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ORIGINAL ARTICLE

Evaluation of the Chromogenic Medium (CPS) in the Isolation and Identification of Urinary Tract Pathogens

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Key words:

Urinary tract infection, Chromogenic media

ABSTRACT

Background: *Urine cultures constitute the majority of the workload for a microbiology laboratory with only 20%-30% of urine sample resulting in significant growth. Chromogenic media (CM) are available for urine specimens to enable rapid identification of common pathogens and also has been reported to increase mixed culture detection, reducing unnecessary workup. Chromogenic media offers the potential to lower costs by providing decreased work time, storage space and identification costs.* ***Objectives:*** *The present study focused on evaluation of the chromogenic medium (CPS) for the diagnosis of UTI in comparison with CLED as a conventional medium.* ***Methodology:*** *Over the period of January to July 2014, fifty urine samples with ≥100 pus cells /HPF were examined. CPS and CLED media were used for direct inoculation in addition to conventional biochemical reactions and/or API as needed.* ***Results:*** *In comparison with CLED, CPS showed a sensitivity of 93.5%, specificity of 100%, positive predictive value of 100%, negative predictive value of 57.1% and total agreement of 94%. The sensitivity of CPS for E. coli was 95%, for KESC was 75%, for Proteeae was 100% and for Enterococcus was 100%. The specificity of CPS for detection of E. coli was 100%, for KESC was 100%, for Proteeae was 97.9% and for Enterococcus was 100%.* ***Conclusion:*** *CPS proved to be a rapid, cost-effective diagnostic method for urinary tract infections. Therefore, CPS can replace the standard primary plating media used in routine diagnosis of urinary tract infection.*

INTRODUCTION

Urinary tract infections (UTI) constitutes one of the most common infectious diseases for which patients seek medical attention, and although many of these infections are treated empirically, urine cultures account for a significant portion of every clinical microbiology laboratory's daily workload[1,2]. Because only 20 to 30% of urine samples result in significant growth, a considerable amount of time is spent in evaluating samples that do not have clinical utility [3]. Therefore any new method or medium with the ability to streamline urine culture processing in a meaningful way should be welcomed. Urine cultures have conventionally been performed using sheep blood agar (BA),as a nonselective medium, and a selective medium such as MacConkey (MAC) agar, cysteine lactose electrolyte-deficient (CLED) agar, or eosin methylene blue (EMB) agar [4].

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Chromogenic media applicable to urine culture processing and reporting had been commercially available for more than 20 years and are intended to perfectly identify more frequently occurring bacteria and yeasts on primary culture with no further testing or a minimum number of confirmatory tests. Substrates present in chromogenic media target specific classes of enzymes produced by certain bacteria and yeasts [5]. Target enzymes hydrolyze chromogenic substrates forming colored products which allow for easy identification of specific organisms [5,6]. The use of these media also allow for substantial upgrading of biochemical identification procedures without a significant rise in total costs [7].

Chromogenic media may facilitate improved sensitivity of identification and may promote more uniform interpretation of urine culture plates by less experienced bench technologists [8], also may provide clinicians with relevant information regarding their choice of empiric antimicrobial therapy [9, 10].

The current study aimed to evaluate the chromogenic medium (CPS) [bioMérieux, Marcy l'Etoile, France]) for routine diagnosis of bacteriuria in comparison with CLED (cysteine-, lactose-, electrolyte-deficient) as a conventional standard media.

METHODOLOGY

Sample collection:

The current study was an observational prospective study over the period from January, 2014 to July 2014. Fifty urine samples were enrolled of which 43 were midstream urine specimens and 7 were catheter urine specimens from patients with UTI, attending the inpatient and outpatient clinics of the department of Urology, Kasr Al Ainy hospitals, Cairo University. A criterion of >100 pus cells/ HPF was utilized in order to increase the chance of isolating clinically significant cultures for the study.

