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Original Article

Use of Chromogenic Agar Media for Identification of Uropathogen

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Abstract

Chromogenic agar media are increasingly being used as versatile tools in early differentiation and identification of Gram positive and Gram negative isolates from clinical specimens. We have evaluated the chromogenic medium as it's use reduces the burden of biochemical characterization and reduces the workload for identification of bacteria. This study included 400 consecutively collected midstream and/or catheter-catch urine samples obtained from patients attending the hospital out patient department (OPD) and also from patients admitted in BSMMU hospital. They were inoculated on blood agar, MacConkey agar, cystine lactose, electrolyte deficient media and chromogenic agar plate for isolation of uropathogen. Out of 400 urine samples tested, 154 (38.5%) yielded significant growth of single organism and 16 (4%) yielded mixed growth. No growth was observed in 230 (57.5%) cases. The chromogenic agar media allowed the growth and primary identification in 171 (92.5%) strains out of 186 strains. The predominant uropathogens were *Escherichia coli, Klebsiella spp. Enterococcus spp.* and *Eneterobacter spp.* (KES group). The different coloured colonies produced by the breakdown of the chromogenic substrate by the specific enzymes of the bacteria were very useful in the presumptive identification of these organisms even from polymicrobial cultures by the color differences of the colonies. The medium also supported growth and differentiation of Gram positive organisms like Staphylococcus and Enterococci. Chromogenic agar media can be used as primary culture medium for isolation and identification of predominant uropathogens like *E.coli*, KES group and Enterococci. It is an easy to use primary screening medium that considerably reduces the daily workload and thus minimizes or limits the use of identification tests.

Key words: Chromogenic agar medium, KES, CLED media, UTI

Introduction

The increase in the resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of pathogens. Early information enable the selection of the appropriate antibiotic prior to the results of susceptibility test and may prevent outbreaks.[1-4] Urine culture and sensitivity tests are advised more frequently than the other clinical specimens for islating the pathogens and to identifying the antimicrobials for appropriate treatment. Blood agar and MacConkey agar media or Cystine Lactose, Electrolyte Deficient (CLED) media are used for primary inoculation of urine in most of the clinical diagnostic laboratories. Though blood agar is used universally as enriched medium but it lacks the ability for primary differentiation between the Gram positive and Gram negative colonies, thereby necessaciating further identification test and causing delay in the final result. It also completely fails to prevent swarming of Proteus spp. colony. Differentiation of lactose fermenters from non lactose fermenters is possible in MacConkey (MAC) medium but it does not support the

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growth of all uropathogen. Though CLED medium support the growth of some additional bacteria but further identification of different genera of lactose fermenters like *Escherichia, Klebsiella, Enterobacter* and *Serratia* spp. can't be identified on primary plate. Additional more biochemical and other tests are required for specific identifican.[5, 6]

Over the last few years' several chromgenic agar medium have been developed and commercialized allowing more specific and direct differentiation of microorganisms on the primary plate itself on the basis of distinct colour and colonial morphology.[6-9] They facilitate direct identification of the bacterial organisms and reduce the burden of biochemical characterization. It has several advantages such as greater ability to differentiate the species of gram negative bacilli and facilitate the detection as well as presumptive identification of Gram negative bacilli.[8]

Chromogenic agar media are designed to isolate and identify uropathogens mainly *Escherichia, Klebsiella* and *Enterococci* spp. It has an advantage that it supports the growth of all bacterial uropathogens unlike MacConkey agar medium. *Escherichia* produces the enzyme ß glucuronidase that attacks ß glucuronide chromogenic substrate and will grow as distinct pink – burgundy colour colonies. *Klebsiella, Enterococci, Enterobacter* and *Seratia* (KES) group produce the enzyme ß glucosides that react with ß glucoside chromogenic substrate and will grow as distinct blue colonies and by colony morphology apparent differentiation of these KES group is possible in primary chromogenic agar media. *Proteus* spp. can also be identified directly if tryphan deaminase reagent is added in the media then it form brown colour. The rest enterobacteriaceae produc colourless colonies as they don't produces specific enzyme. Pseudomonas produces dark green or yellow green flat shining colonies.[10] Another important advantage of chromogenic agar media is that it reduces the burden of biochemical characterization and reduces expenditure for identification of bacteria. With this background the study was carried out to evaluate the usefulness of chromogenic agar media for bacterial isolation and identification in primary culture with other conventional media as there was no such comparison study in our context.

Methods

A cross-sectional study was carried out in the department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU) during the period of July, 2004 to June, 2005.

