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Comparison of chromogenic urinary tract infection medium with cysteine lactose electrolyte deficient media in a resource limited setting

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Abstract

Objectives: To compare the chromogenic UTI medium (CUM) with cysteine lactose electrolyte deficient medium (CLED) in terms of isolation of uropathogens, turnaround time and cost.

Methods: A total of 251 urine samples were selected and inoculated on both CLED and CUM, growth was observed after 24 and 48 hours of incubation. Isolates were identified by colony's colour and biochemical tests. Turnaround time for identification and cost was calculated till final identification of microorganisms.

Results: A discrepancy in isolation was observed in seven samples with growth on CUM in 24 hours while in 48 hours on CLED. There was 100% agreement in identification by both media. Almost 50% samples were identified within 24 hours by using CUM in contrast to CLED where most samples were identified in 48 hours. Total number of reagents used and total cost for processing of a specimen including technologist and consultant time by using CUM is significantly low in comparison to CLED.

Conclusion: CUM can replace CLED as a primary isolation media for urine culture in clinical laboratories in Pakistan as it is user friendly, facilitates early reporting and saves cost.

Keywords: Chromogenic UTI medium; CLED; Uropathogens, Pakistan (JPMA 61:632; 2011).

Introduction

Accurate identification of pathogens in short turnaround time is the primary responsibility of a clinical microbiology laboratory. Various chromogenic media that could identify pathogens in reduced time with precision and ease have been developed to fulfill this task.

Urinary tract infections are one of the most common causes of hospital admissions and clinic visits globally, making urine, the most frequent sample received for culture. In most of the clinical laboratories of the developing world, a combination of blood agar and MacConkey's agar is traditionally used for urine culture.¹ Although, use of Cysteine Lactose Electrolyte Deficient (CLED) agar is a better option for the detection of uropathogens instead of combination of two media.²

Currently a number of chromogenic media are available commercially for the detection of uropathogens such as Cps Id2 Agar, Chromagar Orientation, Uriselect and Rainbow UTI.³ Chromogenic UTI medium (CUM) (Oxoid, United Kingdom) is one of the recently marketed nonselective media which contains essential ingredients to support growth of all common bacterial uropathogens and also provides their presumptive identification. It also contains tryptophan deaminase which facilitates detection of *Proteus*, *Morgenella* and *Providencia* species with inhibition of swarming of *Proteus* spp.⁴

Previous studies comparing chromogenic media with traditional ones have shown advantages which include 20% reduction in time for identification⁵⁻⁷ reduction in workload,^{5,6,8,9} easier recognition of mixed growth^{6,7,10,11} and reduction in number of biochemical test performed for identification of bacteria⁹ which ultimately results in cost reduction.

Most of these studies have been conducted in developed countries; with only one study assessing the feasibility of chromogenic media in a resource limited setting.⁹ In developing countries the apparent higher cost of chromogenic media is a major hindrance for their routine use and their utility has never been explored. Therefore we conducted this study to compare the performance of Chromogenic UTI media with CLED for isolation and identification of common uropathogens, turnaround time and cost consumed by the two media.

The Aga Khan University microbiology laboratory is one of the largest laboratories in the country that processes around 60,000 urine samples for culture annually. Because of non-availability of trained technical staff and improper collection of urine samples interpretation of growth at bench is always challenging. In this scenario introduction of a new user friendly media which provides rapid and accurate results in a cost effective manner would be highly beneficial.

Material and Methods

This was an observational prospective study, performed on 251 urinary samples received in the clinical microbiology laboratory of Aga Khan University hospital, Karachi, Pakistan. The duration of the study was 6 months (August 2007-February 2008). Samples were randomly selected. CLED and CUM (Oxoid, United Kingdom) were prepared according to manufacturer's instruction and 25 ml of media were dispensed in 90 mm petri plates and kept refrigerated at 0-4°C. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains with each batch.

All samples were inoculated simultaneously on both CLED and CUM using 0.001µl calibrated loop and were incubated at 37°C in ambient air for 24 hours.¹² Both plates were checked for growth after 24 hours of incubation and then 48 hours, growth on both plates were observed and noted individually. Colony count of >10⁴ colony forming unit per milliliter (cfu/ml) of single and two uropathogens were considered significant. More than two organisms in a count of < 10⁴ cfu/ml were considered as mixed periurethral flora. Growth of normal skin flora (NSF) was considered as insignificant.