Isolation and identification of microorganisms:

CLED was prepared according to manufacturer's instruction and 25ml of media were dispensed in 90 mm petri plates and kept refrigerated at 0-4ºC. CPS [bioMérieux, Marcy l'Etoile, France] media is ready made media which must be stored at 2 -4ºC for one month after opening the cover. It enables microbial enumeration of the specimen by means of a standardized inoculation method as well as identification of the following bacterial groups: *Escherichia coli, Enterococcus, Klebsiella, Enterobacter, Serratia, Citrobacter (*KESC*), Proteus, Providencia, Morganella (Proteeae)*[11.12.13.14]. ChromID™ CPS® agar consists of a rich nutrient base combining different peptones and 3 chromogenic substrates which enable the detection of activities of specific enzymes. Detection of indole is enhanced by the inclusion of tryptophan in the agar. The high concentration of agar prevents the swarming of *Proteus*. Identification of the bacteria most commonly isolated in urinary tract infections is based on the following principle.[15] *E.coli*: spontaneous coloration (pink to burgundy) of strains producing ß-glucuronidase (ß-GUR) and/or ß-galactosidase (ß-GAL) [11,12,16, 17] .

Enterococcus: spontaneous turquoise coloration of strains producing ß-glucosidase (ß-GLU)[18].

KESC: spontaneous bluish-green to bluish-grey coloration of strains producing ß-glucosidase (ß-GLU); identification of each microorganism must be followed by additional tests [15].

Proteeae: spontaneous brown coloration of strains producing deaminase, then indole detection using the ID Indole-TDA reagent for confirmation [15].

Other organisms: Opaque light pink colonies: Presumption of *Staphylococcus saprophyticus,*; identification of the microorganism isolated must be followed by additional tests.

Bluish-violet to violet colonies: Presumption of *Streptococcus agalactiae*; identification of the microorganism isolated must be followed by additional tests.

Colonies without characteristic colors; identification of the microorganism isolated must be followed by additional tests.

Escherichia coli ATCC 25922 was used as quality control strain.

All samples were inoculated simultaneously on both CLED and CPS using 0.001 mL calibrated loop then incubated at 37ºC in ambient air for 24 hours. Both plates were checked for growth after 24 hours of incubation and then after 48 hours, growth on both plates were observed and recorded.

Morphology of colonies was noted from both media. Colour of colonies were observed on CPS and interpreted as per manufacturer's recommendations. For final identification of isolates, necessary biochemical tests were done. Gram negative organisms were identified using oxidase test, citrate utilization, urea hydrolysis, triple sugar iron (TSI), lysin decarboxylation , indole production and API 20 E identification panel for *Enterobacteriaceae* were performed to reach for a species level. *Staphylococcus* spp. were identified on the basis of positive catalase test, coagulase enzyme and growth on mannitol salt agar. *Streptococcus* spp. were identified by negative catalase test and esculin hydrolysis. Yeasts were identified by Gram's staining and germ tube test (GTT)[19]

Statistical Analysis

Data were statistically described in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and total agreement to compare between CPS as a new medium with CLED medium. Frequencies with percentage were done for categorical variables such as, type of bacteria. Calculations were done using computer programs Microsoft Excel 2010 (Microsoft Corporation, NY, USA).

RESULTS

Bacterial isolates:

In the present study, out of the fifty urine samples included, 46 samples were positive on CLED agar and conventional biochemical and/or API of which 43 samples were positive by culture on CPS.

The incidence of the different organisms in this study on CPS was as follows:

E. coli: 19/46 (41.3%), KESC*:* 6 (13%) including 3 (6.5%) *Enterobacter aerogenes/cloacae* and 3 (6.5%) *K. ornthinolytica, Proteus:* 4(8.7%) including 3 (6.5%) *proteus mirabilis* and 1 (2.2%) gave beige colonies, however API identification showed that it was *E coli, Enteroccocus spp.:* 7 (15.2%), *Staphyloccoci*: 4 (8.7%) including 1 *S. aureus* (2.2%) and 3 *S. saprophyticus* (6.5%), *Streptococcus agalactiae:* 1 (2.2%), *Candida:* 3(6.5%) including 1 *C. albicans* (2.2%) and 2 *Candida non-albicans* (4.4%).

Compared to CLED, CPS showed a sensitivity of 100%, and a specificity of 93.5% with a positive predictive value (PPV) of 100% while the negative predictive value (NPV) of 57.1% and a total agreement of 94%. The comparison of the growth of different urinary isolates by culture on CPS alone in relation to their identification by CLED agar in addition to conventional biochemical reactions and/or API. is summarized in **Table (1)**.

