

Comparative Study Between Different Types of Media used for the Isolation of Uropathogens with Special Reference to *E.coli*

Yashab Kumar, Lata Kunj and Pramod W. Ramteke

Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Deemed to be University, Allahabad - 211007, India.

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Escherichia coli is the greatest cause of primary urinary tract infections. The present study aimed Urinary tract infection in female with special reference *Escherichia coli* and its antibiotic susceptibility pattern. A total of 100 urine samples were collected from different hospitals of Allahabad. The samples were cultured on Cystein lysine electrolytes deficiency media, MacConkey's agar, Nutrient agar and Hicrome UTI agar media. Based on the results it was found that different media had different properties and Hicrome UTI agar was the best and convenient media for the isolation of *Escherichia coli* and other uropathogens. Further it was also observed that *Escherichia coli* was the most common organism for UTI in female.

Keywords: Urinary tract infection, Female, *Escherichia coli*, Nutrient agar, MacConkey's agar, Cystein lysine electrolytes deficiency, Hicrome UTI agar.

Urinary tract infections are among the most common bacterial infections that lead patients to seek medical attention and it has large socio-economic impacts. It is also one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients and can lead to significant mortality. (Adedeji and Abdulkadir., 2009). Urinary tract infection (UTI) is an infection of one or more components of the urinary tract. Many infection resolve spontaneously but other can progress to destroy the kidney. Urinary tract is made up of two sections: the lower urinary tract and the upper urinary tract. Lower urinary tract contains the bladder and urethra. Upper urinary tract contains two kidneys and the tube that connects them, called the ureters. Urine does not normally contain

microorganisms. Most urinary tract infections are due to bacteria that enter the opening of the urethra. Bacteria stick to the walls of the urethra, multiplying and moving up the urethra to the bladder. Most UTIs remain in the lower urinary tract where they cause annoying symptoms, such as a burning sensation during urination. These infections are easily treated in most cases. Subjects were considered to have a culture-confirmed urinary tract infection if they had dysuria, frequency, or urgency (or all three) and e^{10^2} colony-forming units of an uropathogen per milliliter of midstream urine (Hooton *et al.*, 1996).

Escherichia coli has been documented as the most important pathogen associated with urinary tract infections. The organisms isolated were *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Proteus mirabilis* (Adedeji and Abdulkadir., 2009). UTI's more commonly progress to bladder infections in

* To whom all correspondence should be addressed.
E-mail: yashab.kumar@shiats.edu.in

females due to the much shorter length of the female urethra. Among the elderly, UTI frequency is roughly equal proportions in women and men.

Risk factors - Women who experience acute UTI are characterized by both a genetic predisposition and behavioral factors. In healthy pre-menopausal women, sexual intercourse, and a history of recurrent UTI have been shown to be strong and independent risk factors for UTIs. Even recent antimicrobial use, by adversely effecting vaginal flora is considered as a potent risk factor (Giamarellou, 2001). Other behavioral factors include: frequency of urination, aspects of personal hygiene. Despite the frequency of acute uncomplicated UTI, there is little long-term morbidity and no evidence for mortality attributable to this problem. These women are not at increased risk of developing hypertension or renal failure. Short-term morbidity due to acute symptoms may however be substantial, especially for women with frequent UTI. Urinary tract infection is categorized as asymptomatic bacteriuria, acute cystitis ,acute pyelonephritis. Manifestations include burning pain on urination after with turbid foul smelling or dark urine, frequency and lower abdominal discomfort (Nahar et al., 2010).

Table 1. Characteristics of *E.coli* on different agar media

Name of the media	Characteristics
Nutrient agar	Opaque, smooth, flat, moist, white color colonies.
CLED	Opaque, yellow color colonies
MacConkey’s agar	Pink color colonies
Hicrome UTI agar	Magenta color colonies



Plate 1. Growth on nutrient agar plate

In the community, bacterial infection of the urinary tract is one of the common causes for seeking medical attention. Although most UTIs are painful nuisance. The present study is undertaken with an aim to study the effect of media on isolation and identification of uropathogens from female patients attending hospital and clinics.

MATERIALS AND METHODS

100 urine samples, preferably mid stream urine were collected from local hospitals and pathology center in sterile vials from the district Allahabad, Uttar Pradesh. The samples were stored at 4°C for 24 – 48 hrs. The diagnosis of UTI is based on the microscopic findings of more than 10 pus cells/high power field (40X) in urine.

