Original Research Article

To Study the Microbial Agents, Including Bacteria and Yeasts Responsible for UTI in western Uttar Pradesh Population India

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A B S T R A C T

Urinary tract infection (UTI) is a very common infection both in the community and hospital patients. UTI's rank second only to respiratory infections in their incidence. Common etiological agents are Bacteria including most common Escherichia Coli, fungus Candida. Aim is to Study the Microbial Agents, Including Bacteria & Yeasts Responsible for UTI in Patient attending Both OPD and IPD at Santosh Medical College and Hospital Ghaziabad. To Study the Antimicrobial Sensitivity Patterns of bacterial Uropathogens. A total of 300 patients were included for the present study. Their age group ranged from 15 to 45 years of age. Adopted for identification Sample collection, Urine Analysis Wet mount preparation GRAM STAINING, isolation on CLED AGAR, BLOOD AGAR, method and biochemical reaction was done. The most common isolated organism is *E. coli* and *Candida* was isolated. 15%.

Introduction

Urinary tract infection (UTI) is a very common infection both in the community and hospital patients and ranks high amongst the most common reasons that compel a patient to seek medical attention (Gastmeier *et al.,* 1998).

Acute UTIs are associated with substantial morbidity and problems of recurrent infections. Studies have shown that approximately 25 % of women with first UTI will have another attack within 6 Months. A mounting of body of evidence indicates that UTI is responsible for a large proportion of antibiotic consumption in and out of the hospital. No wonder that the infection has a large socio – economic impact and contributes to the emergence of antibiotic resistance in the hospital and the community (Magee *et al.,* 1996).

Urinary tract infection (UTI) is a very common infection both in the community and hospital patients and ranks high amongst the most common reasons that compel a patient to seek medical attention Acute UTIs are associated with substantial morbidity and problems of recurrent infections (Foxman, 1990).

Several studies in the past have shown that *Escherichia coli* is the most common etiological agent of the UTI in hospital and community patients and that hospital-

acquired UTI, in particular, is characteristically associated with a higher prevalence of enterococci and coagulase negative *Staphylococci* (CNS) (Jones *et al.,* 1999).

It was observed that out of 2,379 uropathogens *Escherichia coli* (66.92%) was the most prevalent isolate followed by *Klebsiella* spp. (13.45%), *Proteus* spp. (6.77%) and *Pseudomonas* spp. (6.77%) (Jadav *et al.,* 2004). To optimize the use of empiric antibiotic therapy for UTI, it is important for clinicians to have a working knowledge of the etiological agents and the susceptibility patterns of UTI pathogens in their populations (Vorland *et al.,* 1985).

Since uropathogens and their susceptibility may vary from time to time and from one institution to another, each hospital or institution must have its own evaluation for such infection so that any change in the causative agents and susceptibility pattern can be detected and managed accordingly (Gupta *et al.,* 1999).

Materials and Methods

Urine samples were collected from patients attending the Outpatient Department and admitted in department of general medicine at Santosh Medical College and Hospital, Ghaziabad urine samples from patient showing presumptive symptoms of UTI were collected and processed.

Exclusion criteria

Cases with hypertension

Cases with tuberculosis

Cases with sexually transmitted disease and HIV

Inclusion criteria

Patient of all age groups and both sexes.

Symptomatic patient suspecting urinary tract infection.

Catheterized patient.

Sample collection

Clean-Catch sample to obtain an untainted urine sample midstream (MSU), or clean-catch, urine sample. To provide this, the following steps are taken: Patients must first wash their hands thoroughly, then wash the penis or vulva and surrounding area four times, with front-to-back strokes, using a new soapy sponge each time.

The patient must then begin urinating into the toilet and stop after a few ounces.

The patient then positions the container to catch the middle portion of the stream. Ideally, this urine will contain only the bacteria and other evidence of the urinary tract infection.

The patient then urinates the remainder into the toilet.

The patient securely screws the container cap in place without touching the inside of the rim.

Interpretation

Gram-Negative bacteria – *E. coli, Salmonella, Pseudomonas, Moraxella, Helicobacter, Stenotrophomonas, Legionella Hemophilus influenzae, Neisseria Meningitidis, Moraxella Catarrhalis, Neisseria, Gonorrhoeae, Acinetobacter baumanni* Gram positive bacteria - *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Bacillus*, *Corynebacterium*, *Nocardia*, *Clostridium*, *Actinobacteria*, and *Listeria*.

