Analysis of Uropathogenes among Type II Diabetic Patients in Pakistani Population

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Abstract  Urinary tract infection (UTI) is one of the most common infections in the Pakistani local population that afflicts people and if not treated properly and timely could cause serious damages to the urinary tract. Nowadays increasing antibiotic resistance is one of the problems in managing UTI to check the metal resistance of this disease causing organisms. Urine specimens of in-patients and out-patients in Sheikh Zayed Hospital, Lahore were cultured on blood agar and eosin methylene blue agar (EMB). Isolated bacteria were identified according to standard microbiological tests and then subjected to sensitivity testing according to routine method of disk agar diffusion technique and metal resistance on media containing metal salts. Our findings suggest that there is a method to treat the UTI by antibiotics but nowadays antibiotic resistance is increasing day by day. Results of the present study underline the need for sensitivity tests prior to antibiotic therapy in UTI, which could help and guide in proper choosing of antibiotics and effective treatment and therefore prevention of antibiotic resistance.

Keywords  Urinary Tract Infection (UTI), Disk Agar Diffusion, Antibiotic-resistance

1. Introduction

Urinary tract infections (UTIs) are most common bacterial infectious diseases in humans. UTI affects all age groups, one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime. Diabetes mellitus is a major health problem in developing countries such as in Pakistan. Diabetes type 2 is one of the main causes of kidney disease and end stage renal failure. Vascular complications are the most common cause of diabetic nephropathy, but it is possible that UTIs also contribute to renal insufficiency in patients with DM. Patients with diabetes have an increased risk of infections with the urinary tract being the most prevalent infection site. Escherichia coli, is the organism which 90% of microorganisms possess the adhesive type 1 fimbriae, is the leading uropathogen in patients without DM as well as in those with DM (1).

Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy.

The research was carried out to determine the causative agents of UTIs and their susceptibility patterns to commonly used antibiotics in patients from the Lahore Pakistan. In Pakistan diabetes is the common disease which is may be genetic and some environmental factors also increase the risk of diabetes type 2 in local population of Pakistan and due to this disease the chances of urinary tract infection increases in these patients which is studied in literature. In this study we have identified that which pathogens are responsible for urinary tract infection and what is treatments of this disease. For this investigation the antibiotic sensitivity required and metal resistance is also required to check that is metal salts can inhibit the growth of pathogens in the urinary tract or not.

2. Materials and Methods

2.1. Clinical Samples

The different samples of urine were collected during august and September 2009 from the hospitalized patients of sheikh zayed hospital Lahore which were suspected the UTIs and suspected that they are also diabetic. The pH, color of the urine and the patients history was also noted. The samples were collected directly from the patients. For this purpose they were given sterile, dry, wide-mouthed and leak proof plastic container. These containers were thoroughly washed with detergent water and sterilized in autoclave at 15 lbs pressure (121°C) for 15 minutes. The patients and staff were instructed verbally to clean the perianurethral area carefully with two separate washes with water and soap. They were directed to discard the first portion of voiding and collect the midstream specimen of urine in to sterile container provided. The samples were immediately transported to the laboratory.
where they were processed properly. In case of delay, samples were kept at 4°C and were analyzed in laboratory within two hours of collection (2).

2.2. Laboratory Investigation of Urine Samples

Samples were inoculated on culture media and wet films were performed, microscopy was done. All urine samples obtained were tested by sensitivity method. Biochemicals were put up on the positive cultures. Different urine cultures were prepared such as Mc Conkey agar, nutrient agar, and cysteine lactose Electrolyte Deficient (CLED) Agar. 500µl urine sample was taken and spreaded on these culture plates and plates were incubated at 37°C. Following the appearance of growth, well isolated bacterial colonies were processed for antibiotic sensitivity and biochemical tests. The number of colonies of different media was counted and viable count of bacteria was reported for each specimen. The urine sample also was observed under the microscope by taking a drop of urine on slide smear was prepared cover slip was put on the slide and observed under microscope (40x) (3).

