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**Research Article**

# Laboratory diagnosis of urinary tract infections using diagnostics tests in adult patients

**Akmal Hasan SK[1]*, Naveen Kumar T[2], Radha Kishan N[3], Neetha K[3]**

[1]Department of Medical Microbiology, Mamata Medical College, Khammam, Andhra Pradesh, India
[2]Department of Pharmacology, Apollo Institute of Medical Sciences and Research, Hyderabad, Andhra Pradesh, India
[3]Department of Biochemistry, Apollo Institute of Medical Sciences and Research, Hyderabad, Andhra Pradesh, India

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***Correspondence:**
Dr. Akmal Hasan SK,
E-mail: akmal300@gmail.com



## ABSTRACT

**Background:** The primary aim of this study was to evaluate laboratory diagnosis of urinary tract infection using diagnostics tests in adult patients.
**Methods:** Among the diagnostic tests, urinalysis is useful mainly for excluding bacteriuria. For isolation of pathogenic bacteria semiquantitative culture techniques was used and biochemical tests were done to differentiate Gram +ve and Gram –ve bacteria.
**Results:** The incidence of pathogenic infection caused by Escherichia coli accounts for 216 cases (60%) followed by Pseudomonas, Staphylococcus aureus and Klebsiella.
**Conclusion:** Physicians should distinguish urinary tract infections caused by different organisms for an effective treatment and appropriate clinical information gives clues for better diagnostic evaluation and their susceptibility to antimicrobial agents as well addressing host factors that contribute to the occurrence of infection.

**Keywords:** Urinary tract infection, Urinalysis, Urine culture

## INTRODUCTION

Urinary tract infection is a condition where one or more structures in the urinary tract become infected after bacteria overcome its strong natural defences. Urinary tract infections (UTI) are an important cause of illness in humans.[1] It is a common medical problem with an unpredictable natural history. Many urinary infections resolve spontaneously, but others can progress to destroy the kidney .The infection process may involve the kidney, renal pelvis, ureters, bladder and urethra along with adjacent structures, such as prostate and epididymis in males. Urinary tract infections are important complications of diabetes, renal disease, renal transplantation and structural neurological abnormalities that interfere with urine flow. In addition urinary tract infections are the leading cause of Gram negative sepsis in hospitalized patients and are the origin for about half of all nosocomial infections caused by urinary catheters.[2]

The incidence of urinary infection is greatly influenced by age, sex and by predisposing factors that impair the defence mechanism that maintain the sterility of the normal urinary tract. Females are more prone to suffer from urinary tract infection because of short urethra and is in close proximity of anus and urethral trauma during intercourse. 20-50% of women have urinary tract infection at some time in their life and a significant number have recurrent infections. Although majority of infections are acute and short lived, they contribute to a significant amount of morbidity and health care expenditure in the population.

The prevalence of bacteriuria in females increases gradually with time to as high as 10% to 20% in elderly women. Result of anatomic and hormonal changes during pregnancy can cause urinary tract infection and can lead to serious complications in both mother and foetus. Studies have shown that incidence of bacteriuria (presence of bacteria in urine) among girls aged 5 to 14 is 1-2%. Infections in children are often hard to recognize because of their variable symptology and the difficulty of obtaining suitable urine samples.

In males urinary tract infections are uncommon, except in first year of life. In male patients over 60 years it is specially related to enlargement of prostate or instrumentation interfering with emptying of bladder.

Normal urine is mostly water, salt (sodium and chloride ions) and urea. The yellow color comes from the pigment urochrome which is left over from bilirubin after red blood cells have been recycled and is sterile, but it is free of bacteria, viruses, and fungi. An infection occurs when microorganisms, usually bacteria from the digestive tract, cling to the opening of the urethra, begin to multiply and travel up to the bladder known as ascending route. For urinary tract infections to occur by the ascending pathway, enteric gram negative bacteria and other microorganism that originate in the gastro intestinal tract must be able to colonize the periurethral area. Once these organisms gain assess to the bladder, they may multiply and then pass up the ureters and kidney.

