Research Article

Isolation and Antibiotic Susceptibility of Gram Negative Bacteria Associated with Urinary Tract Infection

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Abstract
Urinary tract infections (UTI) are most commonly observed infections in clinical practice Caused by *Escherichia coli* and other Gram negative bacteria. The present study was conducted to isolate Gram negative bacteria from UTI and non-UTI persons within different age groups. A total of 150 urine samples were collected from UTI patients and non-UTI patients. Urine sample were inoculated on selective media for the isolation of *E. coli, Klebsiella, Proteus* and *Pseudomonas* spp. The isolated Gram negative bacteria were identified on the basis of culture characteristics, morphology and biochemical characteristics. Antimicrobial susceptibility testing was done on the Muller-Hinton agar by disc diffusion method. Associated risk factors with urinary tract infection were determined. The results showed that *E. coli* were 80.77%, *Klebsiella* 10.25%, *Pseudomonas* 6.4% and *Proteus* 2.5% in UTI patients. In this study urinary tract infection was more in females as compared to males. UTI were found more in 18-45 year old patients. The overall results showed that *E. coli* was the major pathogen found in these infections. The results of antibiotic susceptibility testing showed that *E. coli* isolates were resistant to ampicillin and tetracycline, moderately susceptible to Nitrofurantion and Nalidixic acid and Susceptible to Ciprofloxacin and Enoxacin. Among associated factors, stone formation was found in 22% and enlarged prostate gland in 8% cases.
Keywords: Urinary tract infection, *Escherichia coli,* antibiotic resistance.

Introduction

A urinary tract infection (UTI) is an inflammatory process occurring in the kidney, ureter, bladder, or adjacent structures when microorganisms enter through the urethra. Urinary tract infection is the most commonly observed among clinical cases. Urinary tract infections are 35% among all hospitalized patients [1].

Urine is sterile when it reaches to distal urethra. In body there are different defense mechanisms for the prevention of urinary tract infections. Most important defense mechanism is flow of urine which helps to wash bacteria from the body. While in others prostate gland produces secretion that prevent bacterial growth. Bacterial growth is also reduced by acidic pH and low osmolarity within the body [2].

In female's urinary tract, it has important relationship to reproductive organs. In Non-Pregnant state uterus lies just close to the bladder while in pregnant females uterus size increases which affects all the tissues. The chances of urinary tract infections are more in females as compared to males because of short urethral size [3]. There are certain anomalies which increase the prevalence of UTI in males as compared to female's up to age of one year. While in different age groups females more than one year are more susceptible to infection [4].

There are chances of uncomplicated urinary tract infection in structurally and functionally healthy females. Complicated urinary tract infections are those which results in therapeutic failure [5]. Abnormalities like impede urine flow, presence of foreign body in urinary tract are complicated urinary tract infections. Both upper and lower urinary tract may involve in these infections [6].

The most commonly observed pathogens that cause urinary tract infections are from enterobacteriaceae family. Most frequent cause of urinary tract infection is the *E. coli* although some other gram negative bacteria also present in these infections. Other isolated pathogens from urinary tract infections patients are *Klebsiella*, *Enterobacter, Staphylococci, Proteus*, *Pseudomonas* and *Enterobacter spp.* [7, 8].

There are many factors that help in the increase of urinary tract infection. The major factors include are sex, age, kidney tumors, pregnancy, stone, catheterization, neurological diseases, urethral

strictures, suppressed immune system, congenital/acquired anomalies of bladder, enlarge prostrate, diabetes mellitus etc. [9].

Resistance to most of the antibiotics is still a major problem. Resistance to antibiotics is increasing day by day due to wrong or overuse. Major cause of urinary tract infections are bacteria while best treatment for urinary tract infection is use of antibiotics [10]. Cephalosporins, broad spectrum Floroquinolones and Aminoglycosides are antibiotics which are used in routine treatment of UTI. The best antibiotic used to treat the infection against gram negative organism are cephalosporin [11].

Resistance against bacterial pathogen is a worldwide issue. It varies according to geographical and regional location. Antibiotic susceptibility pattern to urinary tract pathogens varies which result in increase in resistance to most of antibiotics. Distribution of uropathogens and their susceptibility to commonly used antibiotics varies from country to country [12].

