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Research Article

Urinary isolates with special reference to Urichrom II

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ABSTRACT

Urinary tract infections (UTIs) are the second most common infections, only after respiratory tract infections. Conventionally, Blood agar (BA), Mac Conkey agar (MAC) and Cysteine Lactose Electrolyte Deficient (CLED) medium are used routinely for processing of urine samples. Several chromogenic media are now available which can be used to allow more specific and direct differentiation of bacterial colonies on the primary plate itself. The following study was conducted to evaluate the advantages of URICHROM II over the conventional media in supporting the growth for routine urinary isolates. The study was conducted over a one year period (January 2012 to December 2012) at Hyderabad. A total of 3094 urine samples were processed during the one year period. The urine samples were inoculated on to blood agar (BA), Mac Conkey agar (MAC) and URICHROM II. The inoculated plates were incubated at 37°C over night (16-20 hr) and examined the next day morning. Samples showing significant bacterial growth (>10^5CFU/ml) were further processed. A total of 3094 urine samples were processed over a one year period. Out of the 3094 urine samples processed, 945 samples were positive (30.54%) and 2149 samples (69.45%) were negative. Among the positive samples (945), Gram positive isolates were obtained from 201 samples (21.22%) and Gram negative isolates from 744 samples (78.82%). Escherichia coli was the predominant Gram negative isolate (51.11%) and Enterococcus feacalis was the predominant Gram positive isolate (14.81%). URICHROM II supported the growth of all routine urinary isolates on par with BA and MAC and can be recommended as a primary plating medium for recovery of uropathogens.

Keywords: Urinary tract infections, Culture media, URICHROM II

INTRODUCTION

Urinary tract infections (UTIs) are the second most common infections, only after respiratory tract infections. UTI is defined as a disease caused by microbial invasion of the genitourinary tract, extending from the renal cortex to the urethral meatus. Presence of bacteria in urine is called bacteriuria, and presence of pus cells in the urine is called pyuria, which often accompanies UTIs.[3] Normally the kidneys, ureters, urinary bladder and proximal urethra are sterile, but bacteria may be present in the distal urethra as transient flora, most often derived from faecal flora. Conventionally, blood agar (BA), Mac Conkey agar (MAC) and Cysteine Lactose Electrolyte Deficient (CLED) medium are used routinely for processing of urine samples.[3] Several chromogenic media are now available which can be used to allow more specific and direct differentiation of bacterial colonies on the primary plate itself. These could help in minimizing the requirement for further biochemical tests, and also could help in giving the clinician a preliminary report, as and when the need arises, for initiation of early therapy. URICHROM II (ELITECH MICROBIO, France), provides the convenience of making a preliminary identification of the organism, after overnight incubation

itself, by producing characteristic coloured colonies of various organisms. The following study was conducted to evaluate the advantages of URICHROM II, over the conventional media, in supporting the growth for routine urinary isolates.

METHODS

The study was conducted over a period of one year (January 2012 to December 2012), a total of 3094 urine samples being processed. The urine samples were inoculated on to BA, MAC[1,4] prepared in-house using commercially available dehydrated media (Hi Media laboratories, Mumbai, India). These media were prepared following the standard instructions from the manufacturer. The autoclaved media were poured into petri dishes (around 25 ml medium per plate and 3-4 mm depth of medium in each plate)[1,4], checked for sterility, and stored at 2-8°C till used, approximately 1 week to 10 days. In addition to the above two media, URICHROM II, a chromogenic medium, was also inoculated. The medium was prepared according to manufacturer's instructions and stored at 2-8^{0}C for 2 weeks. Each fresh batch of medium was tested by inoculating standard strains of Staphylococcus aureus (ATCC 25923), Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853). A standard protocol was followed for carrying out urinary cultures. All the clients were given strict instructions regarding the standard recommendations for transport of urine samples, and only such samples meeting the set criteria were accepted for processing in the lab. All others were rejected and a request for fresh samples was given.[2,5] The urine samples received were inoculated immediately (well mixed and un-centrifuged) onto all the three media (BA, MAC, URICHROM II) with a standard pre- calibrated loop (Nichrome-SWG 24, 4 mm internal diameter holding 10 µl of the urine sample). The inoculated plates were incubated at 37°C over night (16-20 hr) and examined the next day morning. Samples showing significant bacterial growth (>10^{5}CFU/ml) were further evaluated for colony characters, and for further processing and identification. Samples showing lesser colony count were reported as non-significant bacteriuria and considered under no growth. For samples showing mixed bacterial growth, the isolate with growth >10^{5}CFU/ml was further processed and identified. Isolates showing yeast growth were reported as no pathogenic growth, and considered under no growth. A presumptive identification of the organism was attempted based on the colony colours on URICHROM II. Final identification of the organisms was done upto the species level (only upto genus level when not possible) using MicroScan autoSCAN-4 instrument (SIEMENS). For identification of the organism, Prompt Inoculation System-D (from SIEMENS) was used to prepare the inocula for various biochemical tests. The Prompt Inoculation System-D consists of an inoculation wand in a 30ml bottle of diluent (PLURONIC broth). The wand was touched to several bacterial colonies, wiped and then placed in the plastic bottle with broth. The bacteria were suspended by shaking the bottle thoroughly, and this was corresponding to 0.5 Mc Farland's standard according to the manufacturer. The prepared bacterial suspensions were poured into either Gram negative or Gram positive panels depending on the staining characters and morphology of the isolates so obtained. The panels were incubated overnight (16-20 hr) at 37°C in a non-carbon dioxide incubator, and read the next day in MicroScan autoSCAN-4 instrument for final identification of the organism.