For presumptive identification, morphology of colonies was noted from both media. Colour of colonies were observed on CUM and interpreted as per manufacturer's recommendations. For final identification of isolates, necessary biochemical tests were used. Gram negative organisms were identified using sulphide indole motility media (SIM), urea hydrolysis, triple sugar iron (TSI), citrate utilization and oxidation\fermentation (O/F) media. *Staphylococcus* spp. was identified on the basis of presence of coagulase enzyme, detection of DNase and phosphatase enzyme and growth on mannitol salt agar (CDMP). *Streptococcus* species were identified by esculin hydrolysis, growth in 6.5% sodium chloride (BE/NaCl) and Lancefield grouping. Yeasts were identified by Gram's staining, germ tube test (GTT) and API 20 AUX (Bio Murex, France) if required.

To compare the turnaround time for both media, duration from inoculation of individual plates to final identification were noted. To analyze the cost, consumption of media and reagents were recorded. Average time spent by technologist (TL) in organization of bench, plates reading, labeling, and inoculation of media for further identification were noted. Time given by the consultant (CL) was also recorded. Final cost analysis was done by adding the cost of all items mentioned above and cost of TL and CL time.

Sample Size:

The sample size was calculated from software NCSS-

PASS version 2005. Minimum 244 urine samples were required to achieve 80% power in detecting a difference of 0.095 between one media with an area under the ROC curve (AUC) of 0.755 and another media with an AUC of 0.850 using a two-sided z-test at a significance level of 0.05.

Statistical Analysis:

All data was entered into Statistical Package for Social Sciences (SPSS) version 16.0. Frequencies with percentage were generated for categorical variables such as colony count, type of bacteria, total duration required for identification, number of colonies at 24 and 48 hours on CLED and CUM. Mc-Nemar's test was used to compare the difference in growth of organisms on both media at 24 hours.

Results

A total of 251 urine samples were evaluated on both media. There was no growth in 57 samples (28%), 20 (8%) specimens yielded NSF. Uropathogens grew in 174 (69%) specimens of which 155 (89%) had single type of pathogens and 19 (11%) had more than one pathogen.

After 24 hours of incubation, 7 samples revealed discrepant growth, >10⁵cfu/ml of uropathogens were noted on CUM and no growth on CLED. However, growth was found on CLED after 48 hours of incubation. This difference was statistically significant (Mc-Nemar; p = 0.029) (Table-1).

Overall no significant difference was observed in colony count while comparing both the media. Only one sample had >10⁴ cfu/ml on CLED but >10⁵ cfu/ml on CUM. Number of specimens showing mixed growth were same for both media but was easily appreciated on CUM because of variation in colour.

The numbers of isolates identified using CLED media were similar to chromogenic media. Among isolates identified, 125 were *E coli*, 19 *Klebsiella pneumoniae*, 16 *Enterococcus* spp, 11 *Pseudomonas aeruginosa*, 7 *Enterobacter* spp, 3 *Staph aureus*, 2 *Proteus vulgaris*, 2

Table-1: Comparison of bacterial count on CLED and CUM after 24 hours and 48 hours of incubation.

Growth	24 Hours		48 Hours	
	CLED n (%)	CUM n (%)	CLED n (%)	CUM n (%)
NG*	69(28)	62(25)	57(23)	57(23)
>10 ⁴ cfu/ml*	7(3)	6(2) #	10(4)	9(4)
>10 ⁵ cfu/ml	138(55)	146(55)^	145(58)	146(58)
>10 ⁵ cfu/ml two types	17(7)	17(7)	18(7)	18(7)
>10 ⁴ cfu/ml two types	1(0.4)	1(0.4)	1(0.4)	1(0.4)
NSF	19(8)	19(8)	20(8)	20(8)

*Mc-Nemar; p-value=0.029

One sample had growth of >10⁵ cfu/ml on CUM while >10⁴ cfu/ml on CLED.

^Seven samples showed growth of >10⁵cfu/ml in 24hours on CUM while taken 48 hours on CLED.

Table-2: Comparison of cost and turnaround time taken for identification by CLED and CUM media.

