Chapter 3
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The Role of Chromogenic Replica System for Isolation & Identification of Uropathogens in the Era of Molecular Diagnostics

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ABSTRACT

Multidrug resistance in uropathogens is a serious problem in a health care set up. To deal with that, rapid identification of the etiological agent from clinical urine sample is utmost important. Use of replica disc for presumptive identification of uropathogens is an easy to perform, cost effective and rapid method, which can be, adopted in the microbiology laboratories as a primary screening method.

Keywords: Uropathogens; chromogenic; replica disc methods; molecular techniques.

1. INTRODUCTION

Urinary Tract Infections (UTIs) is a second most common infection in a health care facility, the first one being surgical site infection [1]. Incidence of UTIs is at least 250 millions/annum globally [2,3]. Urine is the most frequent sample received in any microbiology laboratory. [4]. Emergence of multidrug resistant microorganisms is a grave concern in all health care facilities. Therefore, rapid identification of the uropathogens is essential to treat the patients and to avoid the serious complications. It also helps the treating clinicians to choose appropriate antibiotics, prior to the results of antibiotic sensitivity test [1].

First reported case of use of chromogenic medium for diagnosis of UTI was in 1995, when the researchers used CPSID2 medium. A substrate for β- glucoronidase was used to identify *Escherichia coli* as pink red colonies. Additional substrate incorporation for β – glucoronidase secreted by enterococci (small green colonies) and *Klebsiella – Enterobacter – Serratia* group (large green colonies) allows its identification rapidly. In other hand, addition of tryptophan and iron salt allow *Proteus – Providentia – Morganella* group to produce brown coloured colonies. This is due to deaminase activity elicited by this group [5,6].

A number of other media has been developed and commercialized since then, such as; CHROMagar orientation, Uniselect medium, Rainbow agar UTI medium, Chromogenic UTI medium, USA agar, Harlequin CLED, Urichrome agar etc. [7,8,9].

Chromogenic replica system is a simplified rapid method to detect uropathogens and this study aimed to access its utility in a clinical microbiology laboratory for the detection of the same. This is also performed to compare the result of chromogenic replica method with the conventional method.

2. MATERIALS AND METHODS

This study was undertaken in a tertiary care teaching hospital of western Maharashtra, situated in a rural background, The study was carried out to compare the utility of conventional method with chromogenic replication system for detection of uropathogens. For this purpose, authors used

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chromogenic replication system for detection of uropathogens. Many researchers reported use of chromogenic agar media but none so far has reported utility of replica system for the same. The study period was 6 months.

All clinically suspected cases of urinary tract infections admitted in this hospital were included in the study. Criteria of inclusion for those indoor patients having UTI were following: >38°C temperature, urgency, frequency of micturition, suprapubic tenderness, presence of more than 3 pus cells/ high power field in urine samples, > 105 colony forming unit/ml of un centrifuged urine and isolation of not more than two organisms on culture [10].

Urine samples were collected from the suspected cases of UTIs admitted in the hospital. Either clean catch mid stream urine samples or from sampling port of indwelling catheter with a sterile syringe and needle were collected [10].

The urine samples were inoculated on Nutrient agar, MacConkey's agar and blood agar. The inoculated plates were incubated aerobically at 37°C for 18 – 24 hours. The significant bacteriuria is detected by counting 100 colonies, which is approximately equal to 105colony forming units/ ml of urine. In conventional method, *Enterobacteriaceae* isolates were identified by the following biochemical tests, such as; motility, indole production, nitrate reduction, glucose fermentation with or without gas production, hydrogen sulphide production, urea hydrolysis, lysine and ornithine decarboxylation and sodium citrate utilization. Gram-negative microorganisms other than *Enterobacteriaceae* were identified by colony morphology, pigment production, gelatin, and catalase test. The oxidase test was performed for non- fermenters. *Staphylococcus aureus* and coagulase negative *Staphylococci* were identified by Gram staining, hemolysis on blood agar, catalase and coagulase tests and novobiocin susceptibility. *Streptococci* were identified up to species level by hemolysis on blood agar, PYR test, bacitracin sensitivity test, bile solubility and aesculin hydrolysis test [11,12,13]. All the media and biochemicals were procured from Hi Media Laboratories, India.

For replication and identification of uropathogens, Hi Detect UTI identification discs (procured from Hi Media Pvt Limited, India) were used. Patient's urine samples were inoculated on nutrient agar, blood agar, MacConkey's agar and soyabean casein digest agar. All the inoculated plates were incubated for 18 to 24 hours. The replication disc of choice for suspected organisms was placed over the bacterial growth on nutrient agar or soyabean casein digest agar for maximum of 30 seconds (following manufacturers instructions). The disc was then put in a sterile petri dish and incubated at 35-37°C for 1-4 hours. Development of colour was observed and interpreted accordingly to manufacturer's instructions. All the tests were run with the control strains side by side.