Isolation and Identification of *E.coli*

Freshly voided midstream urine was inoculated with calibrated loop on to different agar plates, and the plates were incubated at 37°C for 24 to 48hrs. Isolates were further identified by growth characteristics on selective and specific media, microscopic and biochemical tests.(Holt et al.,1994)

RESULTS AND DISCUSSION

Isolation and Identification

Morphological characteristics

100 urine samples were taken. Mid stream urine were cultured on Nutrient Agar, MacConkey’s Agar, Cystine-Lactose-Electrolyte-Deficient, HiCrome UTI Agar media. One loop full of the culture was streaked on different media and colonies on the media were obtained.(tab.No.1)

From the study it was observed that the growth of organism over the media was according



Plate 2. Growth on CLED agar plate

to the characteristics of the media. Mixed cultures were obtained on Nutrient agar, MacConkey's agar, and CLED agar. On MacConkey's agar lactose fermenting organism grows which gives pink to red color colonies. But the organism obtained from the Hicrome UTI agar media was of different colors. *E.coli* gives magenta color colony, *Klebsella spp*

blue to purple, mucoid etc. Chromogenic media is better media than CLED, MacConkey,s agar and Nutrient agar.

The present findings were in concordance with the findings of (Aspevall *et al.*, 2002) observed that the chromogenic media tested in this study was slightly better than CLED agar



Plate 3. Growth on MacConkey's agar plate

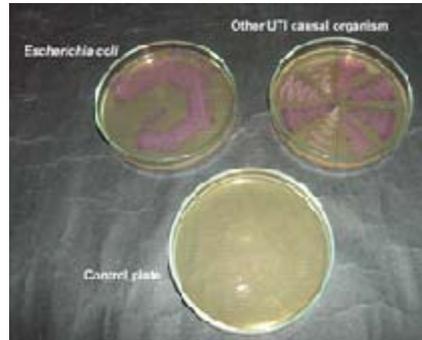


Plate 4. Growth on Hicrome UTI agar plate



Plate 5. Indole +ve, MR +ve, VP -ve, Citrate utilization -ve for *Escherichia coli*

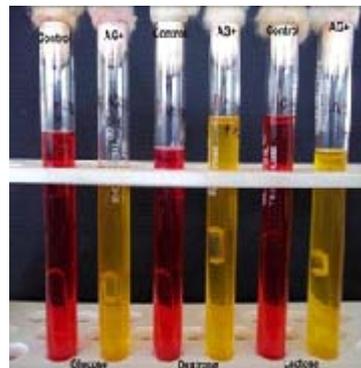


Plate 8. Glucose AG⁺, Dextrose AG⁺, Lactose AG⁺

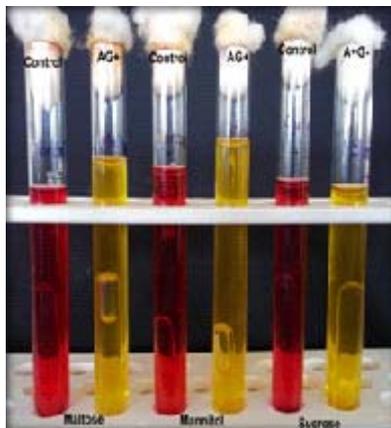


Plate 9. Maltose AG⁺, Mannitol AG⁺, Sucrose A⁺G⁺



Plate 6. Motility test

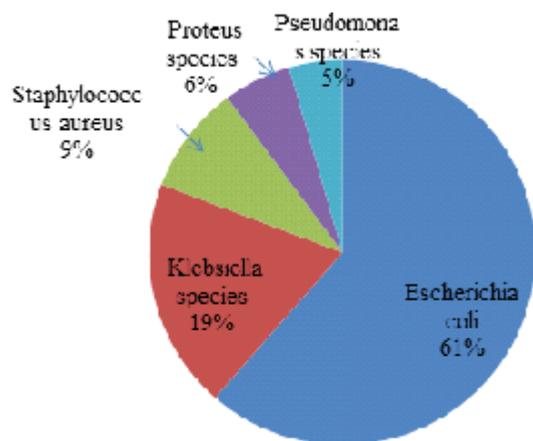


Fig. 1. Percentage of different organism isolated from urine samples

and MacConkey agar. Similar observation was also reported by (Fallon *et al.*, 2003) that use of BBL CHROMagar, UTI medium, or CPS ID2 chromogenic agar as a replacement for cystine lactose electrolyte deficient agar would improve the detection rate of contaminated urine samples. "A cost comparison of the agars suggests that as the use of chromogenic agar in laboratories increases, the purchase cost is decreasing" (Fallon *et al.*, 2003).