RESULTS

A total of 3094 urine samples were processed over a one year period (January 2012 to December 2012). Blood agar supported the growth of all the organisms, while Mac Conkey agar supported the growth of mainly the Gram negative bacilli. But URICHROM II supported the growth of all routine urinary isolates, and also yeasts. The ability of URICHROM II to support the growth of the organisms was concurrent with that of the conventional media. In addition it also provided the advantage of rendering colour differentiation to the various isolates for preliminary identification, which were further confirmed by standard tests using MicroScan autoSCAN-4 instrument. Out of the 3094 urine samples processed, 945 samples were positive (30.54%) and 2149 samples (69.45%) were negative. Among the positive samples (945), Gram positive isolates were obtained from 201 samples (21.22%) and Gram negative isolates from 744 samples (78.82%).

Table 1: Colony characters on URICHROM II.

Organism	Colony colour
Escherichia coli	Pink to burgundy
Enterococcus, KES	Blue to blue green
Proteus, Providencia	Orange to brown
Morganella species	Orange to brown
Pseudomonas aeruginosa	Green
Staphylococcus	Colourless
Streptococcus	Colourless
GNB, Candida	Colourless

KES- Klebsiella, Enterobacter, Serratia group;
GNB- Gram Negative Bacilli

DISCUSSION

Culture media for isolation of bacteria were first devised by Louis Pasteur (Father of Microbiology), where liquid media like urine or meat broth were used.[1,4] Liquid media had several disadvantages like bacteria growing in them could not exhibit specific characteristics and it was difficult to separate the pure isolates from mixed growth. The earliest solid medium discovered was cooked cut potato by Robert Koch (Father of Bacteriology). The universal ingredient in any solid medium, agar, was

Table 2: Urinary isolates obtained.

Organism	No.	%
Gram negative	**744**	**78.82%**
Escherichia coli	483	51.11%
Klebsiella pneumoniae	97	10.26%
Klebsiella species	20	2.11%
Morganella morganii	17	1.79%
Pseudomonas aeruginosa	17	1.79%
Enterobacter cloacae	15	1.58%
Citrobacter koseri	13	1.37%
Proteus mirabilis	13	1.37%
Kluyvera ascorbata	12	1.26%
Enterobacter aerogenes	11	1.16%
Enterobacter species	10	1.05%
Citrobacter fruendii	9	0.95%
Klebsiella oxytoca	6	0.63%
Pseudomonas species	4	0.42%
Serratia species	4	0.42%
Acinetobacter baumannii	3	0.31%
Acinetobacter lwoffii	3	0.31%
Chromobacterium violaceum	3	0.31%
Burkholderia cepacia	2	0.21%
Proteus vulgaris	2	0.21%
Providencia species	1	0.10%
Serratia marcescens	1	0.10%
Gram positive	**201**	**21.22%**
Enterococcus faecalis	140	14.81%
Enterococcus faecium	25	2.64%
Enterococcus species	8	0.84%
Streptococcus species	8	0.84%
Staphylococcus aureus	6	0.63%
Staphylococcus scuiri	4	0.42%
Staphylococcus auricularis	3	0.31%
Staphylococcus epidermidis	2	0.21%
Staphylococcus haemolyticus	2	0.21%
Streptococcus agalactiae	2	0.21%
Staphylococcus xylosus	1	0.10%
Total	**945**	**100.04**

