



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2018; 4(7): 358-366
www.allresearchjournal.com
Received: 23-05-2018
Accepted: 24-06-2018

Kaskar SN
Research Student, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Tendolkar MR
Research Student, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Vaidya SP
Assistant Director, Clinical Pathology Department, Haffkine Institute, Acharya Donde Marg, Parel (East), Mumbai, Maharashtra, India

Angadi SA
Professor, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Dalal AR
Professor and Head, Department of Obstetrics and Gynecology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Karr S
Professor and Head, Dept of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Koppikar GV
Dean, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Correspondence
Kaskar SN
Research Student, Department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India

Comparative evaluation of performance and cost effectiveness of HiCrome UTI agar in detection of urinary tract pathogens

Kaskar SN, Tendolkar MR, Vaidya SP, Angadi SA, Dalal AR, Karr S and Koppikar GV

Abstract

Urinary tract infections (UTI) are important clinical entities, making urine, the most frequent sample received for culture in clinical microbiology laboratory's daily workload. Traditionally conventional media like Blood agar (BA) and MacConkey agar (MAC) are being used in combination by most of the laboratories in developing countries. Moreover, they are unsuitable as an ideal primary isolation medium and chromogenic agar medium like HiCrome UTI Agar is a differential medium recommended for it. The present study was undertaken to validate the usefulness of HiCrome UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to BA and MAC Agar in a Nair Hospital, in Mumbai. Patients included were randomly selected 3300 subjects who were married pregnant and non-pregnant women reporting to ANC clinic of the hospital of age group of 18 to 40 years. Collection, Transportation, Processing of urine, microscopic examination and Urine culture was carried out by standard methods. HiChrome UTI agar, BA and MAC agar were used simultaneously. Antibiotic sensitivity testing of test isolates (200 gram negative and 52 gram positive) bacteria were done, which were separately collected from Hichrome UTI, BA and MAC agar, to check the susceptibility pattern of isolates, isolated from different agar. Both HiCrome UTI agar and BA supported more than 75 % bacterial growth, while MAC agar supported 52.30% growths. For presumptive identification of bacterial species, of 259 bacterial isolates, 99.40% could be differentially identified on HiCrome UTI agar against 49.0% and 81.0% on BA and MAC agar respectively. Comparative results of three culture media for their rate of isolation of polymicrobial growth of uropathogens showed that HiCrome UTI agar supported 100% bacterial growth, while BA and MAC agar supported 40% bacterial growth each. Susceptibility pattern of all the isolates collected from HiCrome UTI™, BA & MAC agar was found to be similar. Cost of HiCrome UTI™ agar per test per urine sample was found to be less than traditional identification methods. Hence, the study shows that HiCrome UTI agar is an excellent and time saving media for the reliable identification of most of the uropathogens and differentiation of mixed bacterial cultures in primary culture plate as compared to conventional culture system.

Keywords: UTI, Hicrome UTI agar, pregnant women, asymptomatic bacteriuria, uropathogens

1. Introduction

Urinary tract infections (UTI) are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infections are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli*, but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* [1]. UTI is one of the most important causes of morbidity and mortality [2]. UTI is second only to the respiratory tract infection in acquiring microbial infection especially in females. It is more common in pregnant than in non-pregnant women [3]. UTI is twice more common in pregnant women than age matched non-pregnant [4]. UTI as group are the most common medical complication of pregnancy [5]. UTI may occur with or without symptoms; the latter is known as Asymptomatic bacteriuria [6]. The most significant adverse effect of bacteriuria is the development of acute upper urinary tract infection or acute pyelonephritis. Other adverse effects that have been reported to be associated with

bacteriuria in pregnancy include prematurity or low birth weight, maternal anemia and maternal hypertension [5]. Studies conducted in India suggest a prevalence of bacteriuria as 2 to 12% among pregnant women that is slightly higher than that from the west [4]. Urinary tract infections are important clinical entities that account for significant outpatients load and hospital admissions globally, making urine, the most frequent sample received for culture [1]. Although many of these infections are treated empirically, urine cultures involve a significant portion of clinical microbiology laboratory's daily workload. The etiological diagnosis of UTI requires quantitative urine culture on standard agar media, because only 20 to 30% of urine samples results in significant growth with predominant causative agents which are *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. *Enterococci* spp. And *Staph. saprophyticus* [7],

For urine culture, the media should ideally be able to support the growth of all urinary pathogens and inhibit possible contaminants. Traditionally conventional media like Blood agar (BA) and MacConkey agar (MAC) are being used in combination by most of the laboratories especially in the developing countries for long and Cystine lactose electrolyte-deficient (CLED) agar has been added later on but none of these media singly or in combination can support the growth and/or identification of possible uropathogens [8]. As a result there is continuous strive by the laboratories to streamline and improve urine culture algorithms. BA can support the growth of majority of uropathogens as an enriched medium but its performance in identification of bacteria is very poor. Similarly differentiation of lactose fermenter and non-fermenter is possible on MAC agar and CLED agar, but further species identification necessitates subculture or different biochemical tests with consequent longer reporting time and cost. Moreover, their limited capacities in maximizing the growth of possible pathogens rendered them unsuitable as an ideal primary isolation medium [9].

