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Research Article

Evaluation of Chromogenic Agar and Direct Antimicrobial Susceptibility Testing in Rapid Diagnosis of Acute Urinary Tract Infection

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ABSTRACT

Introduction: Urinary tract infection (UTI) is the most common type of infection and continues to be a major health problem. The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen. The current detection methods are time-consuming, most typically consisting of 2 to 3 day culture. Aim: In the present study an analysis of chromogenic agar in the rapid identification of bacteria from urine sample was done. Further, the accuracy of the primary antibiotic sensitivity was also evaluated. Materials and Methods: About 100 patients of different age groups and sex with apparent urinary tract infection were selected for the study. The clean catch midstream urine was collected in a sterile container and was transported immediately to the laboratory. HiCrome UTI agar (HiMedia,code No. M1353) was used in the present study. HiCrome agar is evaluated in comparison with the standard agars. Direct susceptibility testing is done as described previously on all urine specimens at the time that they were received in the laboratory. For comparison, the antimicrobial susceptibilities of isolates from pure cultures are determined by a standard disc diffusion method. Results: 64 and 68 specimens showed culture positive in UTI chromogenic agar and conventional media respectively. On statistical analysis, the difference is not found to be significant ($p>0.05$), thus showing that UTI chromogenic agar is equally efficient in the isolation of the microorganisms from the urine of UTI patients. The direct antibiotic sensitivity test has shown to be equally good to the conventional antibiotic sensitivity test ($p>0.05$). **Conclusion:** From the present study, it has been observed that the chromogenic agar and direct antibiotic susceptibility test can be used in conjugation thereby the culture and sensitivity results for urine specimen can be produced within 24 hours. This helps the physician to initiate the appropriate treatment in time. Apart from this, it also reduces the expenditure involved in the conventional culture and sensitivity test.

Keywords: Urinary tract infection, rapid identification, chromogenic agar, direct antibiotic sensitivity test

INTRODUCTION

Urinary tract infection (UTI) is the most common type of infection and continues to be a major health problem[1]. The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen[2-4]. Early information enables the selection of the appropriate antibiotic prior to the results of standard susceptibility tests and may thereby prevent outbreaks[5].

The current detection methods are time-consuming, most typically consisting of 2 to 3 days of culture. A rapid, inexpensive, definitive urine test capable of detecting bacteria and its antibiotic susceptibility would be enormously beneficial in ensuring timely treatment. However, the rapid methods are yet to replace standard bacterial culture method.

In the recent years several chromogenic media have been developed and commercialized, allowing for more specific direct differentiation of microorganisms on primary plates[6]. The chromogenic agar offers simultaneous presumptive identification of gram-positive and gram-negative bacteria and yeasts on a single medium by means of distinct colony colours produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate.

Direct antimicrobial susceptibility testing (otherwise called as primary antimicrobial susceptibility testing) of urine specimens by the disc diffusion assay offers a means of rapidly and inexpensively obtaining the information needed to guide antimicrobial therapy for patients with urinary tract infections (UTIs)[7-9].

However not many works have been conducted to prove the validity of chromogenic agar and the direct sensitivity testing. Hence in the present study an analysis of chromogenic agar in the rapid identification of bacteria from urine sample was done. Further, the accuracy of the primary antibiotic sensitivity was also evaluated.

MATERIALS AND METHODS

Study population and specimen

The institutional ethical committee clearance was obtained before the start of the study. About 100 patients

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Table 1: Results of urine culture among study population (N=100)

Growth	UTI chrome agar	MacConkey agar	Blood agar
Single bacterial growth	64 (64%)	68 (68%)	68 (68%)
Mixed bacterial growth	0	0	0
No growth	36 (36%)	32 (32%)	32 (32%)
Total	100	100	100

Table 2: Distribution of organisms among positive urine culture specimens

Microorganism	Number of isolates			P value
	UTI chromagar	MacConkey agar	Blood agar	
Escherichia coli	19 (29.7%)	23 (33.8%)	23 (33.8%)	>0.05
Klebsiella pneumoniae	25 (39.1%)	25 (36.8%)	25 (36.8%)	>0.05
Pseudomonas aeruginosa	5 (7.8%)	5 (7.4%)	5 (7.4%)	>0.05
Staphylococcus aureus	9 (14.1%)	9 (13.2%)	9 (13.2%)	>0.05
Enterococcus sp.	4 (6.3%)	4 (5.9%)	4 (5.9%)	>0.05
Candida sp.	2 (3.1%)	2 (2.9%)	2 (2.9%)	>0.05
Total	64 (100%)	68 (100%)	68 (100%)	>0.05

of different age groups and sex with suspected urinary tract infection were selected for the study. An informed consent was obtained before the collection of specimen. The clean catch midstream urine was collected in a sterile container and was transported immediately to the laboratory.

Media

HiCrome UTI agar (HiMedia,code No. M1353) was used in the present study. The standard culture media viz., MacConkey, Mueller-Hinton agar, Blood agar, biochemical reagents and antibiotic discs were obtained from HiMedia for both culture and antibiotic sensitivity testing.

Staining

Prior to the culture, the smear was prepared and stained from the urine sample. Based on the gram staining property, the appropriate antibiotic discs were selected for the study.

