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Assessment of antibiotic susceptibility pattern of *Escherichia coli* observed in urine samples of UTI Patients

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Abstract
The infection of urinary tract by *Escherichia coli* are important and serious problems in the clinical field. Moreover the bacterial urinary tract infection is the common danger disease. Therefore, the present investigations were undertaken to study the prevalence and antibacterial susceptibilities of *Escherichia coli* isolated from patients with urinary tract infections.

The study was planned in the Department of Microbiology and Pathology under guidance of Prof. (Dr.) Pramukh Bhattacharya in M.G.M Medical College & LSK Hospital Kishanganj. Total 50 patients were found positive were enrolled in the present study. Each sample was mixed well and aseptically inoculated with 4 mm diameter nichrome wire loop on blood agar and Mac Conkey agar plates and incubated at 37o C for 24 hours aerobically. Significant UTI was defined as the presence of >105 colony forming unit (CFU)/ml in the culture. All positive cultures were further identified by their cultural characteristics, Gram stain and battery of biochemical reaction.

The data generated from the present study suggest that *Escherichia coli* isolates from urinary tract infections have showed resistance too many of the routine antibiotics in our study. This information may directly influence the choice of empirical antibiotic therapy for UTI. Multidrug resistant uropathogenic *E. coli* is an expanding public health threat.

Keywords: *E.coli*, Urine, susceptibility, antibiotic, UTI, etc

Introduction

A urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as kidney infection (pyelonephritis). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder. Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI. Rarely the urine may appear bloody. In the very old and the very young, symptoms may be vague or non-specific [1].

The most common cause of infection is *Escherichia coli*, though other bacteria or fungi may rarely be the cause. Risk factors include female anatomy, sexual intercourse, diabetes, obesity, and family history. Although sexual intercourse is a risk factor, UTIs are not classified as sexually transmitted infections (STIs). Kidney infection, if it occurs, usually follows a bladder infection but may also result from a blood-borne infection. Diagnosis in young healthy women can be based on symptoms alone. In those with vague symptoms, diagnosis can be difficult because bacteria may be present without there being an infection. In complicated cases or if treatment fails, a urine culture may be useful [2].

In uncomplicated cases, UTIs are treated with a short course of antibiotics such as nitro Furantoin or trimethoprim/ sulfamethoxazole. Resistance to many of the antibiotics used to treat this condition is increasing. In complicated cases, a longer course or intravenous antibiotics may be needed. If symptoms do not improve in two or three days, further diagnostic testing may be needed. Phenazopyridine may help with symptoms. In those who have bacteria or white blood cells in their urine but have no symptoms, antibiotics are generally not needed, although during pregnancy is an exception. In those with frequent infections, a short course of antibiotics may be taken as soon as symptoms begin or long-term antibiotics may be used as a preventative measure [3].

Uropathogenic *E. coli* from the gut is the cause of 80–85% of community-acquired urinary tract infections, with Staphylococcus saprophyticus being the cause in 5–10%. Rarely they may be due to viral or fungal infections. Healthcare-associated urinary tract infections (mostly related to urinary catheterization) involve a much broader range of pathogens including: *E. coli* (27%), Klebsiella (11%), Pseudomonas (11%), the fungal pathogen Candida albicans (9%), and Enterococcus (7%) among others. Urinary tract infections due to Staphylococcus aureus typically occur secondary to blood-borne infections. Chlamydia trachomatis

and Mycoplasma genitalium can infect the urethra but not the bladder. These infections are usually classified as a urethritis rather than urinary tract infection.

The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, infection may also occur via the blood or lymph. It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy [6]. After gaining entry to the bladder, *E. coli* are able to attach to the bladder wall and form a biofilm that resists the body's immune response [4].

Escherichia coli is the single most common microorganism, followed by Klebsiella and Proteus spp., to cause urinary tract infection. Klebsiella and Proteus spp., are frequently associated with stone disease. The presence of Gram positive bacteria such as Enterococcus and Staphylococcus increased. The increased resistance of urinary pathogens to quinolones has been reported worldwide and might be the consequence of overuse and misuse of quinolones [5].