The problem of urine culture has been addressed by the introduction of chromogenic agar (CA) medium which is commercially available for two decades. It is being increasingly used as a versatile primary culture tool for better isolation, presumptive identification and differentiation of bacterial species from clinical specimens. [10] HiCrome UTI Agar is a differential medium recommended for presumptive identification of microorganisms mainly causing urinary tract infections. HiCrome UTI agar is such a chromogenic medium that facilitates rapid isolation as well as presumptive identification of most uropathogens including various species from mixed cultures [11]. This Agar is formulated on basis of work carried out by Pezzlo [12] Wilkie *et al* [13], Friedman *et al* [14], Murray *et al* [15], Soriano and Ponte [16] and Merlino *et al* [17]. Chromogenic substrates are incorporated into these media that are broken down by bacterial enzymes imparting a distinct visible colour to the growing bacterial colonies for their identification. [18] This single medium supports not only the growth of all uropathogens but mixed infections can also be diagnosed more easily [19]. In a few studies comparing chromogenic media with traditional ones its advantages including 20% reduction in time for identification, reduction in workload [20], easier recognition of mixed growth [21] and reduction in number of biochemical tests for bacterial identification have

been shown. All these factors have direct impact on ultimate cost reduction. HiCrome UTI Agar is with a slight difference in chromogenic mixture to improve the colour characteristic of media. These media are recommended for the detection of urinary tract pathogens where HiCrome UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colors produced by reactions of genus or species specific enzymes with two chromogenic substrates. The present study was undertaken to validate the usefulness of HiCrome UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to BA and MAC agar in a Nair Hospital, in Mumbai.

2. Material and methods

2.1 Place of work

This prospective longitudinal study was carried out over a period of two years, from January 2003 to December 2004 after taking the permission from Institutional Ethics committee of T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai. It was conducted in the Department of Microbiology in association with the Department of Obstetrics and Gynecology, of T. N. Medical College and B. Y. L. Nair Charitable Hospital.

2.2 Participants

The study included the patients from Out-door and Indoor patients of Gynecology department which were recruited for bacteriologic evidence of asymptomatic bacteriuria by microscopy, culture and chemical examination. Patients included were randomly selected 3300 subjects who were married pregnant and non-pregnant women reporting to ANC clinic of the hospital of age group of 18 to 40 years. Counseling for enrollment procedure in the study was done. Detailed data from the patients were recorded in a specially formulated structured proforma.

2.3 Collection of urine

Before collection, all women were instructed to wash well and rinse periurethral area with water using front to back motion [22]. Saline soaked cotton was provided to wipe and clean periurethral area after washing. Patients were advised to pass urine, discarding the first part of the stream and collecting clean-catch "midstream" urine, in a graduated sterile wide-mouthed container covering around $\frac{3}{4}$ volume.

2.4 Transportation and Processing of urine

Urine samples were transported to the laboratory without delay. If a delay of more than 1-2 hrs was unavoidable, urine were stored in refrigerator at 4 °C. 2ml of homogenized urine were centrifuged at 3000 r.p.m. for 4mins.

2.5 Microscopic examination of urine

a) Microscopic examination

A preliminary inspection of the smears was performed by using a low-magnification (10× to 20×) dry objective in order to locate the material on the slide. Next, with a 100× oil immersion objective, 50 fields were examined, and the shapes and number of microorganisms and cells per field was recorded. The microscopic reading was done

systematically, beginning at the edge of the central region of the smear and continuing across its diameter. A positive microscopic examination was defined as the presence of ≥ 2 microorganisms uniformly distributed per oil immersion field, after observation of at least 20 fields.

b) Gram staining

10- μ l volume of homogenized urine sample was applied, by means of a nickel-chrome loop calibrated to 10 μ l, to the surface of a 25- by 75-mm microscope slide and was allowed to dry, without spreading, at ambient temperature. After air drying, the smears were fixed by passing the slides two or three times through the flame of a Bunsen burner, and then they were stained by the Gram method with Hucker's modification. This method can detect the presence of both bacteria and pus cells in urine specimens [23].

c) Preparation of media: HiChrome UTI agar, MAC agar and base for BA media were obtained as a dehydrated powder from HiMedia Laboratories Pvt. Ltd. Mumbai. All culture Petri plates were prepared in house by following manufacturer's instructions and recommendations. For preparation of Blood agar, 5% defibrinated sheep blood was used. Prepared plates were stored at 2-8 $^{\circ}$ C for a month. Every fresh batch of media was tested for its ability to support the growth of *Escherichia coli* ATCC 25922 to ensure the quality of the media.

d) Urine culture

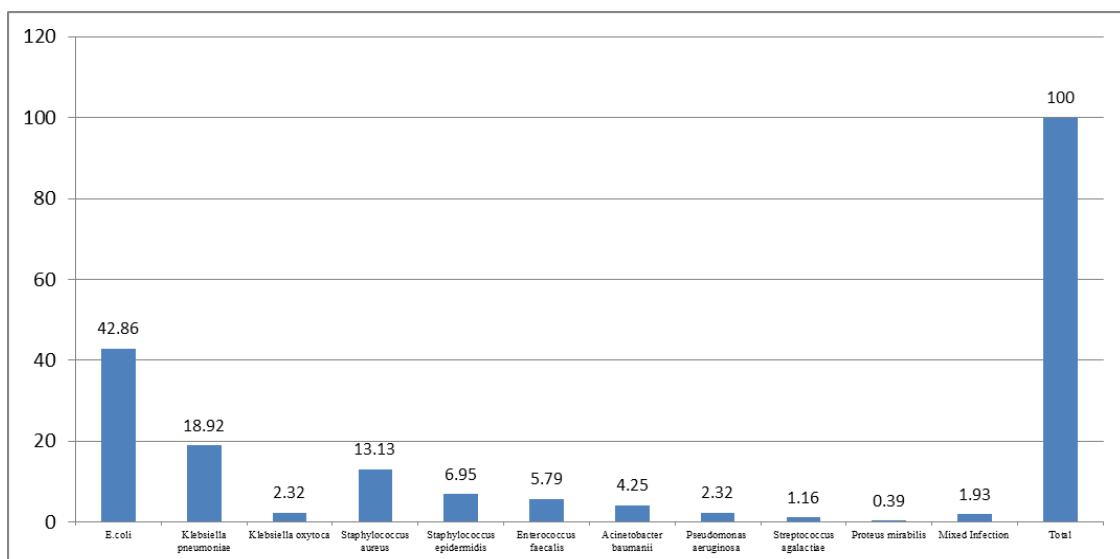
Culturing of un-centrifuged urine specimens was done using standard loop technique. 0.01ml of urine was surface streaked by calibrated loop of 28G with an internal diameter of 3.26 mm holding 0.01 ml of urine on HiChrome UTI agar, BA and MAC agar media aseptically [24]. The plates were incubated at 37 $^{\circ}$ C aerobically and after overnight incubation they were checked for significant bacteriuria as under by enumeration of colonies. If growth was seen, colonies were counted and recorded as colony forming units (CFU) /ml by multiplying count by 100. CFU more than or equal to 10^5 bacteria/ml of one or two organisms on two clean catch cultures in absence of symptoms were considered for significant bacteriuria [24]. Urine specimens containing $\geq 10^5$ or $< 10^5$ CFU of nonpathogenic bacteria /ml or multiple species of gram-negative bacteria, obtained from