2.3. Preparation of Urine Culture

1 µl (0.001ml) wire loop was sterilized and dipped into a urine sample. The loop was then streaked on the plate of cysteine lactose electrolyte deficient (CLED) agar. The plates were incubated overnight at 37°C. Following the appearance of growth, well isolated colonies were processed for antibiotic sensitivity and biochemical tests. The number of colonies on CLED agar plates was counted and viable count of bacteria was reported for each specimen. The colony count was multiplied by 1000 to give an estimate of the number of organisms per ml of urine. According to cheeseburgh (2000) a bacterial count of a 10³ per ml or more indicates infection. Counts between 104 and 105 per ml could mean mean infection or contamination. Counts for less than 104 per ml are nearly always due to contamination unless history of antibiotic intake is present. Keeping this in mind the count was categorized as 105 per ml or more which were regarded as culture negative. The growth obtained after overnight incubation was assessed visually. The culture characteristics noted were colonial morphology (size, shape, margin, color, surface elevation, consistency and translucency), pigment production, odour of colonies, and character of lactose fermentation (4-5).

2.4. Wet Film Examination

50µl (0.05ml) of well mixed uncentrifuged sample of urine was placed with micropipette on the middle of a glass slide. A cover glass of 22X22 mm dimension was placed on it taking care so that no air bubbles were trapped. The film prepared in this way showed a small excess of fluid along the edges of the cover glass. Such film had a depth of 0.1 mm. this examination was carried to find bacterial and pus cells in uncentrifuged urine. The examination for WBCs was done under high power dry objective (40X) and the data was noted (6).

2.5. Identification and Characterization of Bacteria

- Gram’s staining
- Catalase test
- DNase test
- Oxidase Test
- Motility Test
- Indole test
- Urease Test
- Methyl Red test
- Voges Proskauer test
- Citrate utilization Test
- Triple Sugar Iron Test

2.6. Metal Sensitivity

Antimicrobial Sensitivity Test

All organisms isolated were tested against various antibiotics in vitro by Kirby Bauer disk diffusion method. Colonies were picked up by a sterilized wire loop and put on the centre of the Muller Hinton agar plate. Then with a sterile cotton swab the colonies were streaked on plate starting from the centre. The appropriate antimicrobial discs were placed evenly distributed and the plate incubated at 37°C overnight. After overnight incubation the diameter of each zone of inhibition was measured in mm. The end point of inhibition was where the growth starts. Interpretation of zone sizes of each antimicrobial disc was made, as sensitive or resistant using interpretation chart of zone sizes. A standard filter paper disc- agar procedure was also used to check the antibiotic sensitivity it is also known as the kirby–baur method, is frequently used to determine the drug susceptibility of microorganisms. This method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition those results from diffusion of the agent into medium surrounding the disc. In this procedure the stock solutions of the antibiotics were prepared 25 µg/ml, 50 µg/ml, 100 µg/ml were prepared and the specific concentration of antibiotics was taken and then poured the wells of the agar plates (7).

2.7. Protein Isolation and Estimation of Total Proteins

The pathogenic UTI strains were grown in the N-broth culture and in incubated at the 37°C for the 24 hrs. The next day the cultures were taken from the incubator and tubes were vortex to mix the cultures uniformly. The 2ml of the culture was taken and transferred to the sterile eppendorfs in the sterile conditions. The samples were centrifuged at the maximum speed (6000 rpm). Then the supernatant was discarded and the cells were harvest. Then the 1ml phosphate buffer (0.1 N) was added in the harvested cells. Then the sonication was done in the harvested samples for three times. Then the samples were again centrifuged at the maximum
speed (6000 rpm). Then the supernatant was taken for the profiling of the proteins the proteins are present in the supernatant. The proteins which are isolated from the samples are put on the ice for 5 minutes. Then again put on the boiling water bath (first Heat Shock). Then the proteins are put on the ice again for 5 minutes. After it the samples are uniformly mixed by vortex the samples. The samples again put on the boiling water bath (second Heat Shock). Then centrifuged the samples at the maximum speed for 10 minutes. The supernatant was transferred to the pre cool fresh eppendorfs. The samples were loaded in the gel by the ratio of 5µl sample and 10µl 6X sample loading dye. Then a heat shock was again given to the samples at boiling water bath for 5 to 7 minutes (2).