Material and Methods

A total of 150 samples were collected from patients suffering from urinary tract infections both females and males belonging to district Faisalabad. This study was conducted in Feb 2012 to May 2012 from patients of UTI admitted in Allied hospital and District Headquarters Faisalabad. All the Patients were provided with wide mouth, tightly closed sterilized bottle. The patients were advised to collect the clean catch midstream urine. Samples were shifted to clinical microbiology laboratory, Institute of Microbiology, University of Agriculture Faisalabad. With the help of loop samples were collected from centrifuged urine specimens and streaked on MacConkey agar for *E. coli*, Methyl violet agar for *Klebsiella spp.,* Heart infusion agar for *Proteus* and on Citramide agar for *Pseudomonas spp.* Plates were kept in the incubator at 37 °C for 24 hours. Identification of microorganism was done by cultural characteristics, morphology and biochemical testing. Antimicrobial susceptibility testing was done on the Muller-Hinton agar by disk diffusion method. Associated risk factors with urinary tract infection were determined.

Results and Discussion

Bacterial infection is the major cause of urinary tract infection in human beings. It was observed that proper control of bacterial UTI relay on isolation, identification of bacteria that cause the disease and appropriate selection of antibiotic against those bacteria [13].

In our study, a total of n=150 samples were collected from both male and female having different age that were admitted in Allied hospital and District Headquarters Faisalabad. Out of 100 collected samples from UTI patients, 78 were found positive for gram negative bacteria. Out of 50 samples that were collected from Non-UTI patients only 4 samples were found positive against gram negative bacteria.

In present study *E. coli* was found 80.8% which cause the urinary tract infection while *Klebsiella, Pseudomonas* and *Proteus* species were found 10.3%, 6.4% and 2.6% respectively (**Table 1**). A similar study was conducted in which they isolate and determined the antibiotic sensitivity pattern from UTI patients in different reproductive age group. They isolated *E. coli* as 85% while *Klebsiella* as 13%, *Pseudomonas* as 4% and *Proteus* as 1.6% [14].

Table 1: Species wise percentage of isolated Gram Negative bacteria from UTI patients

Sr #	Isolated spp.	Total samples	positive samples	Percentage of G-ve bacteria
1	**E. coli**	100	78	63/78 (80.77%)
2	Klebsiella	100	78	8/78 (10.25%)
3	Pseudomonas	100	78	5/78 (6.4%)
4	Proteus	100	78	2/78 (2.6%)

Similarly in another study 11% *klebsiella* was found [15]. In this study pseudomonas was found 6.4% while in another study pseudomonas was 1.4% [16]. Many studies showed that chances of UTI were more in females as compared to males [8]. In our study, infection was found more in females (63%) as compared to males (37%) which prove the above results (**table 2**). A similar type of results was found having highest incidence of the UTI in females (61%) as compared to males (39%) in all age groups [17].

Table 2: Sex wise percentage of isolated Gram Negative bacteria from UTI patients

Sr #	Isolated spp.	Total	Male %age	Female %age
1	*E. coli*	63	26/63 (41.2%)	37/63(58.7)
2	Klebsiella *Spp*	8	3/8 (37.5%)	5/8 (62.5%)
3	Pseudomonas *Spp*	5	1/5 (20.0%)	4/5 (80.0%)
4	Proteus *Spp*	2	0/2 (0.0%)	2/2(100%)

In an earlier study it was reported that UTI was less in < 9 years of age. While highest prevalence was found in 10-50 year of age group. In the current study, Samples (9%) were collected from 1-18 year while 65% were from 18-45 and 26% from > 46 year of age (**Table 3**). These results were proved in a study conducted to isolate causative agent causes UTI

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and their antimicrobial susceptibility was also determined. In their study girls were showing more prevalence 55% as compared to boys. They divided in four group's 1-4years (16%), 5-10yrs (46%), 11-20yrs (5%) and above 21yrs (73%) [18].