The etiological agents of community-acquired and hospital-acquired UTIs differ (table-1) and only a limited amount of data has been published regarding changes in the frequency of causative agents among outpatients. Enteric bacteria (in particular, Escherichia coli) have been and remain the most frequent cause of UTI, although there is some evidence that the percentage of UTIs caused by E. coli is decreasing.[3,4]

Significant changes in the causes of nosocomial UTI have been reported since 1980. Bronsema et al. reported that, from 1980 through 1991, the percentage of UTIs caused by E. coli, Proteus species, and Pseudomonas species decreased, whereas the percentage of UTIs caused by yeasts, group B streptococci, and Klebsiella pneumonias increased.[5]

Urinary Tract Infections remain common among outdoor and indoor patients and pose a serious challenge to chemotherapy. Antibiotics are usually given empirically before the laboratory results of urine culture are available. It has been estimated that more than 6 millions out patient's visits and 300,000 hospital stays every year are due to Urinary Tract Infections. Approximately 10 % will have urinary tract infections at some time during their lives.

**Table 1: Percentage distribution of etiological agents of urinary tract infection among patients according to the above references 3 and 4.**

| Pathogen | Percentage with pathogen |
|---|---|
| Escherichia coli | 53 - 72 |
| Coagulase–negative staphylococci | 2 - 7.5 |
| Klebsiella species | 6 - 12 |
| Proteus species | 4 - 6 |
| Enterobacter species | 0.6 - 5.8 |
| Morganella morganii | 3.1 - 4.4 |
| Citrobacter species | 0.1 |
| Enterococcus species | 1.7 - 12 |
| Staphylococcus aureus | 2 |
| Staphylococcus saprophyticus | 0.2 - 2 |
| Pseudomonas species | 0.1 - 4 |
| Candida species | - |
| Other | 3 - 8 |

Bacterial examination of the urine is the major aid to the diagnosis of infection. Clinical symptoms may sometimes be a good initial guide to the presence and site of infection, but many infections are symptomless, and genital infection may mimic infections of the urinary tract. The bacterial laboratory thus plays an important role in the investigations of the patients suspected of urinary tract infection whether or not they have symptoms referable to the tract. Urine culture may form 25-40% of the work in average clinical laboratory. Cultural techniques are employed not only to detect bacteria but also enumerate bacteria in the urine. The organisms are identified by semiquantitative or quantitative cultures and their susceptibility to antimicrobial agents is determined. Resistant organisms are most often associated with infection acquired in hospitals and are cause of complicated urinary tract infections.

## METHODS

A population based prospective study of 800 patients of either sex (M&F), suspected to be suffering from urinary tract infection attending medicine out-patient department (over period of one year ) in Mamata General Hospital Khammam, Andhra Pradesh were included in this study after taking permission from Institutional Review Board. Bacterial pathogens were isolated and identified by conventional techniques (Mackie & McCartney, 14th edition).

### *Specimen collection*

Suprapubic aspiration is the best method to avoid contamination of specimens with bacteria in the distal urethra. This collection method is used infrequently because it is not indicated clinically (except in rare circumstances), it is invasive and uncomfortable, and it requires too much time and too many resources to be

practical. Collection of urine by use of a single catheter (straight catheter technique) is the next-best technique for obtaining urine specimens with minimal contamination, but, again, it is not indicated clinically for most patients because it is too labor intensive and costly for routine use and it is invasive. It has added disadvantages, because the process of inserting a catheter through the urethra can introduce bacteria into the bladder (and thereby cause UTI), and rare complications have been reported.