KOH

The potassium hydroxide test is a procedure in which potassium hydroxide (KOH) is used to detect fungi by dissolving human cells in a culture.

The difference in cell wall composition of human cells and fungal cells allows this procedure to help distinguish the two cells. KOH denatures the proteins in the human cell; only the fungal cells remain to be seen under the microscope.

KOH test procedure:

Take scraping from margin (not center) of the lesion

Place on a clean slide

Add 2-3 drops of 10% KOH in water

Warm the slide (don't boil)

Optional - add 1 drop of lactophenol cotton blue

Add cover slip

Examine immediately under high, dry magnification with light microscope

Culture

Semiquantitative culture. A semiquantitative plate counts as described by Barry *et al.,* was used as the reference method.

The method followed is as follows

Mix the urine sample thoroughly and remove the top of the container.

Flame a calibrated wire inoculating loop (inside *diameter* of 4 mm) and allow it to cool without touching any surface. Insert this sterile loop in the urine sample vertically and allows urine to adhere to the loop.

Spread the loopful of urine over the CLED AGAR plate using standard method.

In the similar way, collect a second loopful specimen and inoculate a blood agar plate.

3rd loopful is put on the SDA culture slope

Incubate the plates aerobically at 35-37oc for at least 24 hours. Count the colonies and count the cfu s (colony forming unit) by multiplying with 100 (since 0.01 ml loop was used, *(Hasman et al.,* 2005).

Interpretation: clean voided specimen

Normal: <10,000 organisms per ml
Urinary tract infection

Boys

Organisms >10,000 per ml suggests UTI likely

Girls

Organisms >100,000 per ml suggests UTI

Three specimens: 95% probability of UTI
Two specimens: 90% probability of UTI
One specimen: 80% probability of UTI

Organisms >10,000 per ml needs repeat urine culture

Interpretation: transurethral catheterization

Normal: <1,000 organisms per ml
Urinary Tract Infection

Organisms >10,000 per ml suggests UTI

Organisms >1,000 per ml needs repeat urine culture

Interpretation: suprapubic aspiration in infants

Gram Negative Bacilli: any number suggests UTI (99%)

Gram Positive Cocci: >1-2,000 suggests UTI (99%)

CLED AGAR

Principle

CLED AGAR is a non-selective differential plating medium for the growth and enumeration of urinary tract microorganisms. Omitting sodium chloride inhibits the Proteus swarming and supports the growth of the vast majority of bacteria causing urinary tract infections, and is used to differentiate and identify them.

Procedure

The microorganisms which cause infection in the urinary tract are generally abundant and of only one species. *E. coli* is the organism most frequently isolated. The seeding of the sample can be done by the dilute on method or by streaking on the surface of agar with a calibrated loop. Count the colonies after 24-48 hours of incubation at a temperature of $35 \pm 2°C$. Report the number of colonies per ml of urine. A count of 100.000 (105) /ml or more is an indication of a significant clinical urinary tract infection.

Blood agar

Suspend 40 g in 1 litre of distilled water. Bring to a boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. For blood agar, cool to 45-50°C and add aseptically 6% (5-10% is typically) of sterile defibrinated blood.

Principle and Interpretation

Blood Agar Base formulation has been used as a base for the preparation of blood agar and to support good growth of a wide variety of fastidious microorganisms. Because it is a highly nutritious medium it can also be used as a general purpose growth media without adding blood. For example it was used for studying irradiated Escherichia coli. Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci. Sheep blood gives best results for Group A Streptococci.