patients without clinical evidence of urinary infection, were considered contaminated and were excluded from the study. [25]. Presumptive identification of bacterial growth was done on HiChrome UTI agar according to colony morphology and colour as depicted by the manufacturer. Colonies on the MAC agar and BA were also identified following colony characteristics against each of the uropathogens. The final identification of the isolates was done 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 [24]

e) Antibiotic sensitivity testing

Antibiotic sensitivity testing of the isolate obtained from clinical samples was carried out by Kirby - Bauer disk diffusion method according to HiMedia Manual 1998 [26, 27]. These isolates were separately collected from HiChrome agar and BA and MAC agar, to check the susceptibility pattern of isolates, isolated from different agar. For total 200 gram negative bacterial isolates, antibiotics used were Ampicillin (A)(10 mcg/ml), Cephalexin (Cp)(30 mcg/ml), Nalidixic acid (Na)(30 mcg/ml), Norfloxacin (Nx) (10 mcg/ml), Nitrofurantoin (Nf) (300 mcg/ml), Cotrimoxazole (Co) (1.25 + 23.75 mcg/ml), Augmentin (Ac)(30 mcg/ml), Ceftriaxone (Ci) (30 mcg/ml), Cefotaxime (Ce)(30 mcg/ml), Piperacillin + Tazobactam (Pt) (100 + 10 mcg/ml), Cefuroxime (Cu) (30 mcg/ml), Amikacin (Ak) (30 mcg/ml), Piperacillin (Pc) (100 mcg/ml). And for 52 gram positive bacterial isolates, antibiotics used were Ampicillin (A)(10 mcg/ml) Cephalexin (Cp)(30 mcg/ml), Norfloxacin (Nx)(10 mcg/ml), Nitrofurantoin (Nf) (300 mcg/ml), Cotrimoxazole (Co)(1.25+ 23.75 mcg/ml), Augmentin (Ac)(30 mcg/ml), Ceftriaxone (Ci)(30 mcg/ml), Cefotaxime (Ce)(30 mcg/ml), Piperacillin + Tazobactam (Pt)(100 + 10 mcg/ml), Amikacin-Ak) (30 mcg/ml), Ciprofloxacin (Cf) (5 mcg/ml), Erythromycin (E)(15 mcg/ml), Vancomycin (Va) (30 mcg/ml), Penicillin G-P(10units). Value in bracket indicates abbreviation of antibiotic and its content in disc in mcg/ml. Resistance was ascertained by considering the break point MICs as per the norms. [28] Quality control checks of all the medium was carried out by inoculating standard strains of bacteria.

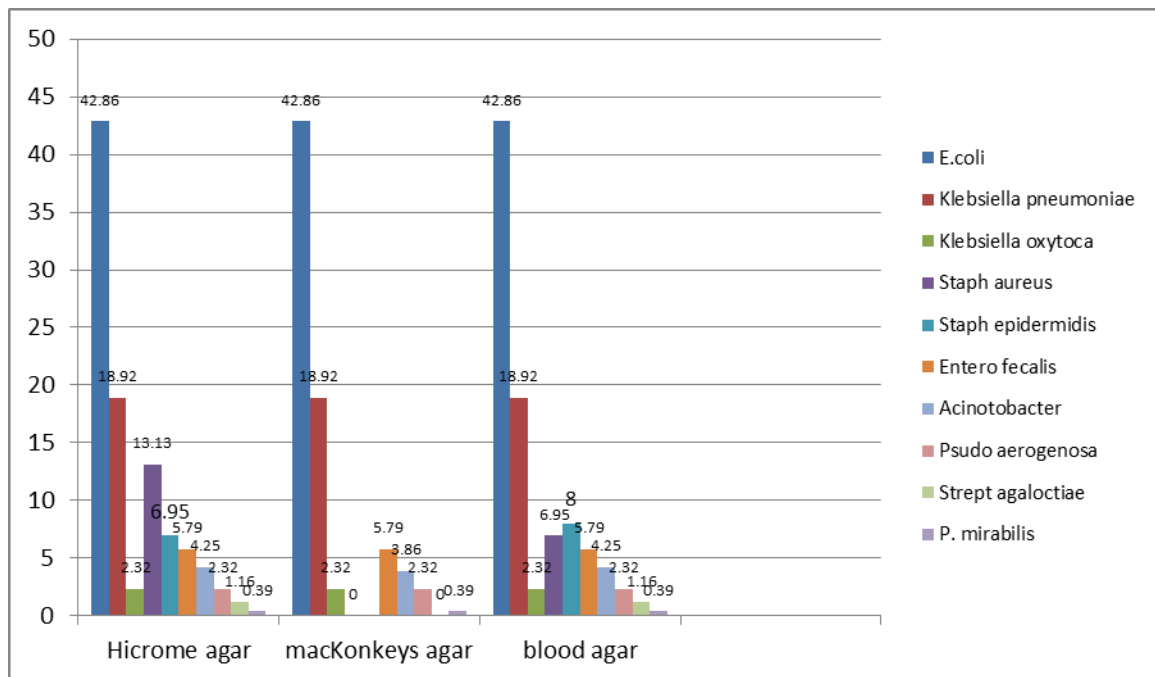
3. Results



Graph 1: Pattern of bacteria (%) isolated from urine culture (n=259).

