An Analytical Study of *Escherichia coli* from Urine Samples with Special Reference to Their Anti Biotic Sensitivity Pattern

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ABSTRACT

A urinary tract infection is an infection that begins in your urinary system. Urinary system is composed of the kidneys, ureters, bladder and urethra. Though Escherichia Coli, which is normally present in the gastrointestinal tract is the commonest causative organism, other gram negative colonic bacteria. Any part of your urinary system can become infected, but most infections involve the lower urinary tract, the bladder and the urethra. Women are at greater risk of developing a urinary tract infection than are men. A urinary tract infection limited to your bladder can be painful and annoying. However, serious consequences can occur if a urinary tract infection spreads to your kidneys. Antibiotics are the typical treatment for a urinary tract infection like (Norfloxacin, Tetracycline, and Ampicillin etc) .By Antibiotic sensitivity pattern we can measure the Zone of diameter in millimeters. The size of zone of inhibition was interrupted by referring to the CLSI (Clinical Laboratory Standard Institute) guidelines and organism was labeled susceptible, intermediate, or resistance accordingly.

Key words: Urinary Tract Infection, Gram negative Rod shaped, Escherichia species Antibiotic sensitivity pattern, Norfloxacin, Tetracycline, Ampicillin, Zone diameters in millimeters.

INTRODUCTION

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and or need to urinate, pain during urination, and cloudy urine (Urinary Tract Infections 2010).

The main causal agent is Escherichia coli. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it (Adult Health Advisor 2005). When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI.

The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Although they cause discomfort, urinary tract infections can usually be easily treated with a short course of antibiotics with no significant difference between the classes of antibiotics commonly used (Zalmanovici Trestioreanu A, Green H et al: 2010).

The most common organism implicated in UTIs (80–85%) is E.coli, (Nicolle LE 2008) while Staphylococcus saprophyticus is the cause in 5–10%.

The bladder wall, in common with most epithelia is coated with a variety of cationic antimicrobial peptides such as the defensins and cathelicidin which disrupt the integrity of bacterial cell walls. (Ali AS, Townes CL et al: 2009). In addition, there are also mannosylated proteins present, such as Tamm-Horsfall proteins (THP), which interfere with the binding of bacteria to the uroepithelium. As binding is an important factor in establishing pathogenicity for these organisms, its disruption results in reduced capacity for invasion of the tissues. Moreover, the unbound bacteria are more easily removed when voiding. The use of urinary catheters (or other physical trauma) may physically disturb this protective lining, thereby allowing bacteria to invade the exposed epithelium.

During cystitis, Uropathogenic Escherichia coli (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs) (Justice S, Hunstad D et al: 2006). By working together, bacteria in biofilms build themselves into structures that are more firmly anchored in infected cells and are more resistant to immune-system assaults and antibiotic treatments. This is often the cause of chronic urinary tract infections.

A doctor can confirm if you have a urinary tract infection by testing a sample of your urine. For some younger women who are at low risk of complications, the doctor may not order a urine test and may diagnose a urinary tract infection based on the description of symptoms.

Antibiotics are the main treatment for all UTIs. A variety of antibiotics are available, and choices depend on many factors, including whether the infection is complicated or uncomplicated or primary or recurrent. For example, if a woman has symptoms, even if bacterial count is low or normal, infection is probably present, and the doctor should consider antibiotic treatment. The following are measures that studies suggest may reduce the incidence of urinary tract infections. A prolonged course (six months to a year) of low-dose antibiotics (usually nitrofurantoin or TMP/SMX) is effective in reducing the frequency of UTIs in those with recurrent UTIs (Nicolle LE 2008).

Cranberry (juice or capsules) may decrease the incidence of UTI in those with frequent infections. Long-term tolerance, however, is an issue (Barbosa-Cesnik, C, Brown et al: 2011 Jan) Subsequent research has questioned these findings (Raz, Raul, Stamm, Walter E 1993).

For post-menopausal women intravaginal application of topical estrogen cream can prevent recurrent cystitis. Breastfeeding can reduce the risk of UTIs in infants (Warren, John W *et al*: 1999).

Escherichia coli (commonly*) E.coli*; pronounced is a Gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E.coli* stains are harmless, but some, such as serotype 0157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls (Vogt *et al*: 2005). The harmless strains are part of the gut, and can benefit their hosts by producing vitamin K2 (Bentley *et al*: 1982).

