Research Article

Utility of UTI CHROMagar Media for the Rapid Identification of the Uropathogens

Jayshri Pethani*,Payal R. Dholaria, Sanjay Rathod*, Afroz Bloch***, Bimal Chauhan****, Parul D. Shah#**
Associate Prof*,3rd Year resident**,Tutor Gujarat Cancer Society***, Assistant Prof****., Prof. & Head#,
Department of Microbiology, Smt. NHL Municipal Medical College, Ahmedabad

ABSTRACT:

Introduction:

Urinary tract infections (UTI) continue to be a common problem. UTICHROMagar, a new chromogenic medium, was evaluated for the detection and differentiation of uropathogens.

Objectives:

To compare the UTICHROMagar medium with standard culture media in terms of rapid isolation of uropathogens.

Material and Methods:

A total of 185 urine samples were tested and inoculated on both standard culture media and UTICHROMagar, growth was observed after 24 and 48 hours of incubation. Isolates were identified by colony's colour, morphology and biochemical tests.

Results:

Of the 185 urine samples tested, 80(43.24%) were found to be culture positive and 105(56.75%) were culture negative. The uni microbial aetiology was found in 60(75%) samples and poly microbial aetiology was found in 20(25%). Colony color and morphology on UTI CHROMagar accurately differentiated Escherichia coli, Proteus spp., Pseudomonas aeruginosa and Acinetobacter spp.

Conclusion:

The accuracy of antibiotic susceptibility determinations according to standard methods was also tested by picking isolates directly from CHROMagar. The results showed excellent correlation with those obtained with microorganisms picked from reference media. Owing to the ease in differentiating mixed flora on CHROMagar, antimicrobic susceptibility tests were performed directly from primary isolates in all cases without the need for subcultures. UTICHROMagar may emerged as a vital aid for primary identification and early presumptive diagnosis of the uropathogens.

Key words: UTI CHROMagar, Chromogenic medium, Uropathogens.

INTRODUCTION:

Bacterial infection of the urinary tract is one of the common causes for seeking medical attention in the community.[1] Among the most common infectious diseases, urinary tract infections (UTIs) are a commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million.[2,3]

The increase in resistance of microorganisms to antimicrobic agents, especially in hospitalized patients, demands rapid identification of the pathogen. Resistance to antimicrobial agents has been noted since the first use of these agents and is an increasing world-wide. Early information enables the selection of the appropriate antibiotic prior to the results of susceptibility tests. In the last few years several chromogenic media have been developed, allowing for more specific direct differentiation of microorganisms on primary plates.

A new, UTI CHROMagar, offers presumptive identification of Gram-positive and Gram-negative bacteria on a single medium by distinct colony colors produced by specific enzymes with a suitable chromogenic substrate.

UTI CHROMagar has a promising scope as alternative culture media in the present set-up of our laboratories. UTI with polymicrobial aetiology being on the rise, use of UTI CHROMagar may prove fast catch as a time saving culture medium detecting majority of pathogens. The study gives an idea on utilization of UTI CHROMagar for early detection of pathogens and implementation of a rational, effective drug therapy.

MATERIAL AND METHODS:

Patients hospitalized at our institute from January 2010 to March 2010 were included in the study.185 urine samples either Midstream or catheter urine, or urine collected by suprapubic bladder puncture from patients in different departments were tested in this study.

MEDIA:

(i) CHROMagar

The principle of this medium is the use of chromogenic substrates revealing metabolic enzymes specific for certain species of bacteria.

Dehydrated powder was provided by the CHROMagar Company, Paris, France. The medium is composed of 16 g each of peptone, meat, and yeast extracts and 15 g of agar per liter and a special chromogenic mixture. The media is prepared as per instruction, and the sterilization process was performed at 120°C for 15 min. The medium was poured into 90-mm-diameter petri dishes, stored at 4 to 6°C, protected from light, and used within 10 weeks.

(ii) Standard reference media:

Standard reference media consisted of Nutrient agar &MacConkey agar.

Media inoculation procedure :

Use of calibrated loops or other techniques commonly used for the plating of urine specimens is mandatory to obtain isolated colonies with their typical colors and shapes. Collect a sample of the undiluted, well-mixed urine using a calibrated loop (0.01 ml). Ensure proper loading of the loop with the specimen. Inoculate the sample down the middle of the plate in a single streak from which additional spreading of the inoculum is performed.

Incubate the inoculated plates in an inverted position at 35 to 37° C aerobically for 20 to 24 hours. Avoid exposure to light during incubation as this might destroy the chromogens.

Once the colors of the colonies have developed, exposure to light is permissible.

(iii) Quality control:

Each batch of medium was tested for sterility, biochemical and chromogenic reactions with American Type Culture Collection (ATCC) strains. *Staphylococcusaureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 4630, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiellapneumoniae* 13883 were used for quality control. The microorganisms were inoculated into 1% peptone broth and incubated overnight at 35 ± 2°C. Serial 10-fold dilutions in sterile saline were performed to reduce the microbial count to the desired inoculum concentration. The number of viable CFU per milliliter in each suspension was monitored.