Out of 3300 urine culture, 259 (7.85%) yielded significant bacterial growths and 3041 (92.15%) showed no growth. Culture-positive samples included 254 (7.7%) growth of single organism and 05 (0.15%) mixed growth of two

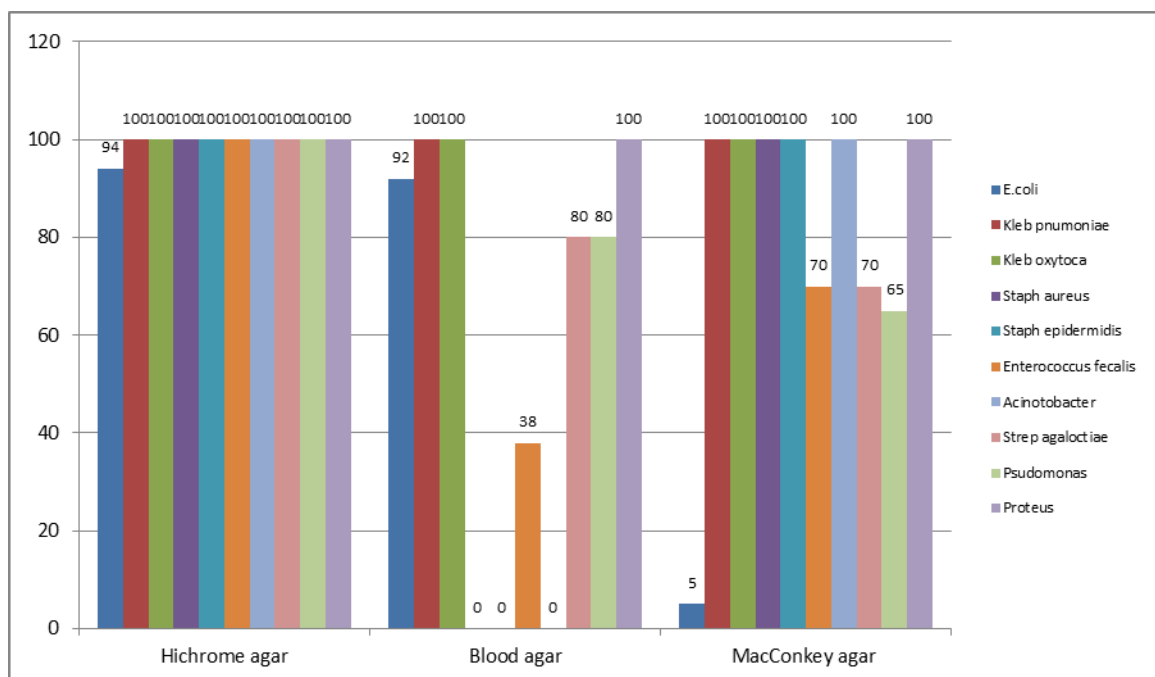
organisms each. *E. coli* was the leading bacteria isolated from 42.86.30% samples followed by *Klebsiella* spp (18.92%) and *Staph. aureus* (13.13%). While least growth was observed was of *Proteus* spp (0.39%).



Graph 2: Comparison of culture media for the rate of isolation (%) of uropathogens

Comparative results of three culture media for their rate of isolation of uropathogens shows that both HiCrome UTI

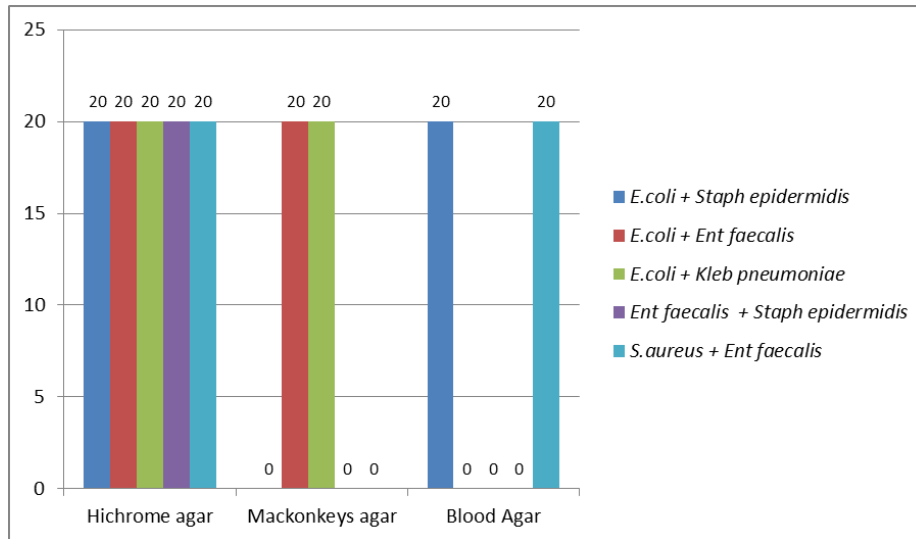
agar and BA media supported more than 75 % bacterial growth, while 52.30% growths were observed in MAC agar.



Graph 3: Comparison of media for rate of presumptive identification as primary culture

For presumptive identification of bacterial species by colony characteristics on primary culture plate, of 259 bacterial isolates, 99.40% could be differentially identified on HiCrome UTI agar against 49.0% and 81.0% on BA and

MAC agar respectively. The rate of presumptive identification of the isolates was found significantly higher on Hi Crome UTI agar than MAC agar as primary urine culture medium.



Graph 4: Comparison of rate of isolation of polymicrobial growth on culture media (n=5).

Comparative results of three culture media for their rate of isolation of polymicrobial growth on of uropathogens showed that HiCrome UTI agar supported 100% bacterial

growth, while BA and MAC agar supported 40% bacterial growth each.