The overall impression of the color changes produced on chromogenic media by *E. coli* (purple – magenta), *Klebsiella spp.*, (blue) and the *Pseudomonas spp.* (colourless) was that they were distinct and easy to perceive. In the study gram positive bacteria were also isolated as one chromogenic substrate is cleaved by β -glucosidase possessed by *Enterococci* resulting in formation of bluish green colonies and *S.aureus* gives golden yellow color colonies. The result was in comparable with the result of (Aspevall *et al.*, 2002). that chromogenic agars effectively supported the growth of gram-negative uropathogens but did not consistently support the growth of gram-positive and fungal uropathogens.

Cultural Characteristics

Cultural characteristics were observed by gram staining method. The cells observed were Gram negative, rod shaped bacteria. Similar result was reported by (Gaafar *et al.*, 2006) *E. coli* are rapidly growing, Gram-negative, rod-shaped cells

Table 4. Mean values of number of colonies on different media

Nutrient Agar media	CLED	MacConkey's Agar media	Hicrome UTI Agar media
25.11	12.53	11.53	10.81

measuring approximately 0.5×2 mm length. Further organisms other than glucose-fermentative and nonfermentative gram-negative rods were identified provisionally by their appearance on Gram stains and colonial morphology on 5% sheep blood agar; identification was confirmed by standard methods (Kodaka *et al.*, 1995).

Biochemical Characteristics

Biochemical characteristics were observed by various biochemical tests which were routinely done for identification of culture.

Similar work was done by (Jha *et al.*, 2009) and (Islam *et al.*, 2010). Biochemical tests were carried out on both reference *E.coli* and isolated strain to compare the result and to confirm the identity of the isolated organism. The tests were urease, oxidase, indole, catalase, TSI agar.

Incidence of uropathogens

Out of 100 samples 88 were found positive. The organism isolated were *Escherichia coli* (61%), *Klebsiella spp.* (19%), *S.aureus* (9%), *Proteus species* (6%), *Pseudomonas species* (4%). (Fig1). Similar finding were reported by (Bashar *et al.*, 2009) which showed that 67% were *Escherichia coli*, 11% *Streptococcus faecalis*, 8% *Pseudomonas aeruginosa*, 2% *Enterococcus faecalis*, 4% *Staphylococcus aureus*, 2% *Hafnia alvei*, 2% *Citrobacter freundii*, 2% *Klebsiella pneumonia* and 2% *Yersinia enterocolitica*. Gram negative isolates maximum were *Escherichia coli* 1937 (41.3%) followed by *Klebsiella species* 622 (15.8%) *Pseudomonas species* 534 (11.4%) *Enterobacter species* 376 (8%) and *Proteus species* 245 (6.2%). Amongst gram positive isolates maximum were *Staphylococcus aureus* 361 (7.8%) followed by *Enterococcus species* 278 (5.9%) (Sonavane *et al.*, 2008).

In the present study, *E. coli* (61%) was the predominant bacterial pathogen followed by *Klebsiella* species, non-lactose fermenters, *Pseudomonas* species and others. Certain strains

of *E. coli* have the ability to persist and predominate in the human intestine, making them more likely than other strains to cause UTI. In contrast to this finding, one study from Tamil Nadu showed *Klebsiella* and *E. coli* as the commonest isolate followed by *P. aeruginosa* and *S. aureus*. This may be due to the fact that the *Klebsiella* is one of the most important organisms causing nosocomial infection (Ramesh *et al.*, 2008).

Comparison between different types of media used for the isolation of uropathogens

The analysis of the data obtained from Nutrient agar with CLED, MacConkey's agar, HiCrome UTI agar for the detection of dependence of tables 4.at 5% level of significance were tested. It was observed that N.A, CLED, MacConkey's agar, Hicrom UTI agar gave significant result when tested for dependence between the media using t-test for paired samples. Result indicated that the growth pattern of the uropathogens were different. It could be due to the different constituents and properties of the media.

The result was similar to the result of Scarparo *et al.* (2002) that urine specimens were considered positive and clinically significant. In all cultures of the group, the isolates developed a significant colony count. But no significant difference was observed in colony count while comparing both the media.

Number of specimens showing mixed growth were same for both media but was easily appreciated on CUM because of variation in color was reported (Qaiser *et al.*, 2011).

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