SDA colony

Gram stain like all fungal media, SDA contains sources of carbon, nitrogen and vitamins. SDA contains the sugar glucose, for carbon, and neopeptone and polypeptone agar (a solidifying agent) for nitrogen. The finished media has a pH of 5.6, which is mildly acidic.
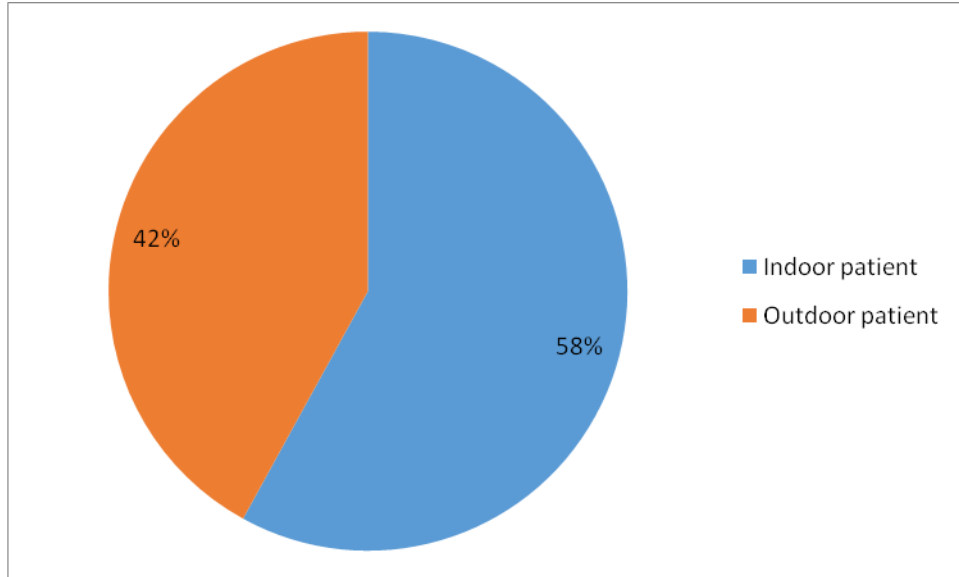
A commercially available form from Conda Pronadisa has a slightly different formulation, using glucose, agar and a mixture of "peptic digest of animal tissue and pancreatic digest of casein." This animal tissue provides the nitrogen and vitamin source that the organisms require.

Candida albicans - Creamy. pasty colony
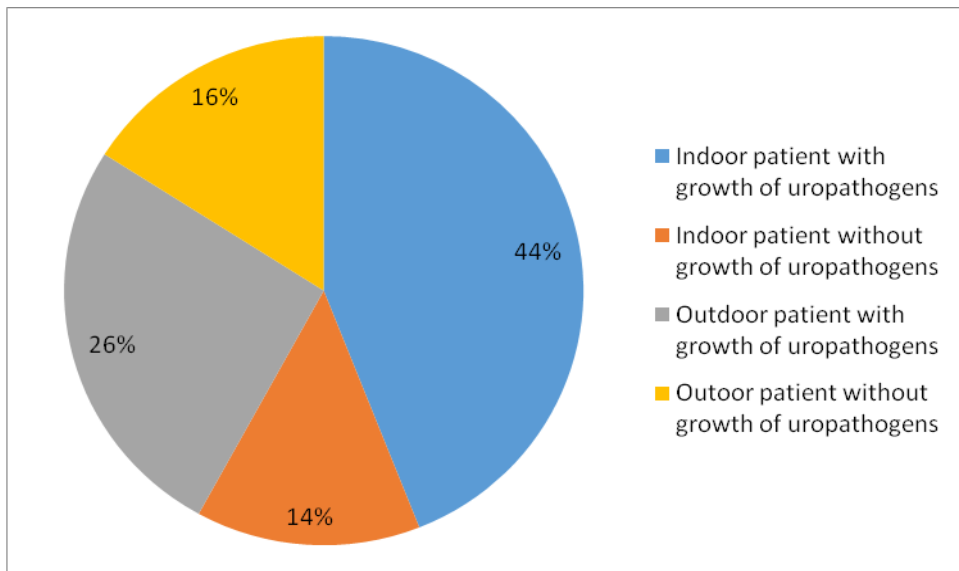
Interpretation of results

Bacteria that ferment any of the three sugars in the medium will produce byproducts.