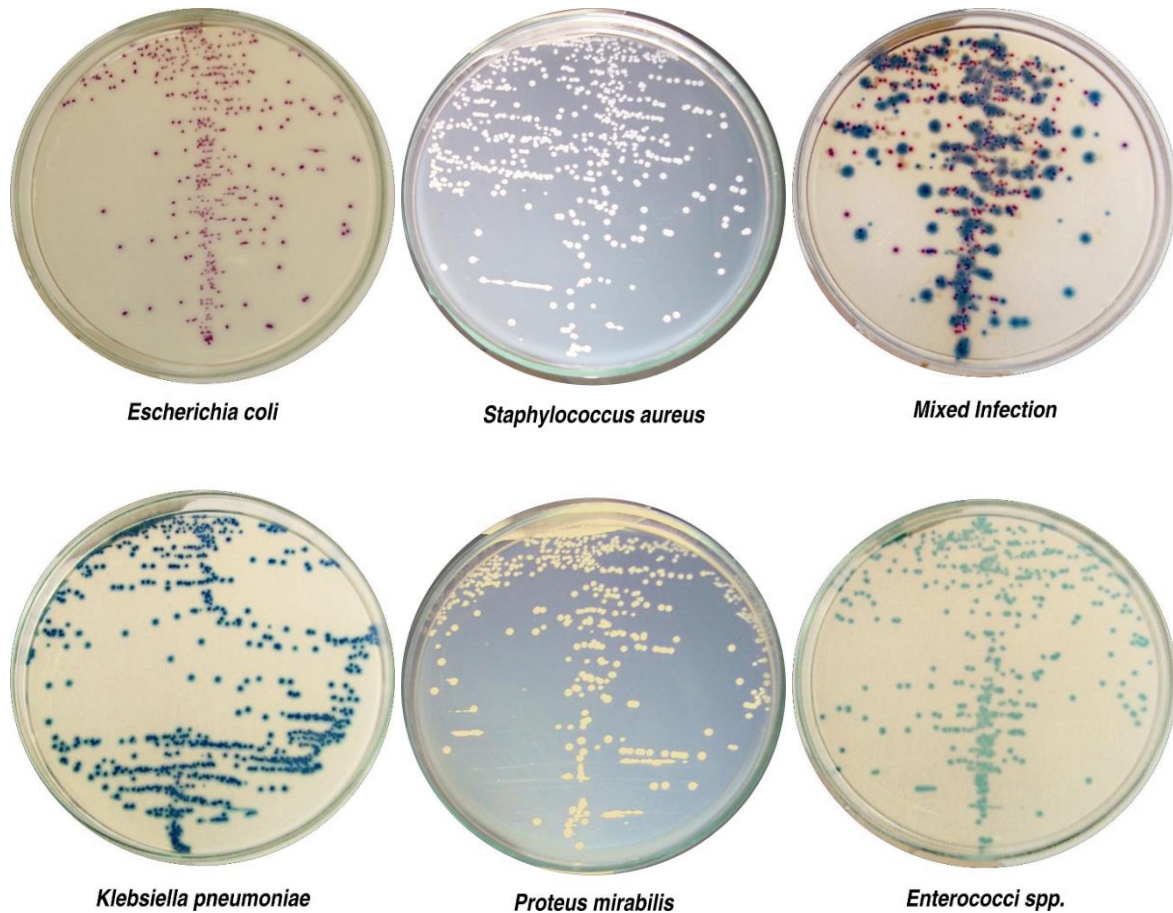
Table 1: identification of uropathogens on HiCrome UTI™ agar (n=259)

No	Organism	Observed color of colonies on HiCrome UTI™ agar	No. of isolates.
1	<i>E. coli</i>	Small pink colony	42.86%
2	<i>Klebsiella pneumoniae</i>	Large blue mucoid colony	18.92%
3	<i>Klebsiella oxytoca</i>	Large blue colony	2.32%
4	<i>Pseudomonas aeruginosa</i>	Transparent yellow to green colored colony with serrated edges	2.32%
5	<i>Acinetobacter baumannii</i>	Nontransparent cream colored colonies	4.25%
7	<i>Proteus mirabilis</i>	Clear diffusible beige color colony	0.38%
8	<i>Staphylococcus aureus</i>	Opaque golden yellow	13.13%
9	<i>Enterococcus faecalis</i>	Pinpoint blue or turquoise	5.7%
10	<i>Staphylococcus epidermidis</i>	Small colourless to yellow color colonies	6.95%
11	<i>Streptococci agalactiae</i>	Pinpoint, translucent diffuse light blue colony with agar	1.14%
	Mixed infection		
12	a) <i>E. coli</i> + <i>Staphylococcus epidermidis</i>	Pink + Colorless	0.39%
	b) <i>E. coli</i> + <i>Enterococcus faecalis</i>	Pink + Pin-point blue	0.39%
	c) <i>E. coli</i> + <i>Klebsiella pneumoniae</i>	Pink + Blue mucoid	0.39%
	d) <i>Staph epidermiditis</i> + <i>Enterococcus faecalis</i>	Colorless + Pinpoint blue	0.39%
	e) <i>Staphylococcus aureus</i> + <i>Enterococcus faecalis</i>	Golden yellow + Pin-point blue	0.39%

Legends



Picture 1: Standard bacterial strains showing colony characteristics specific for that organisms.