Most urine specimens are obtained from adult patients via the clean-catch midstream technique. This technique has the following advantages: it is neither invasive nor uncomfortable, it is simple and inexpensive, it can be performed in almost any clinical setting, there is no risk of introducing bacteria into the bladder by catheterization, and there is no risk of complications. Colony counts from urine specimens collected by this method correlate reasonably well with those of specimens collected via suprapubic aspiration or straight catheterization.[6]

The obvious disadvantage of this technique is that the urine sample passes through the distal urethra and can become contaminated with commensal bacteria. Simple procedures that have been developed to decrease the contamination rate include cleansing of skin and mucous membranes adjacent to the urethral orifice before micturition, allowing the first part of the urine stream to pass into the toilet, and collecting urine for culture from the midstream.[7] The clean-catch midstream method is accepted widely and was used for the study.[8]

***Specimen transportation***

Several studies have demonstrated the adverse effect of delays in transportation or processing of urine specimens on their quality.[9-11]

On the basis of the results of these and other similar studies, it is currently recommended that urine specimens be plated within 2 h after collection unless specimens have been refrigerated or kept in a preservative.[6]

Specimen processing: Routine urine cultures should be plated using calibrated loops for the semiquantitative method. This method has the advantage of providing information regarding the number of cfu/mL (colony forming units) as well as providing isolated colonies for identification and susceptibility testing. The types of media used for routine cultures should be limited to blood agar and MacConkey's agar. For urine specimens obtained from outpatients, it is not necessary to routinely inoculate a medium that is selective for gram-positive bacteria, because nearly all UTIs in outpatients are caused by aerobic and facultative gram-negative bacteria (Table 1) .[12,13]

Urine cultures should be incubated overnight at 35°C-37°C in ambient air before being read. There is no added benefit to incubating routine urine cultures for 48 h, provided that specimens are incubated for a full 24 h and that urine specimens containing $<10^4$ uropathogens or specimens from patients with suspected funguria are incubated for 48 h.[14,15]

Most pathogenic yeasts grow well on blood agar plates, so it is unnecessary to use selective fungal media for urine cultures, even for samples obtained from patients with suspected funguria. Selective fungal media can be used in those rare instances in which there is a high clinical probability that a UTI is caused by a more fastidious yeast or mold. Urine specimens obtained from patients with suspected mycobacterial UTIs should be processed and plated to the appropriate mycobacterial media.[16]

***Detection of pyuria by urine microscopy***

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes with a hemocytometer, counting leukocytes in urine specimens using Gram staining, or counting leukocytes in a centrifuged specimen. The advantages to urine microscopy are that leukocytes, leukocyte casts, and other cellular elements are observed directly. One disadvantage to urine microscopy is that leukocytes deteriorate quickly in urine that is not fresh or that has not been adequately preserved. In addition, each of these methods has disadvantages that limit its usefulness as a routine test.[17]Leucocytes should be found in number of at least greater as $10^4$/ml before pyuria is established.

***Culture and the laboratory diagnosis of urinary tract infections***

Media used was Blood agar & MacConkey agar to isolate causative organisms from urine specimens. Semi quantitative culture techniques of inoculation by standard loop were done to determine whether it contains potentially pathogenic bacteria in significant numbers to identify it as the infecting organism (significant bacteriuria). In Standard Loop Method an inoculating loop of standard dimensions was used to take up fixed and known volume of uncentrifuged urine and it was spread over a plate of agar culture medium. A nichrome wire of SWG 28 was used to make a circular loop of 1mm internal diameter. It can hold 0.002 ml urine.

The number of colonies were counted and this number was used to calculate the number of viable bacteria per ml of urine by following significant bacteriuria (kass concept).

Total viable bacterial count per ml sample = No of colonies X 2000.

***Kass concept (1957)***

Kass and other investigators have established that in the presence of active infection in the urinary tract the urine will contain 100,000 bacteria (or) more per ml. This level is, therefore, considered to represent significant bacteriuria. Counts of 10,000 bacteria (or) less per ml are due to contamination during voiding and are of no significance.

In semiquantitaive culture techniques media used was Macckoneys agar and blood agar. Macckoneys agar was used to help in differentiating lactose fermenting coliforms (E.coli, Klebsiella and Enterobacter) from non-lactose fermenting colonies (Salmonella and Shigella). Blood agar is an enriched medium used for cultivation of fastidious organisms which fail to grow on nutrient agar.

Biochemical tests used to differentiate gram +ve bacteria and gram –ve bacteria. For gram +ve bacteria catalase test and oxidase test was used and gram –ve organisms were identified by methyl red test, Voges-Proskauer test and citrate utilization test.