Control strains used for the study were the following:

Escherichia coli (ATCC 25922) – pink to purple colour on replication disc.
Klebsiella pneumoniae (ATCC 13883) – blue to purple, Mucoid.
Enterococcus faecalis (ATCC 29212) – blue, small.
Pseudomonas aeruginosa (ATCC 27853) – colourless

3. RESULTS

We processed total 625 urine samples from suspected cases of UTIs over a period of six months. Out of 625 urine samples cultured in this study, 419 (67.04%) had significant uropathogens (Table 1a).

The highest number of uropathogens was *Escherichia coli* 198(47.25%) followed by *Enterococcus faecalis* 88 (21.00%), *Klebsiella pneumoniae* 63 (15.03%), *Pseudomonas aeruginosa* 45 (10.73%) and mixed growth 15 (3.57%).

We isolated mixed growth of organisms' in15 (3.57%) urine samples (Table 1b).

The highest uropathogens in mixed isolate is *Escherichia coli* & *Enterococcus faecalis* 7(46.66%) followed by *Escherichia coli* &*Klebsiella pneumoniae* 5 (33.33%). The least number of isolates were *Escherichia coli* &*Pseudomonas aeruginosa* 3 (20.00%).

Distribution of uropathogens identified by conventional and replica system using chromogenic disc were compared. It was observed that conventional method of bacteria isolation showed 100% positive result in all isolates while replica method using chromogenic disc showed *Escherichia coli* 197(99.49%) and other isolates 100% positive result (Table 2).

Table 1a. Distributions of bacterial isolates from positive urine culture (n = 419)

Bacterial isolates	Total number	Percentage
Escherichia coli	198	47.25
Enterococcus faecalis	88	21.00
Klebsiella pneumoniae	63	15.03
Pseudomonas aeruginosa	45	10.73
Mixed growth	15	03.57

The highest number of uropathogens is Escherichia coli 198(47.25%) followed by *Enterococcus faecalis* 88 (21.00%), *Klebsiella pneumoniae* 63 (15.03%), *Pseudomonas aeruginosa* 45 (10.73%) and mixed growth 15 (3.57%).

Table 1b. Distributions of bacterial isolates of mixed growth in positive urine culture (n = 15)

Mixed growth	Total	Percentage
Escherichia coli + *Enterococcus faecalis*	7	46.66
Escherichia coli + *Klebsiella pneumoniae*	5	33.33
Escherichia coli + *Pseudomonas aeruginosa*	3	20.00

The highest uropathogens in mixed isolate is *Escherichia coli* & *Enterococcus faecalis* 7(46.66%) followed by *Escherichia coli* & *Klebsiella pneumoniae* 5 (33.33%) and the least number of isolates were *Escherichia coli* & *Pseudomonas aeruginosa* 3 (20.00%).

Table 2. Number & percentage of identification of uropathogens by conventional & replica system using chromogenic disc

Bacterial isolates & their total numbers	Conventional method No of isolates & (%)	Replica method No of isolates & (%)
Escherichia coli (n= 198)	198(100%)	197(99.49%)
Enterococcus faecalis (n = 88)	88(100%)	88(100%)
Klebsiella pneumoniae (n= 63)	63(100%)	63 (100%)
Pseudomonas aeruginosa (n = 45)	45(100%)	45 (100%)
Mixed growth (n = 15)	15(100%)	15 (100%)

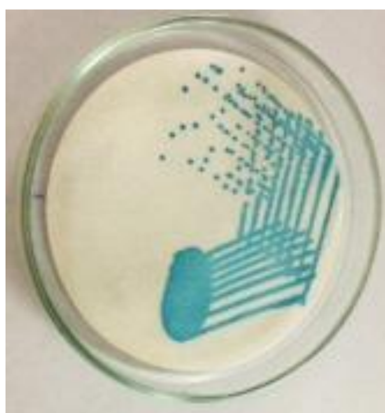

Fig. 1. Replica of small, blue colonies of *Enterococcus faecalis* on replica disc

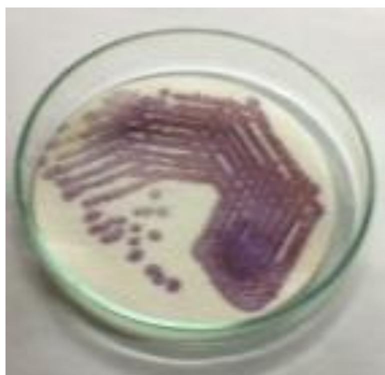

Fig. 2. Replica of purple, low convex colonies of _Escherichia coli_ on replica disc

Table 2 showed distribution of uropathogens identified by conventional and replica system using chromogenic disc. It was observed that conventional method of bacteria isolation showed 100% positive result in all isolates while replica method using chromogenic disc showed _Escherichia coli_ 197(99.49%) and other isolates 100% positive result.

4. DISCUSSION

To rectify the delay in culture – based diagnostic procedures we are passing through era of molecular diagnosis. While adopting the newer methods, one has to consider the drawback of extensive use of molecular methods. Culture- isolates are essential to perform antibiotic- sensitivity test in PCR – positive cases. Some new genes responsible for antibiotic resistance may not be detected by PCR but likely to be recovered on chromogenic media due to phenotypic expression of character [14].