Reagents	CLED		CUM	
	Number of media and reagents	Cost in PKR	Number of Reagents	Cost in PKR
Sulphide Indole Motility Medium	170	3400	17	120
Simmon's Citrate Medium	181	3620	17	120
Christenson's Urea Agar	170	3400	6	300
Triple Sugar Iron Media	170	3400	6	280
Hugh Liefson's oxidation fermentation medium	11	440	11	440
Bile esculin media/6.5%NaCl broth	17	680	1	120
Coagulase, DNase, Mannitol salt agar, Phosphatase agar	3(each)	210	3	210
Germ Tube Test	1	20	1	20
Hanging drop motility	-	-	19	160
Plates of media	270*	4050	251	18825
Plates of MacConkey agar	9*	180	0	0
Total cost of reagents		19400		20595
Cost of technologist time		1722		685
Cost of consultant time		7830		4792
Total		28952		26072
Time for identification				
24 hours	0		127	
48 hours	165		48	
72 hours	9		0	

* Additional media required for further isolation in mixed specimens from CLED.

Proteus mirabilis, 1 *Acinetobacter* spp, 1 *Candida albicans*, and 1 *Streptococcus* Group D. There was no discrepancy of colour in the identification of various organisms on CUM as claimed by the manufacturer and was in 100% agreement with CLED.

One hundred and twenty seven (50.6 %) organisms were correctly identified by CUM in 24 hours and none took more than 48 hours. *E. coli* and *Enterococcus* were fully identified on CUM and no further biochemical tests were required. Presumptive identification of *Klebsiella-Enterobacter-Citrobacter* and *Proteus*, *Morgenella* and *Providencia* group was done by appearance of metallic blue and brown colour respectively but they required further tests for their final identification. Total number of tests used for identification of microorganisms from CUM were less in number in comparison to CLED. In our laboratory, time spent by an average technologist for interpretation of inoculated plate and for inoculating identification tests (ID) is 2minutes/organism. Reading and interpretation of the plate after 24hours of incubation with ID is calculated 1minute/organism. For consultant complete assessment of a case requires 30seconds/organism. According to the salaries, cost of technologist time is 3.3PKR/minute and 45PKR/minute for consultants. Table-2 depicts every important item we considered in costing including TL and CL time. It shows minimal difference in cost if only consumables are considered but there is significant reduction between CUM and CLED after adding the TL and CL time (Table-2).

Discussion

Results of our study showed that CUM is a user

friendly and cost effective medium which allows growth of almost all bacterial uropathogens with presumptive identification within 24 hours of incubation especially *E-coli* which is the major uropathogen. Due to appearance of different colour of various organisms, mix cultures were easily recognized. Therefore, unnecessary delay due to additional work up and isolation was avoided. Another advantage was that specimens growing two morphological variants of same species were easily identified on CUM.

Both media were found comparable as far as isolation of bacteria was concerned, except in seven cases where there was delay of 24 hours in growth on CLED. This difference is statistically significant and is in concordance with previous findings that CUM supported the growth of more urinary isolates than CLED.¹³ Identification of various organisms was in 100% agreement with manufacturer claims in contrast to few other studies,^{9,14,15} that have reported failure to identify 11% *E. Coli* and 2-6% *Klebsiella-Enterobacter-Citrobacter* group by available chromogenic media. We did not face this problem; in fact easier identification of bacteria was appreciated by technical staff and consultant directly from the primary plates.

Turnaround time for final identification was significantly reduced with CUM. *E. coli* and *Enterococcus* spp were identified within 24 hours of inoculation. Rest of the organisms were identified on the basis of colony colour and few additional biochemical tests. We were able to correctly identify 56% of isolates in 24 hours. One of the various reported benefit of chromagar is selection of smaller number of organisms to process for final identification and sensitivities, reported by Baker et al.¹⁶ Reduction in number

of samples to be processed would result in decrease in work load at the bench and staff requirement, improved quality of work and shorter turnaround time. That will be helpful for the physicians to commence empirical therapy as per local susceptibility patterns and avoid inappropriate use of antimicrobials. This will ultimately benefit the patient as well as community at large by impeding the rate of antimicrobial resistance.

In this study number of reagents used was significantly low in case of CUM than CLED. Other studies have reported requirement of fewer biochemical tests for final identification of most of the organisms especially Klebsiella-Enterobacter-Citrobacter and Proteus-Morganella-Providencia group⁷ by CUM and this was also our finding, though we have been able to identify Klebsiella spp within 24 hours on appearance of metallic blue color colony with the help of motility testing. E.coli is the most frequent uropathogen and can be easily identified on CUM resulting in overall reduction in reagents consumption. Comprehensive cost evaluation shows that despite of high cost of chromogenic UTI medium, its use is economically beneficial for the laboratory.

Conclusion

The result of this study showed that CUM can replace use of CLED in clinical laboratory in Pakistan as primary isolation media for urine culture as it is user-friendly , facilitates early reporting, saves time as well as cost.

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