## RESULTS

Of the 800 urine suspected cases of urinary tract infection 360 samples showed significant bacterial growth.

**Table 2: Name of the isolates, number of cases and percentage.**

| Name of the isolates | Number of cases | Percentage |
|---|---|---|
| Escherichia coli | 216 | 60 |
| Pseudomonas | 56 | 16 |
| Klebsiella | 13 | 04 |
| Staphylococcus aureus | 34 | 09 |
| *Mixed growth | 41 | 11 |
| Total | 360 | 100 |

*Mixed growth of organisms**:** E. coli & Staphylococcus aureus, E. coli & pseudomonas, E. coli & Proteus, E. coli & Beta hemolytic streptococcus.

**Table 3: Name of the gram negative isolates, number of cases and percentage.**

| Names of the isolate | Number of cases | Percentage |
|---|---|---|
| Escherichia | 216 | 75 |
| Pseudomonas | 56 | 20 |
| Klebsiella | 13 | 05 |
| Total | 285 | 100 |

Gram positive cocci = Staphylococcus aureus: 34 (9% percent)

**Table 4: Interpretation of urine culture results.**

| Probability of contamination, No. of microorganisms isolated. | Quantitation, cfu/mL | Interpretations |
|---|---|---|
| **Low probability**[a] | | |
| 1 | $<10^2$ | Probable contaminant |
| 1 | $\geq 10^2$ | Significant isolate |
| 2 | $<10^2$ for each | Probable contaminants |
| 2 | $\geq 10^2$ for each | Significant isolates |
| 2 | $\geq 10^2$ for 1 | Significant isolate and contaminant |
| ≥3 | $\geq 10^5$ for 1 | Significant isolate and contaminants |
| ≥3 | $\geq 10^5$ for each | Probable contaminants |
| **High probability**[b] | | |
| 1 | $<10^2$ | Probable contaminant |
| 1 | $\geq 10^2$ | Significant isolate |
| 2 | $\geq 10^5$ for each | Significant isolate |
| 2 | $\geq 10^5$ for 1 | Significant isolate and contaminant |
| 2 | $<10^5$ for each | Probable contaminant |
| ≥3 | $\geq 10^5$ for 1 | Significant isolate and contaminant |
| ≥3 | $\geq 10^5$ for each | Probable contaminants |

Note: cfu, colony-forming units.

[a]Urine specimens obtained via aspiration ( suprapubic, bladder, ureter, renal pelvis, kidney) or single (straight) catheterization, specimens obtained in the operating room, and urine specimens obtained from patients receiving antimicrobial therapy.

[b]Urine specimens obtained via clean catch technique, from indwelling catheters (urinary or suprapubic), or from nephrostomy tubes, ureterostomy tubes, or ileal loops.