There are many upgraded methods currently being applied for detection of uropathogens, such as; MALDI–TOF, FISH etc., but still remaining dependent on initial isolation of bacterial colonies from clinical sample, delaying bacterial identification by approximately 12 hours [15].

Kilian and Bu¨ low (1976) were the first to use a selective chromogenic medium for the direct identification of Escherichia coli. They used it as primary culture medium for urine sample [16].

Introduction of a new chromogenic disc, using replication system provide presumptive identification of uropathogens rapidly. According to some researchers, only 20 to 30% of urine samples sent for microbiological investigations show significant bacteriuria [17,18]. Our research showed that out of 625 urine samples, 419 (67.04%) were positive for culture (Table 1a). Some studies compared the traditional method of identification of uropathogens with chromogenic system and found that chromogenic system reduces 20% in workload time [19]. Other workers found that it reduces media required for identification [20] and helps in faster detection of uropathogens from mixed culture [21,22]. This was very useful for resource limited setting [23,24,25]. Chromogenic substrates are incorporated in this replica disc, which imparts visible colour change of the replica on the colony as a result of breaking down of the substrate by bacterial enzymes. This colour change is specific for a particular microorganism. _Escherichia coli_ produces β – galactosidase enzyme and turns the colour of replica of colonies on replica disc into pink to purple colour (fig. 2.) _Enterococci_ and _Klebsiella, Enterobacter_ and _Serratia_ group produce β – glucosidase and as a result of hydrolysis of glucoside, a chromogenic substrate, impart blue colour on replica disc (Fig. 1). [26] We found the results using replica disc for detection of uropathogens quite satisfactory. We isolated 197 (47.25%) _Escherichia coli_, followed by 88 (21%) _Enterococcus faecalis_, 63 (15.03%) _Klebsiella pneumoniae_ and 45 (10.73%) _Pseudomonas aeruginosa_ in pure growth from urine samples of UTI cases (Table 1a). Nicolle in his work found that 80 – 85% isolates from urine samples of urinary tract infection is Escherichia coli. [27] We isolated 15 (3.57%) mixed growth from urine samples of suspected cases of UTI. Lakshmi et al. [22] found 4% mixed growth in their study. Out of 15 mixed growths, 7 (46.66%) were of _Escherichia coli_ & _Enterococcus faecalis_, 5 (33.33%) _Escherichia coli_ & _Klebsiella_

pneumoniae and 3 (20%) *Escherichia coli* & *Pseudomonas aeruginosa* (Table 1b). Use of chromogenic disc also enabled us to identify mixed growth in urine samples easily by distinct colour change. This easy identification of mixed growth in urine by using chromogenic system was also observed by other researchers [21,22].

All 198 *Escherichia coli* isolates were identified by conventional method but with replica method, only 197 isolates of the same could be identified. One of the isolate of *Escherichia coli* was identified by conventional method but showed cream coloured colony on disc of replica system (Table 2). The absence of β – galactosidase activity (change of colour) of this particular *Escherichia coli* strain in our study was comparable with the studies of other researchers [28].

We identified 88 isolates of *Enterococcus faecalis* in urine samples by both conventional as well as replica method correctly. Hundred percent identification was also achieved in case of *Klebsiella pneumoniae* (63), *Pseudomonas aeruginosa* (45) and mixed growth (15), by both conventional and replica method.

Many researchers reported about usefulness of chromogenic medium for identification of uropathogens in terms of low expenses, less workload and easy identification in mixed culture. Besides, chromogenic media for uropathogens do not contain any antibiotic to make it selective. So this can be used as primary culture media. Yarbrough et al recommended that ChromID CPS Elite agar could be used as an alternative to conventional media for identification of uropathogens [29].

However, some observed that, there is no time- difference of reporting with use of chromogenic media and the conventional one. Besides, a small group of workers reported erroneous identification of *Citrobacter spp* as *Escherichia coli* [7,30].

Newly emerging diagnostic methods including biosensors, microfluidics, and other integrated platforms could improve diagnosis of UTI via direct detection of uropathogens from urine samples, rapid antimicrobial sensitivity testing, and point-of-care testing [31].

5. CONCLUSION

The advanced diagnostic tools, like molecular methods, might not be affordable to all and needs technical skill to perform. Conventional method gives reliable result but time- consuming and workload is more. On the other hand, chromogenic replica method is easy to perform, reliable, cost effective and even can be adopted in resource limited setting. This can also help in improvement of antibiotic stewardship. All our isolates from cases of UTIs were correctly identified by conventional methods, as well as chromogenic system using replica discs, except one. One of the isolate of *Escherichia coli* could not be identified by replica system because of absence of β – galactosidase activity in that particular strain. Use of chromogenic replica disc minimizes workload of a laboratory and helps treating clinicians to initiate the treatment with right drug and the right dose, at the right time, to the right patient.

CONSENT

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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