Escherichia coli also known as *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal microbiota of the gut, and can benefit their hosts by producing vitamin K2, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards [6].

Despite concerning trend in antimicrobial resistance among *E. coli* isolates worldwide, a growing armamentarium of antimicrobial agents provides multiple options for treating *E. coli* infections. In general, monotherapy with trimethoprim sulfamethoxazole, aminoglycosides, cephalosporin or fluoroquinolones is recommended as the treatment of choice for most known infections with *E. coli*, though many broad spectrum agents (such as beta-lactam/beta lactamase inhibitor combinations and the carbapenems) remain highly active.

Pathogenic *E. coli* strains use a multi-step scheme of pathogenesis that is similar to that used by other mucosal pathogens, which consists of colonization of a mucosal site, evasion of host defences, multiplication and host damage. There is an alternate side to *E. coli* afforded through gene gain and loss that enables it to become a highly diverse and adapted pathogen. Pathogenic *E. coli* can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream and central nervous system.

The infection of urinary tract by *Escherichia coli* are important and serious problems in the clinical field. Moreover the bacterial urinary tract infection is the common danger disease. Therefore, the present investigations were undertaken to study the prevalence and antibacterial susceptibilities of *Escherichia coli* isolated from patients with urinary tract infections.

Methodology

The study was planned in the Department of Microbiology and Pathology in M.G.M Medical College & LSK Hospital Kishanganj. Total 50 patients were found positive were enrolled in the present study.

Total 190 sample was examine microscopically, patient with suspected UTI finding was mixed well and aseptically inoculated with 4 mm diameter nichrome wire loop on blood agar and Mac Conkey agar plates and incubated at 37o C for 24 hours aerobically. Significant UTI was defined as the presence of >105 colony forming unit (CFU)/ ml in the culture. All positive cultures were further identified by their cultural characteristics, Gram stain and battery of biochemical reaction.

Antimicrobial susceptibility of *Escherichia coli* isolates was tested by the disk diffusion Kirby Bauer method according to Clinical Laboratory Standards Institute (CLSI) recommendations, using Mueller–Hinton agar. Antimicrobial agents tested were ampicillin, amoxicillin–clavulanicacid, ciprofloxacin, gentamicin, piperacillin tazobactam, cotrimoxazole, imipenem, Nitrofurantoin, cefazolin, cefotaxime, ceftriaxone, amikacin (all disks were chosen as per the CLSI recommendations) [7]. A standard inoculum adjusted to 0.5 Mcfarland was swabbed on the Muller Hinton agar and the antibiotic discs were placed and incubated at 370 C for 24 hours. ATCC reference *E.coli* strain (25922) was used as control.

Following was the inclusion and exclusion criteria for the present study

Inclusion Criteria: Patients with clinical symptoms suggestive of urinary tract infections, who have not received any antibiotic therapy in the previous two weeks.

Exclusion Criteria: The patients with no symptoms suggestive of UTI at the time of sample collection and with history of recent antibiotic usage.

Results & Discussion

Bacterial uropathogens have the potentiality to change tissues of the urinary tract adjacent structures. Early detection and selection of an appropriate effective antimicrobial agent is highly essential for effective management of patients suffering from UTIs to prevent any further complications. Diagnosis and adequate management is only possible by close association between the clinician and microbiologist.

A urine specimen for culture obtained before the initiation of antimicrobial therapy confirms the diagnosis of UTI. The choice of antibiotics depends upon the causative organism and its local expected antibiotic susceptibility pattern. Comparisons of the findings of the present study with those of other recent similar studies indicate similarity.

Antimicrobial resistance is a growing problem and a cause of great concern throughout the world. Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40 % to 50 % of women will suffer from UTI during their lifetime. In the last decades, the number of reports about appearance of bacteria with antibiotic resistance has increased all over the world [8].