Picture 2: Culture plates of various clinical isolates obtained from urine samples

Table 2: Susceptibility results of gram positive isolates (n=52)

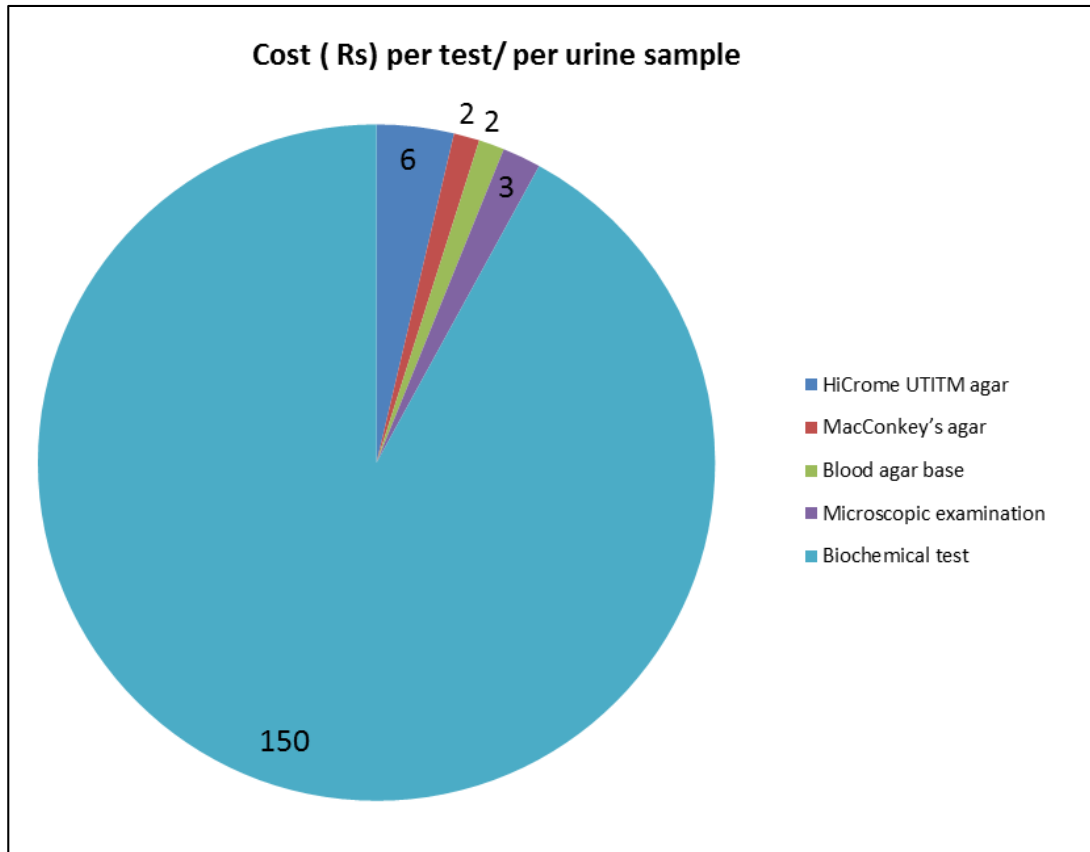
Antibiotic with disc content (µg)	No. of isolates picked from HiCrome UTI™ agar (%)			No. of isolates picked from Blood agar (%)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Penicillin G (10 units)	31(60)	0(0)	21(40)	31(60)	0(0)	21(40)
Ampicillin (10)	37 (72)	1(2)	14(27)	37(72)	1(2)	14(27)
Amoxicillin + Clavulanic acid (20 + 10)	44(85)	0(0)	8(15)	44(85)	0(0)	8(15)
Cephotaxime (30)	18(35)	1(2)	33(63)	18(35)	1(2)	33(63)
Ceftriaxone (30)	20(38)	0(0)	32(62)	20(38)	0(0)	32(62)
Cefuroxime (30)	20(38)	0(0)	32(62)	20(38)	0(0)	32(62)
Amikacin (30)	22(42)	0(0)	30(58)	22(42)	0(0)	30(58)
Erythromycin (15)	27(52)	0(0)	25(48)	27(52)	0(0)	25(48)
Vancomycin (30)	47(90)	0(0)	5(10)	47(90)	0(0)	5(10)
Nitrofurantoin (300)	38(73)	0(0)	14(27)	38(73)	0(0)	14(27)

Susceptibility pattern of all gram positive isolates (52) collected from HiCrome UTI™ agar and BA was found to be similar.

Table 3: Susceptibility results of gram negative isolates (n=200)

Antibiotic with disc content (µg)	No. of isolates picked from HiCrome UTI™ agar (%)			No. of isolates picked from MacConkey's agar (%)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin (10)	32(16)	1(0.5)	167(83.5)	32(16)	1(0.5)	167(83.5)
Cephalexin (30)	67(33.5)	3(1.5)	130(65)	67(33.5)	3(1.5)	130(65)
Nalidixic acid (30)	50(25)	1(0.5)	149(74.5)	50(25)	1(0.5)	149(74.5)
Norfloxacin (10)	73(36.5)	1(0.5)	126(63)	73(36.5)	1(0.5)	126(63)
Nitrofurantoin (300)	117(58.5)	0(0)	83(41.5)	117(58.5)	0(0)	83(41.5)
Cotrimoxazole (1.25/23.75)	60(30)	0(0)	140(70)	60(30)	0(0)	140(70)
Amoxicillin + Clavulanic acid (20 + 10)	57(28.5)	0(0)	143(71.5)	57(28.5)	0(0)	143(71.5)
Ceftriaxone (30)	81(40.5)	0(0)	119(59.5)	81(40.5)	0(0)	119(59.5)
Cephotaxime (30)	79(39.5)	1(0.5)	120(60)	79(39.5)	1(0.5)	120(60)
Piperacillin + Tazobactam (100 +10)	168(84)	0(0)	32(16)	168(84)	0(0)	32(16)
Cefuroxime (30)	80(40)	0(0)	120(60)	80(40)	0(0)	120(60)
Amikacin (30)	101(50.5)	5(2.5)	94(47)	101(50.5)	5(2.5)	94(47)
Piperacillin (100)	88(44)	0(0)	112(56)	88(44)	0(0)	112(56)

Susceptibility pattern of all gram negative isolates (200) collected from HiCrome UTI™ agar and MAC agar was found to be similar.