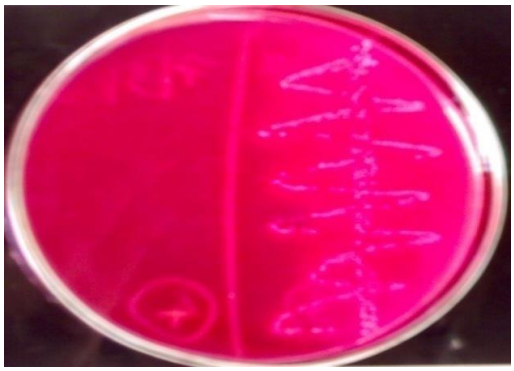

**Figure 1: Mackonkeys agar with lactose fermenting of E. coli.**

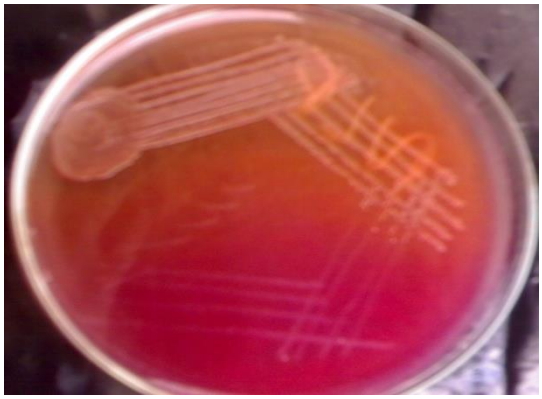

**Figure 2: Mackonkeys agar with non-lactose fermenting colonies of Pseudomonas.**

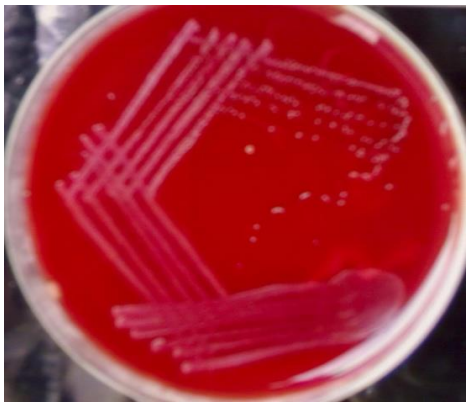

**Figure 3: Blood agar with Staphylococcus aureus colonies.**

**Figure 4: Catalase positive slide of Staphylococcus aureus.**

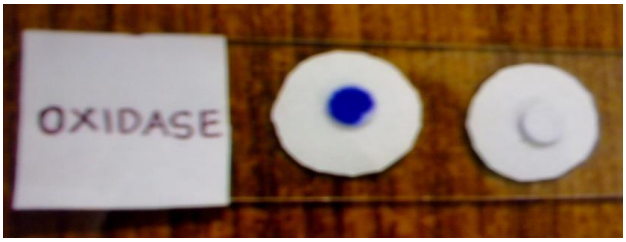


**Figure 5: Oxidase positive slide of pseudomonas.**

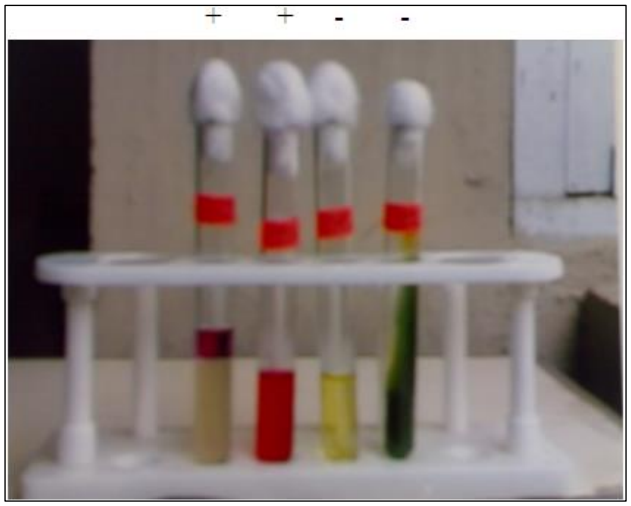


**Figure 6: Indole methyl red Voges-Prosauer and citrate tests of E. coli.**

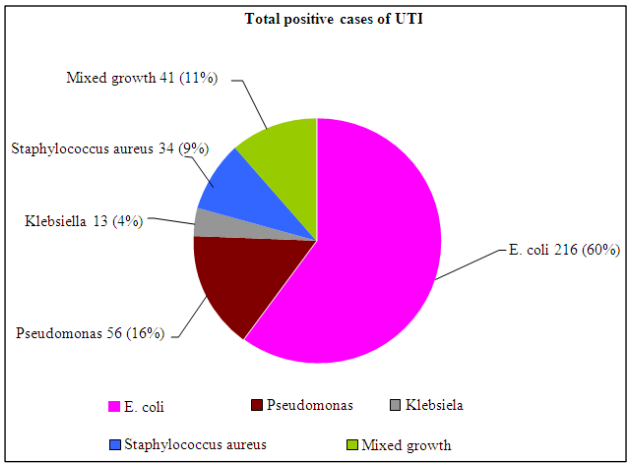


**Figure 7: Demographic comparison of urinary tract infection.**

Total no of Cases = 360

## DISCUSSION

The aim of the present study was to determine the incidence of significant bacterial isolates responsible for causing urinary tract infection by using various culture techniques and bacteriological examination of clean catch midstream urine.

