International Journal of Research in Medical Sciences
Chaturvedi A et al. Int J Res Med Sci. 2017 Aug;5(8):3676-3679
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20173584

Original Research Article

Comparative evaluation of different culture media for the isolation and identification of common urinary pathogens

Aarti Chaturvedi, Ritu Garg*, Varsha A Singh

Department of Microbiology, Markandeshwar University of Health Sciences and Research, Mullana, Haryana, India

Received: 22 May 2017
Accepted: 23 June 2017

***Correspondence:**
Dr. Ritu Garg,
E-mail: dr_rittu07@yahoo.co.in

ABSTRACT

Background: Urinary tracts infections (UTIs) are one of the most common infections encountered in hospital as well as community settings. There is continuous increase in incidence of this infection leading to more consumption of antimicrobial drugs. Urine cultures occupy most of the workload of routine microbiology laboratories in developing country like India. Accurate and rapid identification of pathogens is the primary responsibility of a clinical microbiology laboratory.
Methods: Mid-stream urine and catheterized samples were collected. Cultures were plated on blood agar, MacConkey agar and cysteine lactose electrolyte deficient media and incubated overnight at 35°C-37°C in ambient air. Colonies on the MacConkey agar, CLED agar and blood agar were also identified. The final identification of the isolates was done using standard identification protocol. Antimicrobial susceptibility was performed by Kirby- Bauer disc diffusion test according to the CLSI guidelines.
Results: Out of 500 urine samples processed, 211 samples showed significant growth, 24 samples showed polymicrobial growth and 265 samples were reported sterile. Out of these 211, 199 showed pure growth and 12 showed mixed growths. Out of 199 pure growths, 126 were gram negative bacilli, 56 were gram positive cocci and 17 were yeast. All the gram-negative bacilli grown on all the media but most of the gram-positive cocci and yeast were unable to grow on Mac-Conkey agar and blood agar but grew successfully on CLED agar.
Conclusions: So, in resource constrain laboratories, CLED agar can be used as media of choice for isolation of common uropathogens because it is user friendly, cost effective and decreases work load of the laboratories.

Keywords: CLED agar, Urine samples, Urinary tract infections

INTRODUCTION

Urinary tracts infections (UTIs) are one of the most common infections encountered in hospital as well as community settings.[1] It is the third most common infection found in India which affects people of all age groups. There is continuous increase in incidence of this infection affecting socioeconomic status of the people and leading to more consumption of antimicrobial drugs. It has been reported that more than 60 percent of women experienced UTI once in their life time as UTI is more common in females because of shorter urethra.[2] The infection may involve the kidney, renal pelvis, ureters, bladder and urethra along with adjacent structures, such as prostate and epididymis in males. Both gram positive and gram-negative organisms are responsible for UTI. These infections are the most common cause of gram negative urosepsis in hospitalized patients but delayed diagnosis or misdiagnosis of UTIs in community can also result in urosepsis. So, urinary tract infections if not treated timely can contribute significant amount of morbidity and can be fatal due to increase in

antimicrobials resistance. So that is why accurate identification of pathogens in short turnaround time and generation of authenticated results is the primary responsibility of a clinical microbiology laboratory.[3]

Urine cultures occupy most of the workload of routine microbiology laboratories in developing country like India, therefore these laboratories use different culture media for urine cultures depending upon the resources available. In most of the clinical laboratories of the developing countries, a combination of blood agar and MacConkey agar is used traditionally for urine culture for long time.[4] While, many of them started use of Cysteine lactose electrolyte deficient (CLED) agar which is a better option for the detection of uropathogens instead of combination of two media.[5] Now a day, several chromogenic media are available commercially for the rapid detection of uropathogens from urine cultures.[6] On the other hand in developing countries the apparent higher cost of chromogenic media is a major hindrance for their routine use. As a result, there is continuous strive by the laboratories to streamline and improve urine culture algorithms which are feasible according to their resources available. By keeping above facts in mind, we had planned a study to found out that which media is better that can be used for plating of urine samples and can be able to isolate maximum number of pathogens.

METHODS

A Prospective study of six-months' duration was conducted on 500 urine samples collected from patients with suspected urinary tract infections from outpatient department as well as in patient department of tertiary care hospital of North India after taking permission from institutional ethical committee.

Most of the samples were mid-stream urine and some are from catheterized patients also. Specimens were received in the department of microbiology and processed as soon as possible.[7]

Routine urine cultures were plated using calibrated loop delivering 0.001 ml of urine on blood agar, MacConkey agar and cysteine lactose electrolyte deficient media. Urine cultures were incubated overnight at 35°C-37°C in ambient air. Colonies on the MacConkey agar, CLED agar and blood agar were also identified following colony characteristics against each of the uropathogens. The final identification of the isolates was done using standard identification protocol and other relevant biochemical tests as appropriate for the isolates.[7-9] Antimicrobial susceptibility was performed by Kirby-Bauer disc diffusion test according to the CLSI guidelines.[10]

RESULTS

Out of 500 urine samples processed, 211 samples showed significant growth, 24 samples showed polymicrobial growth and 265 samples were reported sterile. All culture results after 24 hours of incubation shown in Table 1. Out of these 211, 199 showed pure growth and 12 showed mixed growth of more than one pathogen. Out of 199 pure growths, 126 were gram negative bacilli, 56 were gram positive cocci and 17 were yeast. Out of 126 gram negative bacilli, lactose fermenting was 111 and non-lactose fermenting were 15.