Graph 5: Comparison of cost of HiCrome UTI™ agar and other conventional methods.

Cost of HiCrome UTI™ agar (Rs 6) per test per urine sample was found to be less than traditional identification methods.

4. Discussion

HiCrome UTI agar is such a chromogenic medium that facilitates rapid isolation as well as presumptive identification of most uropathogens including various species from mixed cultures [12] The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species. One of the chromogenic substrate is cleaved by β -glucosidase possessed by *Enterococci* resulting in formation of blue colonies. *E. coli* produces pink colonies due to the enzyme β -D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E. coli* can be done by performing the indole test. Coliforms produce purple colored colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus*, *Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptic digest of animal tissue or peptone special provides nitrogenous, carbonaceous compounds and other essential growth nutrients. *Pseudomonas* spp. produces colourless colonies whereas *Staph. saprophyticus* produces white colonies [14] Certain identification tests like the catalase, oxidase and indole production can be done directly from the colonies on HiCrome UTI agar and antibiotic sensitivity testing without sub culturing onto another basic medium is also possible [24] This medium can be made selective by supplementation with antibiotics for detecting

microorganisms associated with hospital borne infections. HiCrome UTI™ agar is reliable and easy to use media. Less trained personnel also can identify the pathogen easily on this media along with mixed infection. The results of the antimicrobial susceptibility test showed 100% correlation of microorganisms picked from HiCrome UTI™ agar and picked from BA and MAC agar. A cost analysis showed that cost of using HiCrome UTI™ agar is lesser than other conventional methods and biochemical test required for identification of isolate. Thus, HiCrome UTI™ agar was recommended as a single medium for direct isolation, presumptive identification and interpretation of culture results which was important for early medical treatment. Therefore it was possible that just one plate could be used for differentiation of urinary pathogen at the genus level by colony color caused by various enzyme activities.

Presumptive identification of bacterial isolates in urine culture is time consuming and requires a expertise by traditional methods. But HiCrome UTI agar medium was found to be much superior over conventional media for its higher rate of isolation and uniform interpretation for identification of uropathogens. As many of the extra tests for bacterial identification associated with conventional culture methods were no longer required, chromomeric medium substantially reduced the laboratory workload with concomitant high bench throughput.

The rate of isolation and pattern of major uropathogens of the present study are in accordance with a few studies carried out on both chromogenic and conventional media. [29] It has generally been noted that only 20 to 30% of urine samples results in significant growth with predominant causative agent being *E. coli* in both community and hospital acquired infections. [7, 18, 26, 28] Regarding rate of uni and polymicrobial growths from urine culture, our results

corroborate with a few studies done here and in India [26-28]. HiCrome UTI agar and BA supported the growths of more than 75% isolates whereas MAC agar yielded 52.00% bacterial growths. BA is an enriched medium and HiCrome UTI agar also contains all essential nutrients to support the growth of possible uropathogens that is why all isolates were possible to be grown on to these two media and similar findings were also reported by others [26]. 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 for members of Enterobacteriaceae.

As far as the presumptive identification of bacterial species is concerned, significantly high percentage of bacterial species were possible to be identified on HiCrome UTI agar by matching with standard colours as opposed to conventional culture system. This high rate of identification could be correlated with the ease of identification technique by seeing the distinct and perceivable colony colour produced by each of the bacterial species on chromogenic agar medium and our findings are consistent with reports published [19, 26]. There was significant difference in rate of presumptive identification especially for *E. coli*, *Klebsiella* spp., *Enterococci* spp. and *Enterobacter* spp. on HiCrome UTI agar and similar results were also observed by other investigators [26-28]. In fact, this differential colour production by individual bacterial species is among the most exciting features of chromogenic agar for which it has been advocated to be used as primary urine 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. 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 [17, 18].

The HiCrome UTI agar also reigned over the conventional media by providing high isolation rate as well as specific identifying characteristics of the organisms in mixed growth thus enabling microbiologists to assess more accurately the clinical relevance of urine culture results. Similar findings for polymicrobial growth in chromogenic agar were also reported by a few investigators [29]. The rate of identification of mixed culture on the BA and MAC agar was very poor due to their limitations in differentiating the colonies. Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics [30, 31]. Now it is obvious from the results of the present and similar studies that chromogenic medium has the right potential to replace both CLED and MAC agar as primary urine culture medium because of its superiority in rate of isolation and ease in identification through characteristic colony colour. Moreover, it also provides an added advantage of requiring less time in mastering the skill in identification of the uropathogens in contrast to conventional media [32, 33].

5. Conclusion

Though chromogenic media on its own are still expensive at the moment but considering the overall costs incurred for the use of multiple media and/or different biochemical tests necessary to identify the organism in the conventional urine culture system, it seems to be cost-effective. The overall

findings of this study shows that HiCrome UTI agar offers 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 compared to conventional culture system. It has the potential to streamline urine culture processing in a meaningful way, reducing technologist workload, improving result turnaround times and reducing costs which together all have considerable laboratory impact [34].

6 Acknowledgement

Authors are grateful to Nair Golden Jubilee Research Foundation for providing financial help to carry out this study and staff of the Microbiology Department of T. N. Medical College and B. Y. L. Nair Charitable Hospital for their moral support.