Out of total of 800 urine samples suspected cases of urinary tract infection, positive cases were 360 and negative cases 440. In Urinary tract diagnosed patients(table -2) number of isolates of gram negative organisms are Escherichia coli 216 and percentage 60%, Pseudomonas isolates 56 and percentage 16%, Klebsiella isolates 13 and percentage 04%, Staphylococcus aureus isolates 34 and percentage 09%, mixed growth isolates 41 and percentage 11% (mixed organisms include E coli and Staphylococcus aureus, E. coli and Pseudomonas, E.

coli and Proteus, E. coli and beta haemolytic Streptococci).

Among the positive case (360) gram negative isolates (Table 3) are Escherichia coli, Pseudomonas, Klebsiella and gram positive isolates is Staphylococcus aureus.

Lactose fermenting coliforms (Figure 1 & 2) formed pink coloured colonies with Macconkeys agar and blood agar (Figure 3) showed haemolytic properties of bacteria (Streptococcus pyogenes, Staphylococcus aureus) indicating potentially significant bacteria ($>10^5$/ml). Positive catalase (Figure 4) test produced bubbles and oxidase test (Figure 5) deep purple color indicating gram + ve bacilli growth. Methyl red test showed red color, Voges-Proskauer test (Figure 6) showed pink color and citrate utilization test showed blue color and streak of growth indicating growth of gram –ve bacilli.

Microbiologists (Table 4) need to interpret the microbiologic relevance of growth on culture plates to determine whether further identification and antimicrobial susceptibility testing are necessary. Most culture results can be interpreted readily; no growth and gross contamination are both unambiguous results, as are pure cultures of common pathogens growing in a quantity of $>10^5$ cfu per milliliter of urine. The interpretation of cultures that yield pure growth in lower quantities is also clear for specimens obtained via suprapubic aspiration or straight catheterization. On the other hand, interpretation of urine cultures that yield mixed flora in varying quantities can be difficult. Although a number of algorithms have been developed to guide the interpretation of urine cultures, the large number of potential combinations of microorganisms in varying quantities and the need to correlate these results with different types of UTIs limits the usefulness of any algorithm.

Antimicrobial susceptibility testing: Each laboratory should have guidelines by which pathogens are tested for antimicrobial susceptibility. These guidelines should be developed and antimicrobial susceptibility tests should be performed and reported according to the most recent version of the NCCLS guidelines (National Committee for Clinical Laboratory Standards).

Bacterial or fungal isolates of uncertain clinical importance should not be tested for antimicrobial susceptibility for purposes of routine patient care.

Correct processing and handling of urine specimens, as well as correct interpretation of test results, is dependent on the method used to collect the specimen. It is therefore, of obvious importance for clinicians to specify the method of collection on the test requisition slip. Other information that should be included on the test requisition slip includes the date and time of specimen collection, patient demographic information and any clinically relevant information (e.g., whether the patient was treated with antimicrobial agents or whether anatomic abnormalities, stones, or an indwelling urinary catheter were present).

## CONCLUSION

Most patients with uncomplicated acute cystitis have cases that are clinically straightforward, and they may not require any laboratory testing beyond urinalysis. For a significant number of patients, however, the clinical history and physical findings alone may be insufficient to make a definitive diagnosis of UTI. For those patients and for patients with complicated UTIs, laboratory tests are necessary to make the diagnosis and to provide specific information regarding the identity and the antimicrobial susceptibility pattern of pathogens. Both the laboratory diagnosis and the clinical diagnosis of laboratory test results must be made in light of the method of collection used; clinicians should specify the method of collection on test requisition forms. Of the available laboratory tests, urinalysis is helpful primarily as a means of excluding bacteriuria, but it not a surrogate for culture. Although cultures identify pathogens, the accurate interpretation of culture results requires clinical information that is usually available only to the clinician. We hope that infectious diseases physicians, in particular, will understand both the strengths and the limitations of the laboratory-based diagnostic studies for UTIs that have been reviewed in this article and we hope that they will incorporate this understanding with current treatment guidelines to optimize patient care.

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