Table3: Age wise percentage of isolated Gram Negative bacteria from UTI patients

Age (Years)	No of Patients	Male	Female	Male %age	Female %age
1-18	9	3	6	33.3	66.6
19-45	65	23	42	35.3	64.6
45-above	26	11	15	42.3	57.6
Total	100	37	63	37	63

Antibiotic resistance of bacteria against different drugs is increasing day by day and it is a worldwide issue. In present study most common isolated bacteria was *E. coli* and that was found resistant to the Ampicillin and Tetracycline, while moderately susceptible to Nitrofurantion and Ceftaxime and their zone of inhibition were 15.3±1.41mm and 16.7±2.03 mm respectively. However, *E. coli* was susceptible to Amikacin, Ciprofloxacin and Enoxacin, and zone formed against these drugs were 17.3±.79 mm, 20.7±1.69 mm and 18.6±1.02 mm respectively (**Table 4**).

Table 4: Mean value of zone of inhibition formed by Gram negative bacteria to different antibiotics in UTI group

Organism	Nitrofurantion	Ampicillin	Amikacin	Tetracycline	Ciprofloxacin	Ceftaxime	Enoxacin	Nalidixic acid
E. coli	15.3±1.41 (M)	0.00 (R)	17.3±.79(S)	0.00(R)	20.7±1.69(S)	16.7±2.03(M)	18.6±1.02(S)	16.1±1.53(M)
Klebsiella	15.2±0.89(M)	0.00 (R)	17.2±.73(S)	0.00(R)	21.5±0.53(S)	16.2±1.04(M)	18.3±.92(S)	14.3±1.06(M)
Proteus	18.0±1.41(S)	0.00 (R)	15.5±.71(M)	0.00(R)	19.5±0.71(M)	16.5±.71(M)	18.5±.71(S)	8.0±0.00(R)
Pseudomonas	19.4±1.14(S)	0.00 (R)	15.0±.71(M)	0.00(R)	21.4±0.55(S)	16.2±1.30(M)	17.8±.45(S)	7.0±4.36(R)

Similar results were found in previous stious studies that support above results. The results which shows different pattern of susceptibility to *E. coli* to ceftaxime as 29% while shown the susceptibility of *E. coli* to Ciprofloxacin 55% [19].

Klebsiella was second major gram negative bacteria isolated in our study and it was resistant to Ampicillin while Amikacin, Cefotaxime and Ciprofloxacin were moderately susceptible 11- 14% from positive samples. A different type of results found which shows that *Klebsiella* to Amikacin, Cefotaxime and Ciprofloxacin >50% resistance [13]. In this study pseudomonas was found resistant to Ampicillin and Tetracycline while susceptible to Amikacin and Ceftaxime. These results were similar to a previous study in which Amikacin and Ceftaxime were 70-90% susceptible [20].

There are many factors which enhance the incidence of UTI. These factors may be intrinsic which include urinary obstruction, enlarged prostate and pregnancy or extrinsic risk factors such as catheterization [21]. UTI obstructions are the major factor which promotes the growth of bacteria. Stone is one of the major UTI obstructions. In this study the major factor associated with UTI that was stone accounts 22% (**Table 5**).

Table 5: Distribution of risk factor in UTI group

Risk factor	Frequency	Percentage
Cathterization	4	4%
Diabetic	3	3%
Enlarged Prostate	8	8%
Non Functional Kidney	1	1%
Pregnancy	7	7%
Stone	22	22%
Tumour in kidney	1	1%
Unknown	54	54%
Total	100	100%

A similar study was conducted to determine the associated risk factor in which stone was present and it showed the 24% prevalence of stone. Another study in which 15% stone was present supports the result of this study [9].

Pregnancy was the second factor associated with UTI. The chance of UTI was increased in the pregnancy due to shorter distance of urethra. In this study 7% patients were found positive for UTI. These results were supported by a study in which it was present 11% [22]. In this study enlarged prostate was present 8%, a similar type of result was found in which it was present 10% [9].

Catheterization was also major factor which promote the UTI. The different methods were used to decrease the chances of UTI in which one to reduce the unnecessary use of catheter in the hospitalized patients. When the catheter was used for a longer period of time different type of microorganism were attach to its wall or enter through its lumen [23]. In our study four samples were associated with Catheterization. In this study 3% patients were having diabetes. Some other factors like tumor in kidney and non-functional kidney were 1%. 54% patients were without any factors. Diabetic was also a major factor associated with UTI that may be due to certain metabolic changes which were taking place in body includes elevated blood sugar levels which results in suppressing the immune system [12].

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