Four hundred midstream urine (MSU) specimens were collected from clinically suspected Urinary Tract Infection(UTI) patients attending in the hospital out patient departments(OPD) and also from the patient admitted in BSMMU hospital. Approximately 20 ml of urine were collected aseptically in a sterile wide mouth container. The clean-catch mid-stream technique was employed to collect urine samples.[11]

Microscopic examination: Five ml of urine sample were poured into a clean and dry 15 ml centrifuge tube and centrifuged at 450 G for 5 minutes. Sediments were transferred to a clean glass slide covered with a clean cover slip and then examined under light microscope using 10X and 40X magnifications.

Culture: Urine were inoculated in Blood agar (BA), MacConkey agar medium (MAC), CLED agar and Chromogenic agar media. Sheep blood was used in Blood agar media. Colony count was done by using 3.26 mm internal diameter loop which hold .004 ml diluted urine and incubated overnight as standard protocol.[12] BA and MAC agar media were considered as single system. A presumptive identification of the isolates was made on the colony colour and morphology on the chromogenic agar, CLED agar and BA and MAC agar plates and was confirmed by standard identification protocol such as Gram's staining, motility test, oxidase test, catalase test and biochemical tests.[12] For quality assurance reference strain of *E.coli* ATCC 25922 was used.

Results

Out of total 400 patients, 80 (45%) were male and rest 220(55%) were female. One hundred and forty eight (37%) urine sample were collected from indoor patients and 252(63%) samples were collected from out patient departments. Among the 400 study population, 85% cases were in more than 18 years age group.

Out of 400 urine sample tested, 154 (38.5%) yielded significant growth of single organism and 16(4%) yielded mixed growth. No growth was observed in 230(57.5%) cases (Table 1)

Table-1: Result of urine culture among study population (n=400)

Growth	Number	Percentage
Single bacterial growth	154	38.5
Mixed bacterial growth	16	4
Total growth	170	42.5
No growth	230	57.5
Total	400	100

Out of these 186 bacterial isolates, *Escherichia* spp. was isolated in highest number of cases 99 (53.23%), followed by *Klebsiella* spp. 20 (10%), *Enterococcus* spp. 20 (10.7%), *Enterobacter* spp. 16 (8.6%), *Pseudomonas spp.* 14 (7.5%), *Staphylococcus. saprophyticus* 5 (2.6%), *Proteous* spp.

4 (2.1%), *Streptococcus* spp. 4 (2.1%), *Sthaphylococcus epidermidis* 2 (1.0%), *Acenatobacter* spp. 2 (1.0%) (Table 2).

Table-2: Bacteria isolated from UTI patients (n=186)

Name of bacteria	Number of bacteria	Percentage
Escherichia coli	99	53.2
Klebsiella spp.	20	10.7
Enterococcus spp.	20	10.7
Enterobacter spp.	16	8.6
Pseudomonas spp.	14	7.5
Staphylococcus saprophyticus	05	2.6
Proteus spp.	04	2.1
Streptococcus spp.	04	2.1
Staphylococcus epidermidis	02	1.0
Acinatobacter spp.	02	1.0

Among the 186 bacterial isolates, all were isolated in Chromogenic agar and BA media but in CLED and MAC agar it was 176 (95%) and 155(84.8%) respectively (Table-3).

Table-3: Number of isolates grown on different media (n=186)

Name of strain	Total no. of strain grown	Chromogenic agar N(%)	CLED	BA	MAC
Escherichia coli	99	99(100)	99(100)	99(100)	99(100)
Klebsiella spp.	20	20(100)	10(50)	20(100)	20(100)
Enterococcus spp.	20	20(100)	20(100)	20(100)	00(00)
Enterobacter spp.	16	16(100)	16(100)	16(100)	16(100)
Pseudomonas spp.	14	14(100)	14(100)	14(100)	14(100)
Staphylococcus saprophyticus	5	5(100)	5(100)	5(100)	5(100)
Proteus spp.	4	4(100)	4(100)	4(100)	4(100)
Streptococcus spp.	4	4(100)	4(100)	4(100)	0(00)
Staphylococcus epidermidis	2	2(100)	2(100)	2(100)	0(00)
Acenatobacter spp.	2	2(100)	2(100)	2(100)	0(00)
Total	186	186(100%)	176(100%)	186(100%)	155(84%)