Table 1: Comparison of identification of different urinary isolates by culture on CPS alone in relation to their identification by CLED agar in addition to conventional biochemical reactions and/or API.

Isolated organism	***CLED plus Biochemical reactions and/or API***	***CPS***	***Sensitivity***	***Specificity***	***PPV***	***NPV***	***Total agreement***
E. coli	20 (40%)	19 (38%)	95%	100%	100%	96.8%	98%
KESC	8 (16%)	6 (12%)	75%	100%	100%	95.4%	96%
Proteeae	3 (6%)	4 (8%)	100%	97.9%	75%	100%	100%
Enterococcus	7 (14%)	7 (14%)	100%	100%	100%	100%	100%
Staph. saprophyticus	3 (6%)	3 (6%)	100%	100%	100%	100%	100%
Staph. aureus	1 (2%)	1 (2%)	100%	100%	100%	100%	100%
Strept. agalactiae	1 (2%)	1 (2%)	100%	100%	100%	100%	100%
Candida	3 (6%)	3 (6%)	100%	100%	100%	100%	100%
Colonies with no characteristic colors on CPS		2 (4%)					
No growth	4 (8%)	4 (8%)					
Total	50	50					

DISCUSSION

Presumptive identification of urinary pathogens and the identification of mixed cultures on CLED medium as well as other conventional media are time-consuming and needs extensive experience. The development of chromogenic agars combining the basal CLED medium with various chromogenic substrates into clinical bacterial diagnostics offered an easier and faster identification of isolates, as well as enhanced identification of mixed culture [10,,20,21,22,23,24].

In the present study, CPS showed a sensitivity of 93.5%, and a specificity of 100% in comparison to conventional standard methods with a positive predictive value of 100%, a negative predictive value of 57.1% and a total agreement of 94%. Results of this study were in accordance with a study by Sekikawa et al.[2] comparing the performance of two chromogenic media; UriSelect 4 (US) and BBL CHROMagar Orientation (CO) with conventional media which showed that the isolation and identification of isolates was found to be best on CO (98.9%), followed closely by US (97.7%) and least favorable by the conventional method (94.4%). They stated that chromogenic media were easier, faster and more reliable than conventional media. Colored colonies also facilitated more accurate detection of mixed cultures which helped to diagnose contaminated specimens, leading to reduced time spent and unnecessary work up of clinically insignificant organisms. Regarding the sensitivity of CPS for detecting the main uropathogens; the present study revealed that sensitivity of CPS for E. coli was 95%, for KESC was 75%, for Proteeae was 100% and for Enterococcus was 100%. This was in accordance of a study by BioMerieux[15] in France using 229 urine samples which showed that the sensitivity of CPS to E coli was 98.3%, to KESC was 97.67%, to Proteeae was 89.49% and to Enterococcus was 97.14%. This was also in agreement with a study by BioMerieux[15] in Germany using 212 samples which showed that the sensitivity of CPS to E coli was 95.61%, to KESC was 90%, to Proteeae was 80.47% and to Enterococcus was 80.47%. This showed that there is an agreement with the sensitivity between the three studies with some difference in the sensitivity of KESC, Proteea and Enterococcus which may be due to the limited number of our samples. Regarding the specificity of CPS for detecting the main uropathogens the present study revealed that specificity of CPS for the detection of E. coli was 100%, for KESC was 100%, for Proteeae was 97.9% and for Enterococcus was 100%. This was in accordance to the study of BioMerieux[15] in France on 229 urine samples which showed that the specificity of CPS to E. coli was 100%, to KESC was 98.92%, to Proteeae was 99.52% and to Enterococcus was 99.5%. This was also in agreement of a study by BioMerieux[15] in Germany on 212 samples, which showed that the specificity of CPS to E. coli was 100%, to KESC was100%, to Proteeae was 99.49% and to Enterococcus was 99.34%.

To make the first, presumptive identification of isolates is a task that needs a great deal of experience when using conventional media, however on chromogenic media this was easier requiring less training. Thus, the use of chromogenic media 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[21] .

The overall impression of the colour changes produced on the chromogenic media by *E.coli, Enterococci,* KESC group and the Proteus-Morganella-Providencia group was that they were distinct and easy to perceive, as previously mentioned by Lakshmi et al.[25].