Total serum protein estimation by Bradford assay was performed for the quantification of microgram quantities of proteins. The dye-binding Bradford assay is based on the interaction of certain basic amino acids residues (primarily arginine, lysine, and histidine) with dissociated groups of Coomassie brilliant blue G-250 (CBB) in an acidic environment. The binding of CBB with proteins results in a spectral shift from the reddish/brown (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 610 nm). The difference between the two forms of the dye is maximal at 595 nm, where the blue color from the Coomassie dye–protein complex is generally measured. This method is rapid, sensitive, and reliable and is subjected to less interference by common reagents and non-protein components of biological samples (2).

2.8. Statistical Analysis and Software Used

All statistical analyses were done with the SPSS statistical software package (version 15.0). Statistical tests were all two-sided (<0.05) using one way ANOVA and Kruskal wallis tests. Significance of difference between mean and median analytes of thiamine and placebo treatment groups was determined using Student’s t-test and Mann-Whitney U test, respectively. Significance of difference from baseline and post-therapy and post-washout period was determined using a independent t-test to the samples. Elution profile obtained from the chromatofocusing column was examined by 32 Karat software which generate the 1st dimension report that tells about information of all peaks separated in the various fractions at particular pH and time interval. ProteoVue and DeltaVue software’s are used to generates the maps based on the pI and compares the maps of control with that of each group.

3. Results

3.1. Microscopy and Culture Results

From the urine culture the gram negative bacteria were identified by the microscopy of the urine cultures which were further characterized (Table 1). The results showed that they are more gram negative bacteria in female patients as compared to the male ones (Table 2).

In urinary tract infection lactose fermenter are more in this as compared to non lactose fermenter.

| Organism                  | Male percentage | female Percentage |
|---------------------------|-----------------|-------------------|
| Gram positive             | 0               | 6                 |
| Gram negative             | 70              | 169               |

| Organism      | Percentage   |
|---------------|--------------|
| Lactose fermenter | 81.7%       |
| Non lactose fermenter | 18.3%       |

Table 2. Gram negative pathogens identification

| Organism | Biochemical Reactions | TSI                  |
|----------|-----------------------|----------------------|
|          | oxidase | Citrate | Motility | Indol | Urease | Slope | Butt | H2S | Gas |
| E.coli   | -       | -       | +        | +     | -      | Y     | Y    | -   | +   |
| Klebsiella | -     | +       | -        | -     | +_{slow} | Y     | Y    | -   | +   |
| Proteus | -       | +       | +        | -     | +      | R     | Y    | +   | +   |
| Pseudomons | +     | +       | +        | -     | d      | R     | R    | -   | -   |

Table 3. Gram positive pathogens identification

| Organism           | Identification Tests |
|--------------------|----------------------|
| Gram staining      | Catalase | Novobiocin | DNase |
| Staphylococcus aureus | Gram +ve cocci | +ve | S | + |
3.2. Biochemical Reactions of Different Pathogens

When the data from 270 different patients was organized in different age groups as well gender wise, it appeared that the overall prevalence of urinary tract infection in females is higher than males. In age group 61-80 the urinary tract infection is high in diabetic patients which is 36% because in the Pakistan the diabetes is genetic diseases as well as environmental factors also play an important role in this disease. So this diabetes appears in the late middle or old age (Figure 1, Table 3-4). Almost all the cases of UTI are due to the gram negative bacillus bacteria (Table 5) from which more found in females as compared to males. Lactose fermenting (81%) bacteria showed predominant frequency in both sexes with 15% higher frequency in females as compared to the males (Table 5-6). Non lactose fermenting is less (6).