(iv) Bacteriological procedures:

UTI CHROMagar was evaluated in comparison to standard reference media: Nutrient agar and MacConkey agar plates. The urine samples were inoculated at the same time on the three agars and were incubated aerobically at 37°C overnight. The antimicrobial susceptibility of the isolates were tested by the disk diffusion technique according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations.[4]

(v) Microorganism identification:

Enterobacteriaceae isolates were identified by the following biochemical reactions: motility, indole production, *o*-nitrophenyl-β-D-galactopyranoside hydrolysis, glucose fermentation with or without CO_2 production, hydrogen sulfide production, urea hydrolysis, and lysine and ornithine decarboxylase and sodium citrate utilization. Gram-negative microorganisms other than *Enterobacteriaceae* were also tested for colony morphology and pigmentation as well as for additional biochemical reactions: gelatin, catalase, and oxidase utilization.

The identification of streptococci was confirmed by hemolysis on 5% sheep blood agar, aesculin hydrolysis, and agglutination tests. Isolates suspected to be *S. aureus* (lack of growth on MacConkey agar, growth of beta-hemolytic colonies on blood agar, and white-to-yellowish colonies on CHROMagar) were Gram stained and checked by the slide coagulase test for final identification.

Candida isolates were subcultured on Candida CHROMagar, a medium allowing the identification of *candida spp.* by their different colony colors.

RESULTS:

Of the 185 urine samples tested, 80(43.24%) were found to be culture positive and 105(56.75%) were culture negative. A single species (uni microbial aetiology) was found in 60(75%) samples and poly microbial aetiology was found in 20(25%).

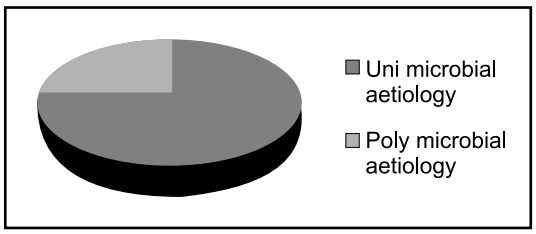

Fig: 1 Uni microbial and poly microbial infections

Furthermore, AST of the bacterial colonies picked up directly from the UTI CHROMagar was done by the Kirby-Bauer method and compared with the AST kept from the routine media. The results were 100% compatible. Due to its specificity with respect to color and colony morphology, CHROMagar made the differentiation of bacterial colonies than did the reference media.

This fact, together with the ability of the medium to limit the spread of bacteria, allowed the presumptive identification of several microorganisms directly, as well as allowing the performance of antimicrobial susceptibility tests without the need for subculture.

Equivalent susceptibilities were obtained in all the cases, and very few differences between zone diameters of 1 to 2 mm were detected randomly. Note that none of these differences were out of the range specified by NCCLS criteria for the disk diffusion method of susceptibility testing.

The numbers of the susceptible isolates picked from CHROMagar were exactly the same as the numbers of those picked from the reference media. No significant differences between gram-negative isolates as well as Gram positive isolates were observed.

Table 1: Colony characteristics on UTI CHROMagar

E. Coli	**Pink transluscent colonies**
Klebsiella sp.	**Dark blue colonies**
Staphylococcus sp.	**Tiny ,cream-white convex colonies**
Enterococci sp.	**Dark green, very tiny colonies**
Proteus sp.	**Yellowish brown,transparent colonies**
Citrobacter sp.	**Magentacoloured colonies**
Pseudomonas sp.	**Pale greentransluscent colonies**

Fig: 2 Colony of *E.coli* and *Citrobacterspp*

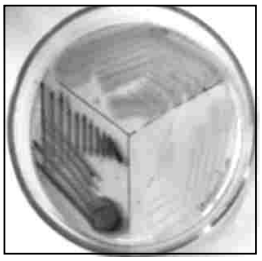

Fig: 3 Colony of *Klebseillaspp*, *Pseudomonas spp.* and *Proteus spp.*

DISCUSSION:

In the present study, UTICHROMagar was evaluated for the first time as a direct isolation medium for clinical specimens. 185 urine samples were tested by parallel inoculation on UTICHROMagar and on reference media, Nutrient agar and MacConkey agar. UTICHROMagar showed the same ability to detect urine pathogens as the combination of the two reference media. The comparative study would clearly point out that the UTI CHROMagar is a better culture media.

The UTICHROMagar offered the advantage of limiting the spread of some isolates, such as *Proteus* spp., *K. pneumoniae*, and *E. coli* mucoid strains which may yield confluent growth on plates. This increased the ability of the medium to detect urinary tract pathogens when mixed flora were present[5]. Colony color and morphological characteristics on UTICHROMagar allowed for easy differentiation of the uropathogens. The UTICHROMagar seems to be very suitable as a differential medium for direct isolation of urine samples.

The results of the study to differentiate the most commonly encountered Gram-negative pathogens in UTI on the basis of color and morphology alone were

favorable for UTICHROMagar compared to MacConkey agar.

It succeeded in detecting all Gram-positive microorganisms also.Treatment of UTIs cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.[6] However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections.[7,10] The results of the AST of microorganisms picked from UTICHROMagar showed an excellent correlation with test results of microorganisms picked from reference media. The only drawback of this medium is that we have to confirm the microorganism by further biochemical tests.

CONCLUSION:

It was observed that UTI CHROMagar detect mixed population & bacteria with low colony count better than routine media used. The medium also supported growth of Gram positive bacteria and *candida* spp. The antimicrobial sensitivity testing done from it matched AST done from the routine media.

To summarise, it is a complete package encompassing the qualities of both Nutrient agar & MacConkey agar. So it is very cost effective medium. UTI CHROMagar may thus be considered as a primary screening medium to use in the laboratory for the ultimate benefit of our patients.

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