COMPARATIVE STUDY OF VARIOUS GROWTH MEDIA IN ISOLATION OF URINARY TRACT PATHOGENS

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ABSTRACT

Background: Since a wide spectrum of bacteria are responsible for causing urinary tract infections. To cover the maximum yield of isolates a study was designed in which a combination of media was used for detection of both gram positive and gram-negative bacteria.

Materials and Methods: A Combination MacConkey agar, Blood agar and Cystine lactose electrolyte deficient medium were used at one and same time to get the positive growth. The identification of responsible pathogens is confirmed by observation of colony characteristics, Gram staining and biochemical screening using different tests.

Results: Out of the one hundred isolates, two isolates showed growth only on MacConkey agar, 19 isolates were detected only on Blood agar and 15 pathogens grew only on CLED medium . Almost 50% of isolates grew on all the three media while 17% isolates showed growth on both blood agar and CLED medium. The combination of CLED medium and MacConkey agar showed the minimum yield.

Conclusion: The use blood agar, MacConkey agar and CLED medium at one and same time have minimum chances of missing cultures. It is proposed that for the isolation of gram-positive urinary tract pathogens blood agar when used singly covers the maximum range.

Key words: Urinary Tract Pathogens, Blood Agar, MacConkey Agar, CLED medium, Gram Staining, Biochemical Screening.

INTRODUCTION

The diagnosis of urinary tract pathogens is based on the quantitation of bacteria in the urine. The culture is taken from midstream, clean catch urine specimen. As rule yielding $> 10^5$ CFU/ml is considered as positive (significant level of organisms).[1] Various growth media such as blood agar, MacConkey agar are used for the culture of urine samples. MacConkey agar is used for the isolation of gram-negative rods. It also inhibits the growth of gram +ve cocci. Blood agar is used for the culturing of the fastidious gram +ve cocci. It is also used to detect the hemolytic streptococci. Instead of using the above-mentioned media, some laboratories use single non-inhibitory medium such as Cystine Lactose Electrolyte Deficient medium (CLED).[2] The main objective of this study was to know the incidence of urinary tract infection in this area of the country. Since this area is located in the situation where the people of NWFP and Punjab both come for investigation and treatment, mixed type of pattern is expected. Since different types of organisms are responsible for urinary tract infection, the whole range of pathogens can not be covered by a single growth medium, therefore we used Blood agar, MecConkey agar and Cystine Lactose electrolyte Deficient medium at one and same time for culturing the urine samples.

MATERIALS AND METHODS

A total 100 patients (both males and females) with urinary tract infection and thirty normal controls were included in this study. All the patients were provided with wide mouthed, tightly closed sterilized bottles. The bottles were sterilized in hot air oven by dry heat at 160 C° for one hour. The patients were advised to collect clean catch, mid stream early morning specimen of urine. The patients were instructed to hand over the specimen to the laboratory within half an hour of the collection of specimen. In any case when it was not possible to reach the laboratory in time, they were asked to put the specimen in the refrigerator. The patients were asked to wash the hands thoroughly with antiseptic (detol) soap, then to clean the urethral meatus in male and to wash the vulva with detol water in female. They were asked to destroy the first part of the urine and to collect the mid stream in the sterilized bottle. With the help of one millimeter sterilized nicrome wire loop, the samples were obtained from uncentrifuged urine specimens and were streaked on Blood agar, MacConkey agar and CLED medium at one and

same time according to standard procedure. After streaking the plates were kept in the incubator at 35 – 37 C° for 18 – 24 hours. When the growth was obtained either on Blood agar, MacConkey agar or on CLED medium, the identification of the responsible pathogen was confirmed by the observation of colony characteristics, Gram staining and biochemical screening using different tests.

RESULTS

The present study was undertaken on one hundred patients of urinary tract infection of either sex (both male and female) and twenty normal males and ten normal female controls. The detail results are given in the proceeding tables. In normal controls the urine samples were also streaked on all the above three media. Only in three cases the growth appeared on blood agar (2/30) and CLED medium (1/30). However, the growth was not significant in any case.

DISCUSSION

Urinary tract infection is one of the most common types of the infection encountered in practice of medicine today. Intensive investigations of these infections has been carried out during the past three decades in an attempt to define more accurately the epidemiology, pathogenesis, Natural history, and prevention of these diseases.[3] There is a great difference between the bacterial flora of the urine in the patients in primary urinary tract infection compared with the bacterial flora of the secondary urinary tract infection.[4,5] The type and frequency of urinary tract pathogens is also a matter of controversary. Staphylococcus saprophyticus is one of the most frequently encountered microorganisms associated with urinary tract infections in young sexually active female outpatients. Orrett and Shurland from West Indies reported in their study the significance of coagulase negative staphylococci in urinary tract infection in a developing country. They found that Staphylococcus saprophyticus and Staphylococcus epidermidis accounted for approximately 90% of isolates from females while in male only 68.7% of isolates were Staphylococcus epidermidis.[6,7] In our study too Staphylococcus sparophyticus was one of the major isolates (43%). It has been reported by Plorade (1984) et al that E. coli is the cause of more than 90% of acute infections of normal urinary tract. Linda (1980) has reported Escherichia coli to account for 85% of urinary tract infections.[8] Some other researchers have reported the incidences of E. coli as urinary tract pathogen in range of 34 – 61%.[4,9,10] From the above findings it appears that the frequency of Escherichia. coli as urinary tract pathogen is decreas-

