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Utility of Chromogenic Disc Using Replication System versus Conventional Method for Detection of Uropathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate utility of replication system using chromogenic disc for detection of uropathogens and to compare the result with conventional method for the same.
Design: A total of 625 urine samples were processed from suspected cases of urinary tract infection, admitted in a rural medical college of Maharashtra.
Methodology: The culture isolates of uropathogens were identified by both conventional method and by chromogenic disc using replica system.
Results: Out of 625 urine specimens, 419 (67.04%) were Culture positive. There was growth of *Escherichia coli* 197(99.49%), *Enterococcus faecalis* 88 (21%), *Klebsiella pneumoniae* 63 (15.03%) and *Pseudomonas aeruginosa* 10.73%. There was mixed growth of organisms 15(3.57%) in urine specimens. All uropathogens isolates were identified correctly by conventional as well as chromogenic disc using replica system, except one. One of the *Escherichia coli* isolate was identified by conventional methods but with replica system it showed colourless colonies instead of purple colonies.

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Conclusion: Replica system is a rapid, cost effective and easy method for detection of uropathogens with satisfactory result. It can be adopted in clinical microbiology laboratory for presumptive diagnosis of uropathogens.

Keywords: Chromogenic disc; replica system; uropathogens; conventional method; β- galactosidase.

1. INTRODUCTION

Urinary Tract Infections (UTIs) is a second most common infection in a health care facility after surgical site infection [1]. Annual global incidence of UTIs is at least 250 millions [2,3]. Most of the isolated microorganisms from clinical samples of hospitalized patients are multidrug resistant. Therefore, rapid identification of the pathogens is the need of the hour. It also helps the clinicians to choose appropriate antibiotics, prior to the results of antibiotic susceptibility test [1].

Rapid detection of uropathogens by routine inoculation and isolation followed by replication and direct identification using a new chromogenic disc is a user friendly and cost effective method. In contrast to the conventional method, this chromogenic replication method minimise time and labour of laboratory workers. A battery of biochemical tests for confirmation of uropathogens can be avoided by this method. The method using chromogenic disc for detection of uropathogens is relatively new. It offers detection of both Gram-positive and Gram-negative bacteria by distinct colour change. This method can also easily and rapidly identifies polymicrobial infections and reduces workload. It does not require any special training. The organisms secrete specific enzymes and an appropriate chromogenic substrate incorporated in the replica disc produce this colour [4].

Aim of this study is to access the utility of chromogenic disc for detection of uropathogens and to compare the result of this method with conventional methods.

2. MATERIALS AND METHODS

This study was undertaken to compare the utility of conventional method with chromogenic replication system for detection of uropathogens. There are many reports on use of chromogenic agar media for detection of uropathogens but non so far has reported utility of chromogenic replication system for the same.

Patients' clinical details were obtained from medical records and from attending clinicians. This work was done with the bacteria isolated in the microbiology laboratory.

2.1 Study Area

This study was carried out in a tertiary care rural hospital situated in western Maharashtra, India.

2.2 Study Period

6 months.

2.3 Study Population

The clinically suspected cases of urinary tract infections admitted in this hospital were included in the study. The criteria for inclusion of patients having UTI were following: >38ºC temperature, urgency, suprapubic tenderness, presence of more than 3 pus cells/ high power field in urine samples, > 10^5 colony forming unit/ml of centrifuged urine and isolation of not more than two organisms on culture [4].

2.4 Sample Collection

Urine samples were collected from the suspected cases of UTIs. Either clean catch mid stream urine samples or from sampling port of indwelling catheter with a sterile syringe and needle were collected [4].

The urine samples were inoculated on Nutrient agar, MacConkey's agar and blood agar. The inoculated plates were incubated aerobically at 37ºC for 18 – 24 hours. The significant bacteriuria is detected by counting 100 colonies, which is approximately equal to 10^5colony 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 also performed for non-fermenters. *Staphylococcus aureus* and coagulase negative *Staphylococci* were identified by Gram staining, haemolysis on blood agar, catalase and coagulase tests. *Streptococci* were

identified by haemolysis on blood agar, PYR test, bacitracin sensitivity, bile solubility and aesculin hydrolysis test [5,6,7]. All the media and biochemicals were procured from Hi Media Laboratories, India.

For replication and identification of uropathogens, Hi Detect UTI identification discs were used, obtained from Hi Media Laboratories, India. 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. Then the disc was put in a sterile petri dish and incubated at 35-37ºC 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 analyzed 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%) and the least isolate *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 isolate 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%)

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

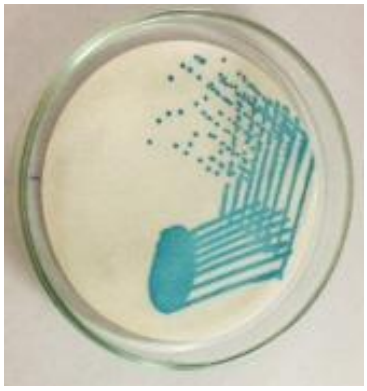

Fig. 1. Replica of small, blue colonies of *Enterococcus faecalis* on replica disc

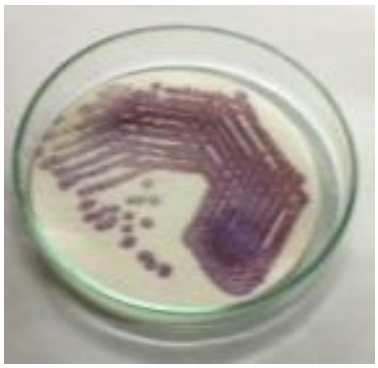

Fig. 2. Replica of purple, low convex colonies of *Escherichia coli* on replica disc

4. DISCUSSION

Emergence of multidrug resistant uropathogens, especially in-hospitalized patients need rapid identification of etiological agents. That helps the clinicians to treat the patients with appropriate antibiotics. Several chromogenic media have been developed recently for rapid identification of uropathogens. 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 [8,9]. 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 [10]. Other workers found that it reduces media required for identification [11] and helps in faster detection of uropathogens from mixed culture [12,13]. This was very useful for resource limited setting [14,15,16]. 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). [17] 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. [18] We isolated 15 (3.57%) mixed growth from urine samples of suspected cases of UTI. Lakshmi et al. [13] 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 [12,13].

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 [19].

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.

5. CONCLUSION

All our isolates from cases of UTIs were identified by conventional methodas 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 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. This also minimizes workload of a laboratory and helps treating clinicians to initiate the treatment with appropriate antimicrobials without further delay.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was obtained from the institutional ethical committee for this research work.

COMPETING INTERESTS

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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