Culture procedure

HiCrome agar was evaluated in comparison with the standard agars. The urine samples were streaked on the agar media and were incubated at 37^0C overnight. The colonies in HiCrome agar were interpreted as per the manufacturer's instructions. The colonies in the standard agar were subjected to various biochemical reactions for the identification of bacteria.

Direct and standardized antimicrobial susceptibility testing.

Direct susceptibility testing was done as described previously[10] on all urine specimens at the time that they were received in the laboratory. A sterile cotton swab was dipped into a sample from a well-mixed, unadjusted urine specimen, and excess fluid was expressed before streaking for confluence onto a Mueller-Hinton agar plate. Commercial antimicrobial discs were distributed and were pressed firmly onto the agar surface with sterile forceps. Plates were read the next day after incubation for 16 to 18 h at 37^0C.

For comparison, the antimicrobial susceptibilities of isolates from pure cultures were determined by a standard disc method according to the guidelines of the National Committee for Clinical Laboratory Standards[11].

Statistical analysis

The predictive values for HiCrome agar is calculated taking the standard culture method as gold standard. The SPSS version 20 software was used to do McNemar's test to compare the results.

RESULTS AND DISCUSSION

The Table 1 shows the results of urine culture among the 100 patients. Out of 100 urine samples, 68 were culture positive and remaining were culture negative.

The Table 2 depicts the distribution of microorganisms among the positive urine culture specimens. The predominant microorganism that was isolated is Klebsiella pneumonia. About 39.1% and 36.8% have been isolated in UTI chromogenic agar and MacConkey agar respectively. This is followed by Escherichia coli which showed 29.7% and 33.8% isolates in UTI chromogenic agar and MacConkey agar respectively.

The antibiotic susceptibility was done directly from the urine specimen (Direct sensitivity test) and also with the clinical isolates obtained from the conventional medium ie. MacConkey agar. The Table 3 shows the antibiotic susceptibility results for gram negative isolates. nitrofurantoin and amikacin showed sensitive for all the isolates and remaining antibiotics showed both sensitive and resistance for both direct sensitivity test and secondary sensitivity test.

The Table 4 depicts the results of antibiotic susceptibility result for gram positive isolates. Here the vancomycin, cefoxitin, nitrofurantoin and amikacin showed sensitive for all the isolates for both the tests. The ciproflox and ampicillin showed both sensitive and resistance to the clinical isolates for both the tests.

DISCUSSION

A total of 100 urine specimens were collected from patients with suspected urinary tract infection. However, 64 and 68 specimens showed culture positive in UTI chromogenic agar and conventional media respectively. On statistical analysis, the difference is not found to be significant ($p>0.05$), thus showing that UTI chromogenic agar is equally efficient in the isolation of the

Table 3: Antibiotic susceptibility result for gram negative isolates (N=49)

Antibiotic disc	Number of isolates				P value
	Direct sensitivity test		Secondary sensitivity test		
	Sensitive	Resistant	Sensitive	Resistant	
Nitrofurantoin	49	Nil	49	Nil	>0.05
Amikacin	49	Nil	49	Nil	>0.05
Ampicillin	7	42	7	42	>0.05
Gentamicin	39	10	39	10	>0.05
Ciproflox	32	17	32	17	>0.05

Table 4: Antibiotic susceptibility result for gram positive isolates (N=13)

Antibiotic disc	Number of isolates				P value
	Direct sensitivity test		Secondary sensitivity test		
	Sensitive	Resistant	Sensitive	Resistant	
Vancomycin	13	Nil	13	Nil	>0.05
Cefoxitin	13	Nil	13	Nil	>0.05
Ciproflox	8	5	8	5	>0.05
Nitrofurantoin	13	Nil	13	Nil	>0.05
Ampicillin	7	6	7	6	>0.05
Amikacin	13	Nil	13	Nil	>0.05

microorganisms from the urine of UTI patients. The isolation of different microorganisms also did not show much difference except for the *E.coli*. The $p>0.05$ in all cases, thus favouring the null hypothesis and thus can be concluded that both the UTI chromogenic agar and conventional media are equally efficient in the isolation and identification of microorganisms from the urine of UTI patients. The present study has confirmed the findings of previous studies[12-15]. In a conventional media like MacConkey agar, the culture and identification takes a minimum of 48 hours. However, the use of chromogenic agar makes it possible to isolate and identify the bacteria in 24 hours.

The direct antibiotic sensitivity test has shown equally good to the conventional antibiotic sensitivity test ($p>0.05$). The direct antibiotic sensitivity test takes the advantage that the urine is normally sterile and during UTI it is mostly monobacterial. The direct antibiotic sensitivity test produced exact results in comparison to conventional antibiotic sensitivity test except for some minor difference in the zone diameter. Several studies have been conducted on direct antibiotic sensitivity test and proved to be efficient in comparison to conventional antibiotic sensitivity test[16-18]. However, it is always prudent to do the conventional antibiotic sensitivity test along with the direct antibiotic sensitivity test to find the correlation of results.

CONCLUSION

From the present study, it has been observed that the chromogenic agar and direct antibiotic susceptibility test can be used in conjugation thereby the culture and sensitivity results for urine specimen can be produced within 24 hours. This helps the physician to initiate the appropriate treatment in time. Apart from this, it also reduces the expenditure involved in the conventional culture and sensitivity test.

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