discovered by Frau Hasse, wife of one of the disciples of Robert Koch. On solid media bacteria exhibit distinct colony morphology and other features like pigment production, haemolysis etc. Media have been traditionally classified into various types like simple, complex, enriched, selective, indicator/ differential etc. Blood agar is an enriched medium which supports the growth of all types of bacteria- both fastidious and non-fastidious. Colonies on this medium can be differentiated into haemolytic and non-haemolytic. It cannot differentiate between the Gram negative isolates, and also completely fails to inhibit the swarming of Proteus species. Mac Conkey agar, a differential medium (differentiates lactose fermenters from non-lactose fermenters) supports the growth of Gram negative bacilli. Gram positive cocci (Staphylococci, Streptococci etc.) causing UTIs do not grow on this medium. Cysteine Lysine Electrolyte Deficient medium (CLED agar) can be used as a primary plating medium, since it supports the growth and differentiation of both Gram positive and Gram negative isolates. It is also inhibitory to Proteus swarming. Even though Mac Conkey agar and CLED agar can be used to differentiate lactose fermenters from non-lactose fermenters, further identification need to be carried out to differentiate among them. Over the past several years, several chromogenic media have been introduced commercially by various manufacturers to allow direct differentiation of various bacteria on the primary plate itself. This will help in reducing the burden of further biochemical testing, and also the time required for reporting to the clinician for initiation of therapy, as when the need arises. Although, final and confirmed report will be given later on, along with a report on antibiotic sensitivity testing. The principle of chromogenic media is based on the fact that specific bacteria possess specific enzymes which can degrade the specific substrates incorporated in these chromogenic media, thereby imparting a distinct colour to the bacterial colony, which can be visually observed. URICHROM II (ELITECH MICROBIO, France) medium consists of substrates for β-glucuronidase, β-glucosidase and tryptophan deaminase. Hydrolysis of these specific chromogenic substrates enables direct identification via the spontaneous colouration of colonies of the principal bacteria found in urine samples. URICHROM II allows a good growth of majority of pathogenic bacteria found in urine, with an inhibition of over growth of Proteus and the revelation of pigmentation of Pseudomonas aeruginosa. Escherichia coli which produces β-glucuronidase enzyme produces pink to burgundy coloured colonies; Enterococcus and KES group positive for β-glucosidase produce blue to blue green coloured colonies; Proteus, Providencia, Morganella group positive for Tryptophan deaminase produce orange to brown colonies. The medium also allows direct biochemical testing on the plate itself for tests like catalase, oxidase, indole production and tryptophan deaminase activity, after looking for colony characters and Gram's staining. Another advantage of URICHROM II was that it allowed easy differentiation of mixed cultures, and the isolate with significant bacterial growth could be picked up easily. The colonies on URICHROM II can be used to perform antibiotic sensitivity testing without sub culture on to another medium. But URICHROM II has some limitations like it does not support the growth of fastidious organisms, few strains of β-glucuronidase negative Escherichia coli give colourless colonies, and colour variations could be seen with Pseudomonas aeruginosa and group B Streptococci, hence preliminary identification may not be possible.

Recovery of uropathogens from URICHROM II medium used in our study was well correlating with that on Blood agar and Mac Conkey agar in terms of their colony counts, as was also reported by others.[8,9] The total culture positivity in our study was 30.54%, which correlates with others.[11,13] V Lakshmi et al reported a low culture positivity of 19.7%, while Mansour et al reported much lower isolation rate of 8.7% only. Gram positive isolates were seen in 21.22% and Gram negative isolates in 78.82% in our study, which correlates with others.[8,11] Escherichia coli was isolated in 51.11% of the positive samples in our study which correlates well with others.[8,10,13] Klebsiella species was the second predominant Gram negative isolate (13%), which correlates with others.[6,7,10,12,14] Enterococcus species were isolated in a high percentage in our study compared to many other studies: Shanti et al- 9.8%, Mahesh et al- 1.53%, V Lakshmi- 4.55%, Manikandan et al- 5.84%. The reason for this could be because of the automated identification system used.

CONCLUSIONS

UROCHROM II (ELITECH MICROBIO) is a satisfactory medium, and can be used as a primary plating medium for recovery of uropathogens. URICHROM II considerably reduces the daily work load, and thus minimizes the use of biochemical identification tests later on. Rapid results on URICHROM II can help in facilitating early initiation of therapy, and thus can help the clinician and the patient, and the society at large.

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