Table 1: Frequency distribution of total Urinary Tract Isolates on different media

S. No.	Name of Organism	Total No. of Isolates	Growth on Blood agar	Growth on CLED Medium	Growth on MacConkey Agar
1	Staph. saprophyticus	43	34	30	17
2	Staph. aurius	19	17	16	7
3	Micrococcus	4	3	2	1
4	Streptococci	5	4	4	2
5	Escherichia coli	11	11	11	10
6	Enterobacter Spp.	4	4	4	4
7	Klebsiella Spp	2	1	2	1
8	Serratia Spp	9	5	7	5
9	Proteus mirabilis	1	1	1	1
10	Pseudomonas	1	1	1	1
11	Candida	1	Nil	1	Nil
	Total	100	81	79	49

Table 2: Frequency distribution of Urinary Tract Isolates on three and two medium at one and same time.

S. No.	Name of Organism	No. of cases	Growth on B+C+M	Growth on M+C	Growth on B+C	Growth on B+M
1	Staph.saprophyticus	43	15	1	7	Nil
2	Staph. Aurius	19	7	Nil	7	Nil
3	Micrococcus	4	1	Nil	Nil	Nil
4	Streptococci	5	2	Nil	1	Nil
5	Escherichia coli	11	10	Nil	1	Nil
6	Enterobacter Spp.	4	4	Nil	Nil	Nil
7	Klebsiella Spp.	2	1	Nil	Nil	Nil
8	Serratia Spp.	9	3	1	1	Nil
9	Proteus mirabilis	1	1	Nil	Nil	Nil
10	Pseudomonas	1	1	Nil	Nil	Nil
11	Candida	1	Nil	Nil	Nil	Nil
	Total	100	45	2	17	—

B = Blood agar, M = MacConkey agar, C = CLED medium

Table 3: Frequency distribution of different Urinary Tract Isolates on Single Medium only.

S. No.	Name of Organism	No. of cases	Growth only MacConkey agar	Growth only Blood agar	Growth only CLED Medium
1	Staph. saprophyticus	43	1	12	7
2	Staph. aurius	19	Nil	3	2
3	Micrococcus	4	Nil	2	1
4	Streptococci	5	Nil	1	1
5	Escherichia coli	11	Nil	Nil	Nil
6	Enterobacter Spp.	4	Nil	Nil	Nil
7	Klebsiella Spp.	2	Nil	Nil	Nil
8	Serratia Spp.	9	1	1	2
9	Proteus mirabilis	1	Nil	Nil	Nil
10	Pseudomonas	1	Nil	Nil	Nil
11	Candida	1	Nil	Nil	1
	Total	100	2	19	15

ing gradually and it is being replaced by other pathogens. In our study E. coli was found to be present in 11% patients. This difference might be due to environmental, seasonal and other intrinsic and extrinsic factors responsible for urinary tract infection.[8]

We used Blood agar, MacConkey agar and Cystein Lactose Electrolyte Deficient (CLED) medium at one and same time. However such combination is rarely used in local laboratories. Most laboratories use MacConkey agar or CLED medium, or combination of MacConkey agar and Blood agar.[2] Nicolle (1988) and his associates used Blood agar and MacConkey agar for the culture of urinary tract pathogens.[11] Linda et al (1985) has used CLED medium for the inoculation of urine samples.[8] Kamran and K U Nissa (1989) have used Nutrient agar and MacConkey agar for the isolation of urinary tract pathogens.[12] Some other workers used only CLED medium for the culturing of urine.[4,10] We have observed that if Blood agar, MacConkey agar and CLED medium is used at one and same time, then the detection rate of isolates approaches to 100%. From table No 1 it is clear that if CLED medium is used alone for the culture of urine samples, there are 21% chances of missing positive culture. If Blood agar alone is used for the culturing of urine samples, there are 19% chances of missing positive cultures. Similarly, if MacConkey agar alone is used there are 51% chances of missing positive cultures. If Blood agar and MacConkey agar are used in combination, then there are 15% chances of missing positive cultures. (Table 2)

CONCLUSION

This study indicates that although it is expensive but it good to use Blood agar, MacConkey agar and CLED medium at one and same time to have minimum chances of missing positive cultures. We therefore propose that at least for research purposes, a combination of different media should be used to detect both gram negative and gram-positive bacteria. In rare and resistant cases the above protocol can also be used routinely in clinical laboratories.

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