E.coli are not always confined to the intestine ,and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination (Fang p *et al*: 2000) and is now classified as part of the Enterobacteriaeae family of gamma-probacteria.

MATERIAL AND METODS

Sample were collected from different wards (Medicine, NICU & OPD) Ganesh Shankar Vidyarthi Memorial Medical College, *Kanpur*. Specimen of urine generally collected in sterile plate universal container. These samples were culture on Luria Bertani agar plate and incubate at 37oc for 18 hours. Plates were ready for presence of any growth. Positive culture processed in a usual manner for identification. Examination of gram stain films and relevant by Biochemical test for the purpose of identification of *Escherichia coli.*

The medium had the following composition (g/L): Peptone-10g; Yeastextract-5g; Nacl-5g; Agar- 20g; pH-7.2.

Biochemical Assays

Biochemical test were performed for the conformation of *Escherichia* species.

IMViC Tests

Enterobacteriaeae: (enteric) are Gram-negative bacteria that grow in the intestinal tract of humans and other animals. The IMViC tests are frequently employed for identification of this group of microbes which includes such microorganisms as *Klebsiella*, *Enterobacter* and *Escherichia coli*. The presence of *E.coli* is used by public health officials as an indicator of fecal contamination of food and water supplies. While Enterobacter and *Klebsiella spp*. Resemble *E.coli* in being lactose fomenters, their presence does not necessarily indicate fecal contamination because they are widespread in soil and grass. The IMViC tests can be used to differentiate these three organisms.

The IMViC series includes four tests

- Indole production.
- Methyl red test.
- Voges-Proskauer test.
- Citrate utilization.

Evaluation of UTI by Antibiotic Sensitivity Pattern

The following isolated samples of *Escherichia coli* were examined in the liquid culture for the Antibiotic sensitivity pattern.

Suspension of an overnight growth culture of *E. coli* was made in normal saline/nutrient broth. Bacterial suspension of *Escherichia coli* was spread on Luria Bertani agar plates with the help of sterile cotton swab uniformly and finally antibiotics were placed at regular distance after 5 minutes.

The plates were incubated at 37°C for 18-24 hours. Zone diameter was measured in millimeters. The size of zone inhibition was interrupted by referring to the CLSI (Clinical Laboratory Standard Institute) guidelines and organism was labeled susceptible, intermediate, or resistance accordingly.

RESULTS AND DISCUSSION

Total no. of isolates from urine samples: 50
No. of isolates of Escherichia coli: 15
Percentage of Escherichia coli: 30%

A total no. of 50 urine samples was processed. Out of these samples, 15 isolates were confirmed as E.coli by Gram's staining and biochemical tests. Rest of these samples showed growth of other microorganism like as *Klebsiella* species, *Staphylococcus* species, *Bacillus* species etc. Out of 15 *E.coli* the isolation rate in urine samples was high in females in comparison to males. All the 15 isolates of *E.coli* had given positive test for Indole, methyl-red test, catalase and for lactose fermenting, and negative biochemical test are Voges-Proskuaer, citrate, Urease, there we confirmed that these organism are *E.coli.*

During our study we observed that most

Table 1:

Bacteria	No. of bacterial isolates	Percentage of bacterial isolates
Escherichia coli	15	30%
Staphylococcus aureus	5	10%
Bacillus subtilis	5	10%
Klebsiella pneumoniae	3	6%
Sterile	10	20%
Contaminated	12	24%

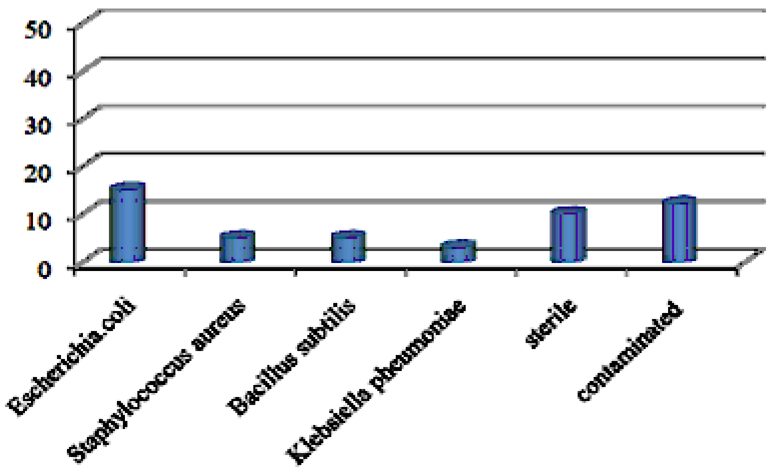
Table 2:

Antibiotics	Sensitve	Moderate Senstive (%)	Resistant (%)
Ampicillin	1 (2%)	7 (14%)	6 (12%)
Penicillin	0 (0%)	4 (8%)	11 (22%)
Tetracycline	11 (22%)	0 (0%)	4 (8%)
Cephalosporin	0 (0%)	5 (10%)	9 (18%)
Norfloxin	9 (18%)	2 (4%)	3 (6%)

of the urine samples were infected by *E.coli*. So they are the major cause of urinary tract infection (UTI). So for the detection of this pathogenic microorganism phenotypic characterization is an inevitable part.

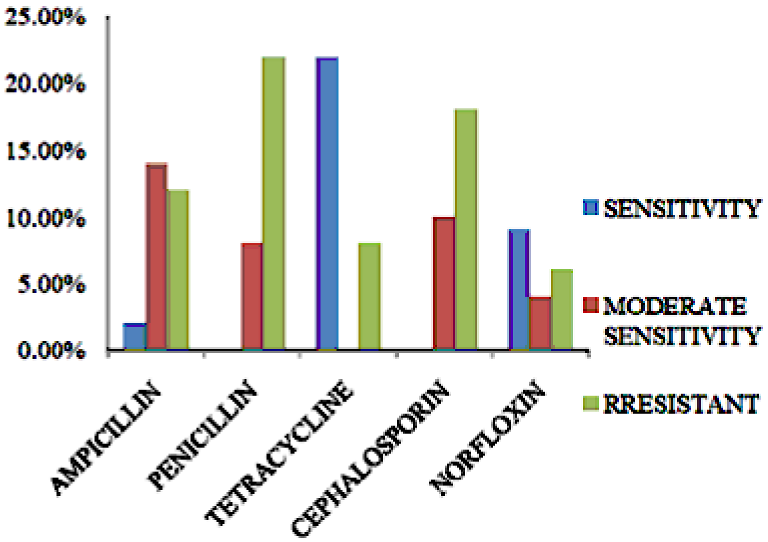
We processed 50 urine samples out of these 15 isolates were confirmed as E.coli. On the Luria Bertani agar plates the colonies were gram negative because it had taken pink stain (i.e. counter stain) and occur Gram negative rods under microscope i.e. Escherichia coli. All the isolates of E.coli had given positive test for the indole, methyl-red, catalase test; there we confirmed that these organisms are *E.coli.*

All the isolates of *E.coli* were tested for antibiotic sensitivity pattern against common drugs like Ampicillin, Tetracycline, Norfloxcin, penicillin, Cephalosporin.



(Bar graph showing bacterial percentage isolated from urine samples)

Fig. 1: Isolation Rate of *Escherichia Coli* and Other Microorganisms Isolated from Urine Samples



(Graph showing antibiotic sensitivity pattern of *E.Coli* isolated from urine samples)

Fig. 1: Antibiotic Susceptibility Pattern (%) of *E.coli* Isolated from Urine Samples

After isolation and confirmation test of the *E.coli* we processed antibiotic sensitivity test. Thus, we concluded that it show high sensitive pattern of common drugs like Tetracycline (22%), Norfloxacin (18%).

It show high Moderate sensitive pattern of common drugs like Ampicillin (14%), Cephalosporin (10%).

It show high Resistant pattern of common drugs like Penicillin (22%), Cephalosporin (18%).

CONCLUSION

In Patients with urological abnormality, *E.coli* with lower virulence can cause infections. Uropathogenic E.coli have virulence properties, that are absent in non-pathogenic *E.coli* we processed 50 samples out of 15 were confirmed as E.coli. In Urology the isolation rate of E.coli was very high in comparison to the other wards *G.S.V.M. Medical College, Kanpur*. Isolates from these patients exhibited greater drug resistance. In community acquired Infections simple antimicrobial drugs Tetracycline, Norfloxacin, Ampicillin might still be useful. Antimicrobial susceptibility patterns varied in isolates from different categories. This need to be considered while developing guidelines for treatment of Urinary Tract Infection which showed high prevalence of antimicrobial resistance among Uropathogens.

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