Table 1: Different type of growth on culture of urine samples.

Type of growth	Number
Pure growth	199
Mixed	12
Polymicrobial	24
Sterile	265
Total	500

Among lactose fermenting, Escherichia coli were most commonly isolated. Rate of isolation of gram negative bacilli are shown in Table 2. Among 56 gram positive bacteria isolated, most common was *Enterococcus species* 25 (*Enterococcus faecalis* is 23 and *Enterococcus faecium* 2) followed by *Staphylococcus aureus* 24, *Staphylococcus saprophyticus* 5 and *Staphylococcus epidermiditis* from 2 cases that was too from young females of age group 15-25 years. Among yeast isolates all the 17 cases showed *candida* species (*Candida albicans* 12 and *non albicans candida* 4).

Table 2: Rate of isolation of gram negative bacilli from all the media.

Name of the organism	Total number
Escherichia coli	51
Klebsiella species	**36**
Klebsiella pneumoniae	33
Klebsiella oxytoca	3
Citrobacter species	**15**
Citrobacter kosri	09
Citrobacter frundii	06
Enterobacter species	07
Proteus mirabilis	02
Pseudomonas aeruginosa	07
Acinetobacter CBC	08
Total	126

Culture of 500 urine samples on three different media simultaneously i.e. on CLED agar, Mac-Conkey agar, blood agar, the rate of isolation of gram negative bacilli were 100% but there is difference in rate of isolation of gram positive cocci and yeast which are shown in Table 3. Same results were observed in mixed culture growth of gram negative bacilli and gram-positive cocci grown together i.e. all the gram-negative bacilli grown on all the media but most of the gram-positive cocci were unable to grow on Mac-Conkey agar and blood agar but grew successfully on CLED agar.

Table 3: Comparison of three culture media for the rate of isolation of gram positive cocci and yeast.

Name of the organism	Total number	CLED	MA	BA
Staphylococcus aureus	24	20	10	20
Staphylococcus saprophyticus	05	5	3	01
Staphylococcus epidermidis	02	02	00	02
Enterococcus species	25	25	03	15
Candida species	17	17	05	09
Total	73	69	21	47

DISCUSSION

The present study was conducted for comparative evaluation of culture media like MacConkey agar, blood agar and cysteine lactose electrolyte deficient agar used routinely in microbiology laboratories of developing countries for isolation and identification of uropathogens.

In the present study, out of 500 samples, pure growth was shown in 199 samples while 12 showed growths of mixed growth with two organisms and 24 showed polymicrobial growth and rest 265 were sterile. Our findings correlate with the study done by Manjusree BS and Sharmin et al.[11,12] Out of 199 pure growths, 126 were gram negative bacilli and 56 were gram positive cocci and 17 were *Candida species*. In the present study, gram negative bacilli outnumbering gram positive cocci. *Escherichia coli* are the most commonly isolated organism. Findings are in comparison to the studies done by Singh VP and Gupta V.[13,14] In this study, isolation rate of gram negative bacilli from three different media i.e. CLED agar, MacConkey agar and blood agar was same, all 126 gram negative bacilli were grown on three media. In a study done by Manjushree BJ showed the same results.[11] But out of 56 gram positive cocci, rate of growth on CLED agar was 52 while on MacConkey agar only 16 were grown and on blood agar only 38 were grown. By literature search, there are several studies which showed that gram positive cocci like *Staphylococcus aureus* and coagulase negative *staphylococcus* like *Staph. saprophyticus* and *Staph. epidermidis* and *Enterococcus species* are the common uropathogens now a day responsible for UTI. Among *Candida species*, in current study all the 17 isolates are grown on CLED agar, but on Mac-Conkey agar, isolation rate was 5 and on blood agar it was 9. These findings are in accordance with study done by Ciragil et al and Manjusree BJ.[15,11]

Microbiology laboratories play key role for the isolation of specific pathogens responsible for urinary tract infections. However, isolation and identification of the pathogen should be rapid, as delayed diagnosis can lead to delayed antimicrobial therapy which can further lead to fatal complications like urosepsis as well as can emerge drug resistance among pathogens. In this study, almost all the pathogens responsible for UTI were isolated from CLED agar alone as compared to Mac-Conkey and blood agar together, which further cuts the cost as well as workload of the laboratory. In a study done by Quiser S, they observed that the rate of isolation by using CLED media were like rate of isolation by using chromogenic media i.e. both media were found comparable as far as isolation of bacteria was concerned the difference was that identification was rapid by chromogenic media but the cost of chromogenic media is very high as compared to CLED agar.[6]

CONCLUSION

So, in resource constrain laboratories as well with more than average workload, where high cost chromogenic media are out of reach, CLED agar can be used as media of choice for isolation of common uropathogens because it is user friendly, cost effective and also decreases work load of the laboratories.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Chaturvedi A, Garg R, Singh VA. Comparative evaluation of different culture media for the isolation and identification of common urinary pathogens. Int J Res Med Sci 2017;5:3676-9.