Most of the isolated organisms in the present study were correctly presumptively identified based on colour production as described by the manufacturers. This result came in agreement with a study in Baghdad[26] in which 375 midstream urine samples were cultured on CHROM agar Orientation medium. All E coli isolates grew on CHROM agar Orientation in pink red colonies and were very easy to distinguish, Proteus spp. were easily distinguished on the primary plates because of their characteristic brown halo; however the medium failed to differentiate Klebsiella spp. and Enterobacter spp. owing to similarity of colour produced and final identification among them required additional biochemical tests.

The most common isolate responsible for UTI was *E. coli* which can be identified a day earlier than conventional methods, allowing the clinician to commence an initial course of antibiotics based on the organism's most common susceptibility pattern. This is important especially in the setting of a nosocomial UTI when patients are often immunocompromised [27].

Regarding the recovery of E. coli in the current study, CPS showed a sensitivity of 95% and a specificity of 100%. Our results were consistent with the finding of BioMerieux[15] in France using 43 pure E. coli strains with different enzymatic profiles which showed that on CPS medium; direct detection of 97.7% of strains was possible owing to the combination of several chromogenic substrates in the medium. This was also in agreement with a study by Perry et al. [20] which showed that most *E. coli* strains (97.1%) for Uriselect 4 and (96.8%) for CPS ID2 generated red or pink colonies. The remainder generated white colonies as a result of the absence of either β-galactosidase or β-glucuronidase activity, respectively. There was also matching between the results obtained in the present study and those of the studies of BioMerieux [15] in France and in Germany which showed a 100% specificity for the detection of *E. coli.*

In the present study, organisms from the KESC group were recovered well and were easily distinguished from other Gram negative bacilli and API was performed to reach a species level. This observation was consistent with the findings of Sekikawa et al. [2] who showed that most KESC isolated in their study were correctly presumptively identified based on colour production with no errors. Regarding the recovery of *Enteroccocus;* 7 specimens (14%) were positive by conventional culture and biochemical reactions for Enteroccocus and also were recovered by culture on CPS followed by Gram stain as a confirmatory test, however, Gram staining may be unnecessary as the colonial morphology was very distinctive and there was no confusion with other blue colony producing organisms (KESC). Moreover, many studies did not perform any type of confirmatory test for *Enteroccocus*[21,22,10,24,7]. Our results were also matching with the study of Sharmin et al.[8] in Bangladesh using 186 strainsw hich revealed that 100% of Enterococci were identified from the growth on chromogenic agar media but only 95% of CLED agar which was explained that on CLED medium the presence of *Enterococci* were identified on CLED agar which was explained that on CLED medium the presence of *Enterococci* was frequently masked by larger colonies of Gram negative species.

Definitive identification of beige colonies with a brown halo or brown coloration of bacterial growth were suggestive of the *Proteeae* group and were confirmed by indole test. All strains of *P. mirabilis* and one strain of *Proteus penneri* were indole negative, whereas other members of the *Proteeae* group were indole positive[2,28].

Regarding the recovery of *Streptococcus agalactiae,* CPS showed a sensitivity of 100% and a specificity of 100%, however this result may not be significant as only a single isolate of *Streptococcus agalactiae* was recovered. However, Moore[30] observed discrepancy regarding the recovery of *Streptococcus agalactiae* in favour of the conventional method. This could be explained by the larger inoculum size on the conventional media compared to the chromogenic media which would increase the chance of growing Streptococcus agalactiae if a patient had a low level of colonization. Comparing the detection rates of the different urinary isolates on chromogenic UTI agar, USA agar and CPS ID2 agar; Carricajo et al. [14] showed that the detection rates for the three media were similar, with a lower detection rate for *Staphylococcus* spp. on chromogenic UTI agar and USA agar. Concerning the abilities of the three media to directly identify *E. coli, Enterococcus spp., P. mirabilis and P. penneri,* only slight differences were observed[14].

The present study confirmed the following advantages of the CPS medium: the greater differentiation of colonies in mixed cultures based on different colour production of uropathogens, the capability to prevent swarming of *Proteus* spp. and the

direct identification of most commonly encountered UTI pathogens, which saved reagents, time and bench work.

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