Knowledge of antimicrobial resistance trends among isolates of uropathogens is essential to provide clinically appropriate

and cost-effective therapy. Guidelines for the empirical treatment of patients with UTI suggest it is important to consider local resistance patterns of commonly isolated pathogens in selecting the antibiotic agents. This study provides an update on *E. coli*, the main cause of UTI in outpatients. Ampicillin or amoxicillin were once standard therapy for UTI, but resistance of *E. coli* to ampicillin has been on the increase in most regions of the world [9].

Table 1: Demographic details of the patients.

Factors	No. of Cases
Age	
20-35	8
36-45	12
46-55	16
≥56	14
Sex	
Male	18
Female	32
Family history of diabetics	
Yes	41
No	9
Pancreatic disease	
Yes	29
No	21
Weight in kg	
30-40	4
41-50	3
51-60	4
>61	39
Exercise	
Yes	13
No	37
Marital status	
Married	31
Single	8
Widow	11

Table 2: Antibiotic susceptibility pattern of *Escherichia coli* isolates

	No. of Susceptible *E.coli* isolates	No. of resistant isolates
Ampicillin	6	43
Amoxycillinclavulanic acid	8	41
Ciprofloxacin	22	28
Gentamicin	26	14
Cotrimoxazole	15	35
Nitrofurantoin	48	2
Cefazolin	14	36
Cefotaxime	25	24
Piperacillintazobactam	46	3
Imipenem	50	0
Amikacin	44	9
Ceftriaxone	24	23

E. coli, the most common uropthogen isolated more commonly from female patients comparatively to the male patients and isolation of *E. coli* more among female patients. The reason for the higher prevalence of CoNS in males is not clear, though lack of circumcision, receptive anal intercourse and HIV infection recognised as risk factor for males [10]. The study revealed that females (65.3%) were more susceptible to UTI than males (34.7%), which is also similar to other studies [11-12]. The increased incidence of the urinary tract infection in women is conditioned by favouring anatomic factors, by hormonal changes and by the urodynamic disturbance occurring with age [13].

Multiple antibiotic resistance (MAR) index is a tool that reveals the spread of bacterial resistance in a given population [14]. An MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used [15]. The MAR indices of *E. coli* obtained in this study is a possible indication that a very large proportion of the bacterial isolates have been exposed to several antibiotics. Higher resistance rate to all antibiotics used in this study except nitrofurantoin may be explained by high and uncontrolled use of these antibiotics in our institutions. Most *E. coli* isolates are highly resistant to commonly prescribed antibiotics (ampicillin, cephalexin, quinolones and co-trimoxazole), but are still susceptible to nitrofurantoin which should be considered as preferred therapeutic agent once the organism is identified.

Bacterial biofilms are associated with long-term persistence of the organisms in various environments. Biofilms make the organisms impermeable to antibiotics and bind the agents at the outer surface of the matrix layer which protects the bacteria from penetration of the antibiotics. This causes recurrent infection and results in the organism developing multidrug resistance. These strains respond poorly or not respond at all to conventional and routine antimicrobial therapies.

The study showed that positive urine culture with the antibiotic sensitivity of the isolates is very important for antimicrobial therapy, as antibiotic resistance is a worldwide problem which causes ineffectiveness of treatment. Early and proper treatment can decrease the antibiotic resistance. This study will also help in assuming the emerging trends in resistance at the local level to support clinical decision making, infection - control interventions, and antimicrobial - resistance containment strategies.

Conclusion

The data generated from the present study suggest that *Escherichia coli* isolates from urinary tract infections have showed resistance to many of the routine antibiotics in our study. This information may directly influence the choice of empirical antibiotic therapy for UTI. Multidrug resistant uropathogenic *E. coli* is an expanding public health threat. Knowledge of antimicrobial resistance trends among isolates of uropathogens is essential to provide clinically appropriate and cost-effective therapy. Guidelines for the empirical treatment of patients with UTI suggest it is important to consider local resistance patterns of commonly isolated pathogens in selecting the antibiotic agents.

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