Chart.1 Proportion of urine samples received in the lab from indoor and outdoor patients
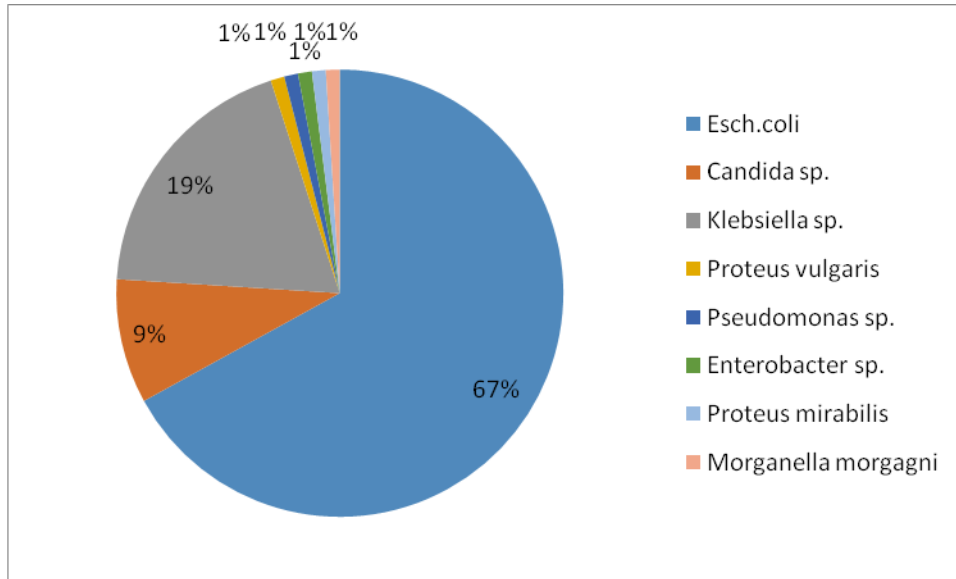

Indoor patient	Outdoor patient
174(58%)	126(42%)

Chart.2 Proportion of urine samples from indoor and outdoor patients with and without growth of pathogen
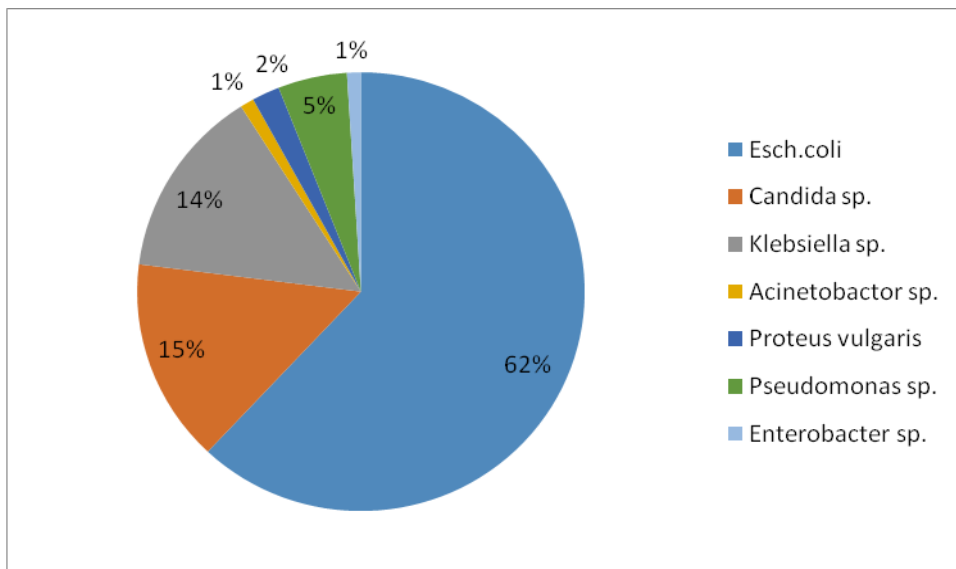

Indoor patient with growth of uropathogens	Indoor patient without growth of uropathogens	Outdoor patient with growth of uropathogens	Outoor patient without growth of uropathogens
(44%)132	42(14%)	78(26%)	48(16%)

Chart.3 Types of uropathogenis organism isolated from outdoor patients

E. coli	*Candida sp.*	*Klebsiella sp.*	*Proteus vulgaris*	*Pseudomonas sp.*	*Enterobacter sp.*	*Proteus mirabilis*	*Morganella morgagni*
(67%) 84	(9%) 11	(19%) 24	(1%) 12	(1%) 12	(1%) 12	(1%) 12	(1%) 12