| Table 3 | Prevalence of UTI pathogens |
|---------|-----------------------------|
| Organism | %age | Male %age | Female %age |
| E.coli | 76.8% | 22 | 10.73 | 82 | 40 |
| Klebsiella | 10.0% | 7 | 2 | 20 | 8 |
| Enterobacteriaceae | 9.6% | 10 | 3.73 | 15 | 5.55 |
| Pseudomonas | 4.40% | 2 | 0.74 | 9 | 3.33 |
| Staphylococcus aureus | 2.20% | 1 | 0.92 | 4 | 1.48 |
| Candida | 1.50% | 0 | 0 | 4 | 1.48 |
| Proteus | 1.40% | 0 | 0 | 3 | 1.11 |

| Table 4 | Sex wise and Socio-economic status distribution of UTI pathogens |
|---------|---------------------------------------------------------------|
| Sex | No of patients | %age |
| Male | 70 | 37.03% |
| Female | 170 | 62.96% |
| Socio-economic status | No. of patients | %age |
| Poor | 98 | 29% |
| Middle class | 176 | 52% |
| Upper class | 63 | 18.6% |

| Table 5 | Age wise distribution of UTI in diabetic patients |
|---------|-----------------------------------------------|
| Age group | No. of patients | %age |
| 1-20 | 2 | 0.74 |
| 21-40 | 34 | 12.5 |
| 41-60 | 75 | 27.7 |
| 61-80 | 98 | 36.29 |
| 81-100 | 29 | 10.74 |

| Table 6 | Clinical manifestations of diabetic patients with UTI. |
|---------|--------------------------------------------------------|
| Signs and symptoms | Total No. | %age Male %age | Female %age |
| Fever | 87 | 87% | 50% | 49% |
| Chills | 68 | 68% | 43% | 55% |
| Lethary | 80 | 80% | 44.5% | 53% |
| Urine retention | 24 | 24% | 58% | 38% |
| Frequency of micturation | 67 | 67% | 23% | 64% |
| Burning micturition | 50 | 50% | 36% | 64% |
Figure 1. Prevalence of urinary tract infection (UTI) pathogens and age wise distribution in the diabetic patients.

Figure 2. Sensitivity of various antibiotics in the different micro-organisms in the Urinary tract infection of diabetic patients.
The most frequent causative agents in the studies were found to be \textit{E. coli} and \textit{Klebsiella} which are 76% and 10% respectively followed by \textit{Pseudomonas} (4.40%), \textit{S. aureus} (2.20%), \textit{Candida} (1.5%), and \textit{Proteus} (1.40%).(Table 7) Among the uropathogens, the frequency of \textit{E. coli} was about 40% in females as compared to males (10.0%), while \textit{Klebsiella} showed 6% more in females as compared to males (8%) (Figure 2). Among the different signs and symptoms the frequency of fever was found to be 50% in both sexes. Chills found to be 11.7% higher in females (55.9%) than that in males (46.5%). The frequency of urine retention was found to be 29.2% higher in females (64.6%) than that of the males (35.4%). Burning micturation was found to be the highest (64%) than that of the males. Among all the antibiotics used in UTIs cephalexin was the most resistant (80%) followed by Naladixic acid (76%), cefixime (68%) and Pipemidic Acid (45%). The most sensitive were Imipenem (98.6%) followed by Cefixime (77%), Naladixic Acid (73%), Augmentan and Ceftazidime (48% each Table 8).