Out of total 186 isolates, 171 (92%) were identified by chromogenic agar media, 134(73%) and 137 (73%) were identified by CLED and BA & MAC media respectively. Out of 99 *Escherichia coli* isolated, 93 (94%) were identified in chromogenic agar media, 72 (73%) were identified by each of CLED, BA & MAC agar media respectively. Out of 20 strain of *Klebsiella* spp. 19 (95%) were identified by all three groups of media. Among the 20 *Enterococcus* spp., all (100%) were identified by chromogenic agar media, 10 (50%) by CLED, 12(60%) by BA & MAC media. All (20) *Enterobacter spp*. were identified by chromogenic agar media, 4(25%) were identified by other two group of media. (Table -4)

Table-4: Rate of identification of bacteria in primary culture plates

Name of strain	Number of strain	Chromogenic agar N(%)	CLED N(%)	BA & MAC N(%)
Eschericia coli	99	93(94)	72(73)	72(73)
Klebsiella spp.	20	19(95)	19(95)	19(95)
Enterococcus spp.	20	20(100)	10(50)	12(60)
Enterobacter spp.	16	16(100)	4(25)	4(25)
Pseudomonas spp.	14	10(71)	13(93)	13(93)
Staphylococcus saprophyticus	5	3(60)	5(100)	5(100)
Proteus spp.	4	4(100)	3(75)	4(100)
Streptococcus spp.	4	3(75)	4(100)	4(100)
Staphylococcus epidermidis	2	1(50)	2(100)	2(100)
Acinatobacter spp.	2	2(100)	2(100)	2(100)
Total	186	171(92%)	134(72%)	137(73%)

Out of 186 total isolates 171(92%) of the isolates matched with the standard colour as explained before. 100% of *Enterococcus spp., Enterobacter spp., Proteus spp*. and *Acenatobacter spp*. matched with the standard colour (Table 5).

Table-5: Matching of colony colour of isolates in chromogenic agar and standard media

Name of strain	Number of strain	Match with std. N(%)	Mismatch with std*. N(%)
Escherichia coli	99	93(94)	6(6)
Klebsiella spp.	20	19(95)	1(5)
Enterococcus spp.	20	20(100)	00(00)
Enterobacter spp.	16	16(100)	00(00)
Pseudomonas spp.	14	10(71)	4(29)
Staphylococcus saprophyticus	5	3(60)	2(40)
Proteus spp.	4	4(100)	00(00)
Streptococcus spp.	4	3(75)	1(25)
Staphylococcus epidermidis	2	1(50)	1(50)
Acinetobacter spp.	2	2(100)	00(00)
Total	186	171(92%)	134(72%)

*Std: Standard

Sixty five bacteria could not be identified in primary culture plate by any of the three group of media used. 15(23%) strains could not be identified on primary culture media by Chromogenic, 41(65%) by CLED and 49(75%) by BA and MAC agar media (Table-6).

Table-6: Organisms that could not be identified on primary culture plate of different media

Name of isolales	Total organism that could not be identified	Chromogenic agar N(%)	CLED N(%)	BA & MAC N(%)
Escherichia coli.	33	6(18)	27(82)	27(82)
Klebsiella spp.*	2	1(50)	1(50)	1(50)
Enterococcus spp.	8	0	0	8(100)
Enterobacter spp.	12	0	12(100)	12(100)
Pseudomonas spp.	5	4(80)	1(20)	1(20)
Staph. saprophyticus	2	2(100)	0	0
Proteus spp.	1	1(100)	0	0
Streptococcus spp.	1	1(100)	0	0
Staph. epidermidis	1	1(100)	0	0
Acinetobacter spp.	0	0	0	0
Total	65	15(23%)	42(65%)	45(75%)

* One failed to grow in Chromogenic agar and another failed to grow in CLED, BA and MAC agar media

Out of 16 polymicrobial growth which showed significant growth of two organisms, all (100%) were detected by Chromogenic agar media, 5(31.2%) by CLED, and 2(12.5%) by BA and MAC agar media (Table -7).

