uncomplicated cystitis and pyelonephritis may also be caused by other species of Enterobacteriaceae rods (Klebsiella spp., Proteus spp., Enterobacter spp.) or, less frequently, by coagulase-negative staphylococci and enterococci.

Laboratory diagnosis of UTI usually includes urine analysis, dipstick tests, culture and antibiotic susceptibility testing. Urine microscopy for the diagnosis of UTI, though easy to perform, has its own limitations. In fact, microscopy and dipstick test can only provide preliminary information about bacterial infection, but isolation of the causative organism by culture is the gold standard for the diagnosis of UTI [5]. But selection of culture media is somehow inconvenient. 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, Streptococci 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 [6, 7]. Therefore, it cannot be used alone as a primary isolation medium [8]. CA medium is increasingly being used as a versatile tool in early differentiation and identification of bacterial isolates from clinical specimens [9]. This single medium supports not only the growth of all uropathogens but mixed infection can also be diagnosed more easily [10]. Since Chromogenic agar medium facilitates direct identification of the organism on the basis of distinct color production and characteristic colonial morphology thus it reduces the burden of biochemical characterization of the bacterial species in most instances. 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 [11-14]. HiCrome UTI agar is such a chromogenic medium designed to isolate and identify all uropathogens. An important aspect of HiCrome UTI agar is that it allows an easy differentiation of various species from mixed cultures due to specific colony color [14, 15]. The aim of the present study is to make assessment of the Chromogenic Agar Medium and Conventional Culture System for the Isolation of Uropathogens

OBJECTIVES

General Objective

- To assess the efficacy of Chromogenic Agar Medium and Conventional Culture System for the Isolation of Uropathogens

Specific Objectives

- To evaluate the performance of a chromogenic agar medium (HiCrome UTI agar) and conventional culture system (Blood agar, MAC, CLED agar media)
- To identify the urinary isolates.
- To define the pattern of bacterial growth from urine culture

METHODOLOGY AND MATERIALS

Three hundred 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 were included in this study. This study was carried out in the Department of Microbiology, Rajshahi Medical College, Rajshahi. It was conducted between 1st July 2007 to 31st June 2008.

Inclusion Criteria

- Clinically suspected patients of UTI having pus cells ≤5/ HPF detected on microscopy of centrifuged deposit of urine.

Exclusion Criteria

- Evidence of haematuria and chyluria.
- Patients on antimicrobial therapy.
- Patients refused to participate in the study.

RESULTS

A total of 300 patients of different age and sex suffering from UTI were included in this study. Among the patients, 121(40.33%) were male and 179(59.67%) were female with male-female ratio of 1:1.48. Study cases were divided into three major age groups; <15 yrs., 15-45 yrs. and >45 yrs**.** Majority of the UTI patients 222(74.00%) was in the 15-45 yrs. age group followed by >45yrs 44(14.67%) and below 15 yrs. 34(11.33%). Regarding rate of bacterial growth, slightly higher number of culture positive cases was noted among female (29.33%) than male (17.00%) as a whole and male aged >45yrs have had more incidence of significant bacteriuria than their female counterpart (4.33% versus 2.33%) (Table I). Out of 300 urine samples, 245(81.67%) samples were collected from outpatient department (OPD) with 37.55% culture-positive cases and 55(18.33%) from hospital admitted patients with 85.45% culture-positive cases (Table-4.2). Results of urine culture are shown in Figure I**.** 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. Patterns of bacterial isolates from urine culture are shown in Table III. 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%). Evaluation results of three culture media for the rate of isolation of uropathogens are shown in Table IV. It was seen that both HiCrome UTI agar and Blood agar media supported 100% bacterial growth, while MAC agar yielded 133 (91.72%) bacterial growth out of 145 total isolates. 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. Distribution of bacterial isolates according to the sources of patients is shown in Table VI described that, E. coli was the leading bacterial isolate found in both OPD (75.53%) and hospital admitted cases (39.22%) of UTI. The percentages of Klebsiella (09.58%) and Enterococcus spp. (07.45%) Pseudomonas spp. (01.06%), S. saprophyticus (04.26%), Enterobacter spp. (01.06%) and Proteus spp. (01.06%) isolated from OPD cases. While, the percentages of Klebsiella (17.65%) and Enterococcus spp. (17.65%%) Pseudomonas spp. (15.68%), S. saprophyticus (01.96%), Enterobacter spp. (05.88%) and Proteus spp. (01.96%) isolated from hospital UTI cases.

Table-I: Culture positive cases detected in chromogenic agar medium in relation to the age and sex of the patients (N=300)

Age (yrs.)	Male		Female		Total	
	Cases Studied	Culture positive	Cases Studied	Culture positive	Cases Studied	Culture positive
<15	16 (5.33%)	05 (1.67%)	18 (6.00%)	06 (2.00%)	34 (11.33%)	11 (3.66%)
15-45	78 (26.00%)	33 (11.00%)	144 (48.00%)	75 (25.00%)	222 (74.00%)	108 (36.00%)
>45	27 (9.00%)	13 (4.33%)	17 (5.67%)	07 (2.33%)	44 (14.67%)	20 (6.67%)
Total	121 (40.33%)	51 (17.00%)	179 (59.67%)	88 (29.33%)	300 (100%)	139 (46.33%)

Table-II: Sources of patients (N=300)

Sources of Patients	Number of patients	Number of Culture-positive cases	Percentage of culture positivity (%)
OPD cases	245	92	37.55
Hospital admitted cases	55	47	85.45
Total	300	139	100