### Table 7. Sensitivity pattern of various antibiotics in UTI's caused by various bacteria.

| Antibiotic        | Sensitivity E.Coli | Sensitivity Klebsiella | Sensitivity Pseudomonas | Sensitivity Proteus | Sensitivity S. aureus |
|-------------------|--------------------|------------------------|-------------------------|---------------------|-----------------------|
| Augmentin (AMC)   | 74.8               | 55                     | 39                      | 100                 | 100                   |
| Cephalexin (CL)   | 28                 | 19                     | 13                      | 0                   | 0                     |
| Cefixime (CFM)    | 40                 | 15                     | 39                      | 0                   | 0                     |
| Ciproflexin (CIP) | 73                 | 69.3                   | 91.3                    | 100                 | 0                     |
| Cefazidime (CAZ)  | 64.9               | 68                     | 82.6                    | 100                 | 0                     |
| Nladixic Acid (NA)| 20                 | 23                     | 34.8                    | 0                   | 0                     |
| Nitrofuration (F) | 19                 | 98                     | 69.6                    | 0                   | 100                   |
| Pipemidic acid (PIP) | 51.6           | 21                     | 65.2                    | 0                   | 0                     |
| Cefizox (ZOX)     | 85                 | 93                     | 69.5                    | 100                 | 100                   |
| Imipenem (IPM)    | 100                | 98                     | 95.5                    | 100                 | 100                   |
| Penicilline (P)   | 80                 | 12                     | 67                      | 23                  | 100                   |
| Chloramphenicol(CHL) | 65               | 15                     | 50                      | 32                  | 100                   |
| Ampicilline(AMP)  | 44                 | 2                      | 14.2                    | 28                  | 28                    |
| Gentamycine(GEN)  | 30                 | 20                     | 26.2                    | 45                  | 34                    |
| Carbencilline(CAR)| 94                 | 55                     | 57                      | 34                  | 25                    |

### Table 8. Sensitivity against Different heavy metals

| Sr. No. | Organism               | 10µg/ml | 50µg/ml | 100µg/ml | 150µg/ml |
|---------|------------------------|---------|---------|----------|----------|
| 1       | \textbf{Chromate}      |         |         |          |          |
|         | \textit{E. coli}       | +       | +       | +        | -        |
|         | \textit{Klebsiella}    | +       | +       | L        | -        |
|         | \textit{Enterobacteriaceae} | +    | +       | L        | -        |
|         | \textit{Pseudomonas}   | +       | +       | L        | -        |
|         | \textit{Staphylococcus aureus} | +  | +       | +        | L        |
|         | \textit{Candida}       | +       | +       | -        | -        |
| 2       | \textbf{Iron chloride} |         |         |          |          |
|         | \textit{E. coli}       | +       | -       | -        | -        |
|         | \textit{Klebsiella}    | +       | -       | -        | -        |
|         | \textit{Enterobacteriaceae} | +  | +       | -        | -        |
|         | \textit{Pseudomonas}   | +       | -       | -        | -        |
|         | \textit{Staphylococcus aureus} | +  | -       | -        | -        |
| Sr. No. | Organism        | Silver nitrate          | Copper sulfate         | Cadmium chloride       | Zinc chloride          | Cobalt chloride       |
|---------|-----------------|-------------------------|------------------------|------------------------|------------------------|-----------------------|
|         |                 | 10µg/ml  | 50µg/ml  | 100µg/ml | 150µg/ml | 10µg/ml  | 50µg/ml  | 100µg/ml | 150µg/ml | 10 µg/ml | 50 µg/ml | 100 µg/ml | 150 µg/ml |
| 1       | E.coli          | +        |          |          |          |          | L        |          |          |          |          |          |          |
| 2       | Klebsiella      | +        |          |          |          |          | +        |          |          |          |          |          |          |
| 3       | Enterobacteriaceae | +    |          |          |          |          | +        |          |          |          |          |          |          |
| 4       | Pseudomonas     | +        |          |          |          |          | +        |          |          |          |          |          |          |
| 5       | Staphylococcus aureus | +          |          |          |          |          | L        |          |          |          |          |          |          |
| 6       | Candida         | -        |          |          |          |          | -        |          |          |          |          |          |          |

*L= low growth, + = growth, -= no growth*
3.3. Antibiotic Resistant

The study showed that E.coli was the most resistant to Naladixic Acid (80%) Cephalexin (73%) and Cefixime (56.8%) and most susceptible to Imipenem and Nitrofurion (100%) followed by Clizox (86.5%). The most sensitive antibiotics for Klebsiella were Imipenem and Nitrofurion (97.3%) followed by Cefizox (92%) and Ciprofloxacin (70.3%). Pseudomonas showed maximum resistance to Cephalexin (87%), Nalidixic Acid (65%) and Cefixime (61%) and sensitivity to Imipenem (95%), Ciprofloxacin (91.3%) and Ceftazidime (82.6%). The other bacteria Proteus and S.aureus were low in number, their sensitivity pattern is given in tables.