7. References

1. Nicolle LE. Epidemiology of urinary tract infections. *Clin Microbiol News*. 2002; 24:135-140.
2. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterisation of uropathogenic *Escherichia coli* in urinary tract infections. *Indian Journal of Medical Microbiology*. 2003; 21(2):102-107.
3. Lavanya SV, Joglekshmi D. Asymptomatic bacteriuria in Antenatal women. *Indian Journal of Medical Microbiology*. 2002; 20(2):105-106.
4. Nath G, Chaudhary M, Pandey JPL, Singh TB. Urinary tract infection during pregnancy and fetal outcome. *Indian Journal of Medical Microbiology*. 1996; 14(3):158-160.
5. Gilstrap III LC, Ramin SM. Urinary tract infections during pregnancy. *Obstetrics and Gynecology Clinics of North America*. 2001; 28:581-591.
6. Graham JC, Galloway A. The laboratory diagnosis of urinary tract infection. *Journal of Clinical Pathology*. 2001; 54:911-919.
7. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P *et al.* Urinary tract infections in women. *Eur J Obstet Gynecol Reprod Biol*. 2011; 156(2):131-136.
8. Fung JC, Lucia B, Clark E, Berman M, Goldstein J, D'Amato RF. Primary culture media for routine urine processing. *J Clin Microbiol*. 1982; 16:632-636.
9. Sekikawa E, Vidicki M, Bilkey M. Comparison of two chromogenic media and conventional media in the primary isolation and identification of urinary tract pathogens. *N Z J Med Lab Sci*. 2011; 65:77-82.
10. Perry JD, Freydiere AM. The application of chromogenic media in clinical microbiology. *J Appl Microbiol*. 2007; 103:2046-2055.
11. Ciragil P, Gul M, Aral M, Ekerbicer H. Evaluation of a new chromogenic medium for isolation and identification of common urinary tract pathogens. *Eur J Clin Microbiol Infect Dis*. 2006; 25:108-111.
12. Pezzlo M. Detection of urinary tract infections by rapid methods. *Clin Microbiol Rev*. 1988; 1(3):268-80.
13. Wilkie ME, Almond MK, Marsh FP. Diagnosis and management of urinary tract infection in adults. *British Medical Journal*. 1992; 305:1137-1141.
14. Friedman MP. Universal Differential Chromo Select Medium, *J Clin. Microbiol*. 1991; 29:2385-2389.
15. Murray P, Traynor P, Hopson D. Evaluation of microbiological processing of urine specimens:

- comparison of overnight versus two-day incubation. *J. Clin. Microbiol.* 1992; 30:1600-1601
16. Soriano F, Ponte C. Biogroups of Enterobacteriaceae Isolated from Clinical Specimen, *J. Clin. Microbiol.* 1992; 30:3033-3034.
 17. Merlino. UTI chromo select media. *Abstr. Austr. Microbiol.* 1995; 16(4):17
 18. Lakshmi V, Satheeshkumar T, Kulkarni G. Utility of Urichrom II – A Chromogenic Medium for Uropathogens. *Indian J Med Microbiol.* 2004; 22(3):153-158
 19. Fallon D, Ackland G, Andrews N. A comparison of the performance of commercially available chromogenic agars for the isolation and presumptive identification of organisms from urine. *J Clin Pathol.* 2003; 56:608-61
 20. Manickam K, Karlowsky JA, Adam H, Lagace-Wiens PRS, Rendina A, Pang P *et al.* CHROM agar Orientation Medium Reduces Urine Culture Workload. *J Clin Microbiol.* 2013; 51(4):1179-1183
 21. Aspell O, Osterman B, Dttmer R, Sten L, Lindback E, Forsum U. Performance of four chromogenic urine culture media after one or two days of incubation compared with reference media. *J Clin Microbiol.* 2002;40:1500-1503
 22. Koneman EW, Allen SD, Janda WM. Urinary tract infections. In: *Color atlas and textbook of diagnostic microbiology.* Edn 14, J. B. Lippincott Company. 1997, 136-141.
 23. Isenberg HD. Urine Culture Procedure. In: *Clinical Microbiology Procedures, Vol 1: American Society for Microbiology Press, 1992, Section: 1.4.10- 1.4.11.*
 24. Collee JG, Marr W. Specimen collection, culture containers and media. Edn 14, n: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie & McCartney Practical Medical Microbiology.* New York: Churchill Livingstone, 1996, 85-111.
 25. Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P *et al.* Urinary tract infections in women. *Eur J Obstet Gynecol Reprod Biol.* 2011; 156(2):131-136
 26. *The Himedia Laboratories Manual of Microbiology Lab. Practice, 1998*
 27. Delost MD. Urinary tract infections. In: *Introduction to Diagnostic Microbiology a text and workbook.* Mosby, 1997, 312-345.
 28. Rani L, Pinnelli VBK, Hemavathi, Belwadi S, Rajendran R. Utility of HiChrome Urinary Tract Infection (UTI) Agar Medium for Identification of Uropathogens: A Comparative Study with Other Conventional Media. *J Chem Pharm Res.* 2012; 1(4):95-105.
 29. Ciragil P, Gul M, Aral M, Ekerbicer H. Evaluation of a new chromogenic medium for isolation and identification of common urinary tract pathogens. *Eur J Clin Microbiol Infect Dis.* 2006; 25:108-111
 30. Gaillot O, Wetsch M, Fortineau N, Berche P. Evaluation of CHROM agar, a new chromogenic medium, for isolation and presumptive identification of *S. Aureus* from human clinical specimens. *J Clin Microbiol.* 2000; 38:1587-1591
 31. Sharmin S, Alamgir F, Begum F, Jaigirdar Q. Use of chromogenic agar media for identification of uropathogen. *Bangladesh J Med Microbiol.* 2010; 4:18-23.
 32. Parveen R, Saha SK, Shamshuzzaman SM, Rashid AL, Chowdhury A, Muazzam N. Detection of Uropathogens by Using Chromogenic Media (Hicrome UTI agar), CLED agar and other Conventional Media. *Faridpur Med Coll J.* 2011; 6(1):46-50.
 33. Perry JD, Butterworth LA, Nicholson A, Appleby MR, Orr KE. Evaluation of a new chromogenic medium, Uriselect 4, for the isolation & identification of urinary tract pathogens. *J Clin Pathology.* 2003; 56:528-531
 34. Laila Akter, Rezwana Haque, Md. Abdus Salam. Comparative evaluation of chromogenic agar medium and conventional culture system for isolation and presumptive identification of uropathogens. *Pak J Med Sci.* 2014; 30(5):1033-1038.