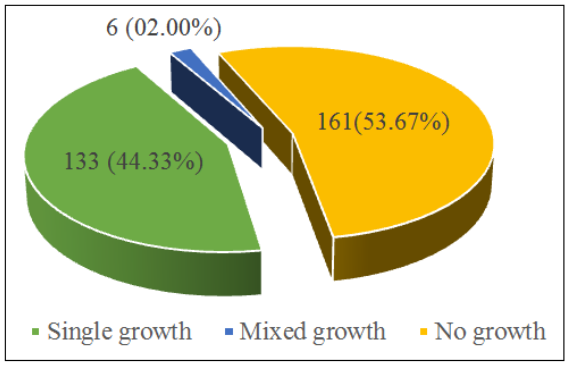

Fig-1: Pattern of bacterial growth from urine culture (N=300)

Table-III: 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

Table-IV: Evaluation of three culture media for the rate of isolation of uropathogens (N=145)

Bacteria	Total isolates	HiCrome UTI agar	Percentage (%)	MAC agar	Percentage (%)	Blood agar	Percentage (%)
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	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

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

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

Table-VI: Distribution of bacterial isolates according to sources of patients

Bacterial isolates	Number of patients	OPD	Percentage (%)	Hospital admitted	Percentage (%)
E. coli	91	71	75.53	20	39.22
Klebsiella spp.	18	09	09.58	09	17.65
Enterococcus spp.	16	07	07.45	09	17.65
Pseudomonas spp.	9	01	01.06	08	15.68
S. saprophyticus	5	04	04.26	01	01.96
Enterobacter spp.	4	01	01.06	03	05.88
Proteus spp.	2	01	01.06	01	01.96
Total	145	94	100	51	100

DISCUSSION

UTI accounts for approximately 23% of all hospital acquired infections [16]. Worldwide about 150 million people are diagnosed with UTI each year costing the global economy in excess of 6 billion US dollars [17]. To reduce the morbidity and even mortality in some cases due to UTI, microbiology laboratories must provide services for identification of clinical isolates accurately within the shortest period of time [18]. 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. The study population constituting both male and female was divided into three different age groups- above 15yrs, between 15-45yrs and below 45yrs. It was observed that, majority of the study population (74.00%) was in the second age group i.e. 15-45yrs with higher number (36.00%) of culture positive cases. Also, female had higher number (29.33%) of bacterial growth than male (17.00%). Additionally, male aged below 45yrs have had more incidence of UTI than their female counterpart (4.33% versus 2.33%). It is assumed that, the significant difference in the incidence of UTI between male and female is due to anatomical difference between sexes, the length of the urethra, the antibacterial properties of prostatic fluid etc. The sex distribution of samples and incidence of UTI in the present study is consistent with reports by others [19]. The increased incidence of UTI in male below 45yrs of age might be due to prostatism, which is more often noticed in this age group that favors urinary tract infections. 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. This result resembles other two similar studies carried out by Sharmin [20]; Fatema [22]. In a study, conducted in the UK, showed 54.20% single growth and 21% polymicrobial growth [7] which defines that the rate of polymicrobial growths may vary in different geographical locations. A study conducted in India, showed the frequency of bacteriological culture positivity in urine to be 19.79% with 95.12% unimicrobial and 04.87% polymicrobial growths, where

urine samples were cultured randomly without screening for pus cell under microscope [9]. 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. In the present study, 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%). The similar pattern and rate of isolation of uropathogens were also observed by other investigators [20, 21]. An extensive study finding also pinpointed *E. coli* as the predominant isolate followed by *Klebsiella* spp. and *Enterococcus* spp [22]. Similar studies conducted in developed countries like Israel and USA, also reported high rate of isolation of *E. coli* from urine cultures [14, 23]. The high prevalence of *E. coli* in UTI cases may be due to its existence as a normal flora in the large intestine and colonization in the perineal area. However, HiCrome UTI agar failed to produce expected colony colors for 3(03.33%) of the *E. coli* and 01(11.12) of Pseudomonas spp. This behavior of certain bacterial species on chromogenic agar media has also been reported by other investigators Sharmin *et al*., [20], Aspevall *et al*., [6]. Though chromogenic media are still prohibitively expensive at present 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 redundant many routinely used to identify biochemical tests. This might be a striking, easy to use primary screening medium that significantly lessen the daily workload and thus diminish the use of difficult biochemical tests [9].

LIMITATIONS OF THE STUDY

This was a prospective type of study in a single community with comparatively small number of sample size. So, the study result may not reflect the exact scenarios of the whole country.

CONCLUSION AND RECOMMENDATIONS

Conventional bacteriological culture media like Blood agar, MacConkey agar and CLED agar cannot support the growth of all uropathogens when used alone. We conclude that, chromogenic UTI agar medium can be an attractive, easy to use primary isolation and identification medium that substantially reduce the daily work load associated with urine culture in microbiology laboratory. It can be recommended as primary urine culture medium to be used by the clinical microbiology laboratories. It is also recommended that more studies with chromogenic UTI agar medium are required for assessing its usefulness and suitability in comparison to routine urine culture media in terms of primary isolation and identification, polymicrobial growths and also the cost effectiveness. The commercial companies engaged in production of culture media should come forward to supply chromogenic agar media in a competitive price, so that its cost effectiveness can be improved further by lowering the current price of these media.

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