Biochemical reactions used in the identification of gram negative bacilli were Oxidase, Citrate, Motility, Indole, Urease, and TSI (Figure 3).

Metal Resistance

In these results it is shown that all the pathogenic strains are sensitive at the 150 µg/ml concentration of the metal salts while some pathogens are sensitive at 100 µg/ml concentration such as in the case of Silver Nitrate. In the case of Chromate all the pathogenic strains showed the sensitivity at the 150 µg/ml concentration while the S. aureus showed the low growth at this concentration while the Candida yeast is more sensitive to chromate metal salt. In the iron chloride all the strains are sensitive at 100 µg/ml concentration while the Candida showed no growth at any concentration. In the silver nitrate case all pathogens are
sensitive at all concentrations except 10µg/ml while the Candida yeast showed no growth at 10µg/ml.

In cooper sulfate all the strains showed sensitivity at 100 µg/ml concentration except the Enterobacteriaceae and Pseudomonas showed low growth at this concentration. In cadmium chloride all pathogens showed the sensitivity at 150µg/ml while they showed low growth at the 100µg/ml concentration and Candida showed no growth at this concentration. In the case of Zinc the pathogen strains showed the sensitivity at 150µg/ml concentration while showed low growth at 100 µg/ml concentration. But Candida showed no growth at this concentration. In cobalt chloride sensitivity is observed at 100µg/ml while low growth is observed at the 50µg/ml.

3.4. Estimation of Total Cell Proteins

Bradford assay was performed for the quantification of microgram quantities of the protein while optical densities were taken at 595nm. Then protein concentration of every sample was calculated from the standard curve of bovine serum albumin. Protein concentration of every sample was also determined by kit method by using Pyrogallol red method. In both Bradford and kit method increase serum protein concentration was observe for diabetic patients. We observe an increasing trend in the serum protein concentration from normal to diabetic UTI. The average protein content of diabetic patients UTI is loaded as 10 µg/µl in SDS PAGE analysis.

4. Discussion

The diabetes mellitus type 2 is commonly observed in the old age. A recent WHO publication states that urinary tract infections (UTI) are one of the most common bacterial infections in children and that in the first year of life its rates are 2.7% in males.(27) Age wise distribution of UTI was determined and the graph 4.4 shows that the UTI is more prevalent in age group of 61-80 followed by 41-60 and equally distributed in age groups of 21-40 and 81-100 while least prevalent in 1-20 years of age group (16).

Urinary tract infections are among the commonest infections seen in day-to-day practice during the dealing of patients (8). It involves the urethra, bladder, and kidney. Escherichia coli isolated from 80–90% of these infections. (10) UTIs are an important complication of pregnancy and when associated with structural or neurological lesions of the urinary tract at any age, often lead to severe incapacity and death (11). There is also a higher prevalence of genitourinary structural abnormalities (cystocele, cystourethrocele and rectocele). There are many intrinsic (such as urinary obstruction and pregnancy) and extrinsic risk factors (such as catheterization and other invasive procedures), which are the leading causes of urinary tract infection (12-15).

All the urinary tract infection patients were observed in the microbiology lab of the Sheikh Zaid Hospital, Lahore Pakistan and the sampling and urine analysis was done there. The urine sample was collected and inoculated on CLED media. The positive sample were inoculated on Mac-Conkey agar, these isolates were then selected for morphological, biochemical characterization, antibiotic and metal susceptibility determination and protein profiling.

The growth on Mac-Conkey agar was showed that maximum strains were lactose fermenter. Table3.2 showed that 81.7% were lactose fermenter and 19.3% were non lactose fermenters. The graph 4.1 also represent that maximum organisms are lactose fermenters while NLF are very low in number. The results of gram staining showed that 13% were gram positive staphylococi and 77% were gram negative rods.