Chart.4 Types of uropathogenic organisms isolated from indoor patient

E. coli	*Candida sp.*	*Klebsiella sp.*	*Acinetobacter sp.*	*Proteus vulgaris*	*Pseudomonas sp.*	*Enterobacter sp.*
62% (108)	15% (26)	14% (24)	1% (2)	2% (4)	5% (10)	1% (2)

These byproducts are usually acids, which will change the color of the red pH-sensitive dye (phenol red) to a yellow color. Position of the color change distinguishes the acid production associated with glucose fermentation from the acidic byproducts of lactose or sucrose fermentation. Many bacteria that can ferment sugars in the anaerobic butt of the tube are enterobacteria. Some bacteria utilize thiosulfate anion as a terminal electron acceptor, reducing it to sulfide. If this occurs, the newly-formed hydrogen sulfide (H_2S) reacts with ferrous sulfate in the medium to form ferrous sulfide, which is visible as a black precipitate. Examples of sulfide-producing bacteria include *Salmonella, Proteus, Citrobacter* and *Edwardsiella* species.

The blackening of the medium is almost always observed in the butt (bottom) of the medium. All lactose fermenters result in yellow slant/yellow butt (acid/acid reaction), whereas non-lactose fermenters may result in pink/yellow or yellow/yellow (if sucrose is fermented). Blackening of the butt due to H_2S production may mask the acid reaction (yellow) in the butt. *Salmonella enterica* serovar *Typhi* may result in blackening of the medium at the interface of butt and slant.

For yeast

Sabouraud Dextrose AGAR W/ Chloramphenicol Intended use SDA plates were incubated at $26\pm1°C$ to enhance the growth of fungi, and the remaining plates were incubated at $36\pm1°C$ Primary isolation and other two were compared with it.

Results and Discussion

A total of 300 patients were included for the present study. Their age group ranged from 15 to 45 years of age.

Proportion of urine samples received in the laboratory from indoor pt. was 174 (58%) and outdoor pt. was 126 (42%) (chart no-1). Number of indoor patients showing growth of uropathogens were 132 (44%) and outdoor pt with growth of uropathogen were 78(26%) (Chart no-2).

Among out door patients commonest uropathogens isolated was *E. coli* 84 (67%) followed by *Candida* sp. (9%), *Klebsiella* sp. (19%), *Proteus* (1%), *Pseudomonas* sp. (1%), *Enterobacter* sp. (1%), Proteus mirabilis (1%) (Chart No.-3, 4).

A total number of 300 urine samples from OPD and IPD patient of Santosh Medical College and Hospital were processed. Out of these samples 240 urine samples shows growth of uropathogens, highest number was *E. coli* (61.9%), *Klebsiella* (16.1%), and *Candida* (12.3%). that *E. coli* is the most common isolates is well known fact and is evidence form different studies. in a study conducted at Apollo hospital Delhi, India (Bal, 2006) *E. coli* was the most prevalent uropathogen (61%) followed by *Klebsiella* (22%) 56.6% of uropathogens isolates were from indoor patient and rest 43.4% were from outdoor patient. among the patients hospitalized with UTI.

The usual isolation rate of uropathogens in this study used to be 22.7% which is notably decrease than the quotes mentioned from Ethiopia and Nigeria (Biadglegne *et al.,* 2009), (El-Mahmood *et al.,* 2009). However, the charge was higher than different studies (Kashef *et al.,* 2010; Beyene *et al.,* 2011; Demile *et al.,* 2012). *E. coli* was once the most predominant bacterium reported from urine, observed via *Klebsiella* spp., *Proteus spp., Pseudomonas spp.,* CNS, *S. aureus, Enterobacter spp.* and *Citrobacter spp.* The isolation rates of *E. coli* and different pathogens in this study were similar to the prices documented in the past (Demile *et al.,* 2012; De Francesco *et al.,* 2007). Bacterial etiologies of UTI can show geographic versions and may even range over time within a populace (Demile *et al.,* 2012; El-Mahmood *et al.,* 2009).

A substantial percentage of hospitalized patients had nosocomial UTI. *E. coli* I was found to be

the most common urinary isolate both from inpatient and outpatient. There was a high proportion of ESBL production strain among the uropathogenic *E. coli* isolates in the present study.

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