Table-7: Detection of polymicrobial growth on Chromogenic agar,CLED and BA and MAC agar media

Polymicrobial growth	**No. of cases**	**Detected by**		
		Chromogenic agar	**CLED**	**BA and MAC**
Escherichia coli & Enterococci spp.	8	8(100%)	3(37.5%)	Nil
E.coli & Enterobacter	3	3(100%)	Nil	Nil
Enterobacter & Acinatobacter	1	1(100%)	Nil	Nil
Enterobacter & Pseudomonas	1	1(100%)	Nil	Nil
E.coli & Klebsiella	1	1(100%)	Nil	Nil
E.coli & Pseudomonas	1	1(100%)	1(100%)	1(100%)
E.coli & Proteus	1	1(100%)	1(100%)	1(100%)
Total	**16***	**16(100%)**	**5(31.3%)**	**2(12.5%)**

16 cases yielded a total of 32 bacteria

Discussion

Rapid bacterial identification and susceptibility in the microbiology laboratory can have a demonstrable clinical impact as well as provide significant cost saving. One of the most recent advances in the rapid presumptive identification of pathogenic organisms is the use of different colony colours that are produced by the reactions of genus and species-specific enzymes with Chromogenic substrate.[13-17] Current study was done to evaluate the chromgenic agar media in respect to isolation frequency and presumptive identification of uropathogens on primary culture plate.

Females attending in the hospital out patient department and aged more than 18 years were the larger part of our study population which is the usual finding of such kind of studies. Out of 400 urine samples tested, 154 (38.5%) culture yielded single growth, 16(4%) yielded mixed growth of bacteria. Lakshmi et al. in India found 20% single growth and 4% were mixed growth.[10] In their study, urine samples were collected irrespective of pus cell count which may the reason for lower percentage of growth than that of ours study results. A study done in California reported single growth in 24.5% and mixed growth in 17.5% cases.[18] A similar study in UK conducted by Perry et al., found single growth in 24.5% and mixed in 17.5% cases.[23] In their study, urine sample having pus cell >200 /cmm were only included which is the reason for accordance with present study.

Out of 186 culture positive cases, all were identified from the growth on Chromogenic agar, BA agar and MAC media but from CLED agar media 176(95%) cases were identified . On CLED medium the presence of Enterococci is frequently masked by larger colonies of Gram negative species, for this reason isolation of the organism may be less in CLED.[19]

Of these 186 strains isolated, *Escherichia coli* was isolated in highest number of cases 99(53.2%) followed by Klebsiella spp 20(10.7%), Enterococcus spp. 20(10.7%), Enterobacter spp. 16(8.6%) and Psedomonas spp. 14(7.5) respectively. *Escherichia coli* and Klebsiella spp. were major isolates in most the studies conducted by others.[21,18] Increased number of Enterococci and Enterobacter in our study may be due to higher isolation rate of these organism in chromogenic agar media. In our study, only 5 (2.6%) cases yielded *Staphylococcus saprophyticus* which is relatively lower than most the other studies reviewed. The reason may be the low number of < 18 years population in our study population in which age group *Staphylococcus saprophyticus* infection occurs predominantly. Chromogenic agar media does not have any advantage over conventional media for isolation and has poor performance in identification of Gram positive cocci except Enterococci spp.

The reason for highest percentage of identification of *E.coli,* Klebsiella spp*,* Enterococci spp. and Enterobacter spp. on Chromogenic agar media in comparison to other two media is that these organisms produce specific colour on chromogenic

agar media which were distinct and easy to perceive.

Difficulties in differentiating lactose fermenters from non lactose fermenting *E.coli* are probably the reasons responsible for poor identification (73%) of *E.coli* in CLED compared to chromogenic agar media where *E.coli* were identified in 94% cases. Six percent of *E.coli* did not give expected colour in Chromogenic agar media, probably because of the absence of glucoronidase in those strains. Similar were the cause of 5 Klebsiella spp. which did not give proper colour due to lack of glactosidase. Identification of these variant was done by further biochemical tests. Lakshmi el al. also have similar finding.[10] They failed to identify 10% of *E.coli* and 2% of Klebsiella spp. in primary culture plate of Chromogenic agar media. Aspevall et al. in Sweden found that *E.coli* didn't give proper colour in 5% cases and Klebsiella spp. in 2% cases.[25]Almost similar was the finding by Perry et al. in United Kingdom.[23]

Although BA, MAC and CLED are good media for isolation of single pathogens, they do not have the differential capacity to distinguish between some mixture of species. These findings reaffirm those from other studies by Perry et al. and Aspevall et al. which shows that chromogenic media offers far superior means of differentiation of polymicrobiol cultures, enabling microbiologists to asses more accurately the clinical relevance of urine culture results.[23.25]

The result suggests that the chromgenic agar media can be used as a single medium for the isolation of uropthogens. To make the first, presumptive identification of isolates is a task that requires a great deal of experience when using traditional media. On chromogenic media this is easier thus requiring less training. Though expensive the use of chromogenic agar may improve the quality of urine culture by contributing to a more uniform interpretation of urine culture plates by the different personnel engaged in this task at the laboratory.

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