After gram staining biochemical characterization was done and the Table 3.3 indicated that maximum strains were E.coli, Klebsiella and Proteus and Pseudomonas. While only gram positive isolated strain was Staphylococcus aureus. The principal causative agents accounting for 85% of cases of UTI are enteric gram negative bacteria. (17). Escherichia coli is the primary urinary tract pathogen, accounting for 75 to 90% of uncomplicated urinary tract infection isolates Staphylococcus saprophyticus, Klebsiella spp., Proteus spp., Enterococcus spp., and Enterobacter spp. Are pathogens less commonly isolates (18). Escherichia coli is the most common cause of urinary tract infection (19). Other microorganisms Include Klebsiella spp., Enterobacter spp., Serratia sp., Pseudomonas aeruginosa and other Pseudomonas spp., Enterococci, Staphylococcus saprophytics, Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter spp., and Streptococci spp. (20).The Graph 4.3 represent that most prevalent isolate among all pathogen is E.coli followed by Klebsiella,Enterobacter, Pseudomonas, Staphylococcus aureus, Candida and Proteus. (21)

All women experience UTI at some point in their life. Women taking insulin were mainly those at higher risk, possibly because of more severe diabetes, since the use of insulin may be a marker for disease severity (22). Results also suggested that UTI is more prevalent in females as compared to the males the Table 3 shows that females are 62.96% while males were 37.03%.

Table 3 represents that the symptoms of UTI seen in this study were found to be the highest (56%) in the middle class followed by 38% in the poor and were the lowest in the upper class (6%). The poverty and poor hygienic conditions are the major factors that are responsible for UTIs in the poor and the middle class.

The goal of treatment of UTIs are to eliminate pathogens to prevent urosepsis and reduce the risk of renal scarring (Madrigal et al., 1988). Therefore investigation of the rate of bacterial resistance is of paramount significance (23). In the United States fluoroquinolones such as Ciprofloxacin is used as a preferred initial agent for empiric intravenous and/or oral therapy of UTI in both hospitals and emergency
department settings (24). However Ciprofloxacin has shown a stepwise >3 folds increase in the resistance from 1995 (0.7%) to (2.5%). In a cross sectional survey of urine cultures obtained in the emergency departments of urban tertiary care centers in the United States, Scientists have (25) reported the microbial resistance 13% to nitrofuration. After determining the prevalence of UTI antibiotic susceptibility was determined against different antibiotics and graph 4.5 showed that Cephalaxin was the most resistant (80%) followed by Nitrofurantoin (76%), Cefixime (68 %) and Pipemidic Acid (45 %), Gentamycine (68 %). The microorganisms were most sensitive to the antibiotics such as Imipenem (98 %), Nitrofurantoin (91 %), Cefizox (85 %), Ciproflexin (76 %) and Augmentin (60 %), penicillin (89 %), ampicillin (70 %) and some extent Chloramphenicol (65 %), so these antibiotics can be given safely to the patients suffering from UTIs.

Every strain exhibit different pattern of antibiotic resistance. While in the reported case E. coli exhibited resistance to the commonly used antibiotics, and the most effective in-vitro agents were found to be gentamicin (94%) among the injectables; and ciprofloxacin (78%), among the orally administered ones. Other useful oral antibiotic is Nitrofurantoin (89%). The organisms showed resistance to common used oral antibiotics like ampicillin (59%).

Our strains showed the antibiotic resistance in the nosomial infection such as UTI. The strain isolated from this are then further tested for the metal resistance for this purpose the resistance against the different metal was observe at the concentration of 150µg/ml of concentration.

Iron chloride, Chromate, Silver nitrate, Copper sulfate, Cadmium chloride, Zinc chloride was checked and it was found that the most of the pathogen strains showed the sensitivity at the highest concentration at 150µg/ml as reported earlier that the organisms which are antibiotic resistant are also the metal resistant. Some enterobacteria isolated from nosocomial infections harboured a conjugative plasmid (>56.4 kb) encoding resistance to antibiotics and heavy metals.

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Conflicts of Interest

There is no conflict of interest associated with this research work and project.

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