Antimicrobial Resistance Pattern of Uropathogens Causing Urinary Tract Infection (UTI) Among Diabetics

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Abstract

Urinary tract infections (UTI) are very often encountered in patients with diabetes mellitus. UTI due to emergence of resistant bacterial strains in hospitals increases the cost of treatment, morbidity and mortality in diabetics. Therefore, this study was undertaken to determine the prevalence of UTI, the causative pathogens and the status of multi-drug resistance organism causing UTI among diabetics, which help to monitor the spread of resistant strains involved in UTI. Mid Stream Urine (MSU) samples were collected in the sterile, clean, dry wide mouthed bottle from diabetes patients. Standard protocol was followed to isolate and identify organism which was followed by Disc Diffusion antibiotic sensitivity tests. The data were analyzed using SPSS 16.0 version and Microsoft Excel 2007. The overall prevalence of UTI in diabetics was 50.66% with female predominance of 61.84 % and in male with 37.66%. The UTI was common in age group between 21-40 years. E. coli was the most predominant bacterial isolates which showed 78.6% multi-drug resistance. Bacterial isolates showed more resistant towards tobramycin, nalidixic acid, penicillin-G and amoxycillin. This study highlights that the prevalence of UTI among diabetics was significantly high and the responsible pathogens had developed resistance to several potent antibiotics. Regular monitoring of susceptibility pattern of uropathogens should be essential for optimal empirical therapy of diabetic patients with UTI.

Keywords: UTI, Diabetes, MSU, E. coli, Amoxicillin, Resistance.

1. Introduction

Diabetes mellitus (DM) causes several abnormalities of the host immune system. The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2005). The incidence of diabetes is ever-increasing throughout the world and is becoming a serious public health threat particularly in the developing countries. It is associated with many complications and in the long run it has some major effects on the genitourinary system which makes diabetic patients more vulnerable to UTI, particularly to upper urinary tract infections (Ribera et al., 2006; Patterson et al., 1997). Urinary tract infection (UTI) is associated with multiplication of organisms in urinary tract and is defined by the presence of more than 10⁵ organisms per ml in a midstream sample of urine. (Yadav et al., 2014).

Urine may be static or even bactericidal against uropathogens under certain situations. In diabetes mellitus modification of chemical composition of urine occurs which alters this capability of urine and sustain the growth of pathogens. It has been experimentally shown that osmotic diuresis secondary to glycosuria predispose to ascending Escherichia coli infection in laboratory animals. Autonomic neuropathy in diabetes mellitus impairs bladder emptying and successive urological exploitation prejudice to UTI (Brauner et al., 1993).

Several factors have been implicated in the higher prevalence of asymptomatic bacteriuria and incidence of UTIs in patients with DM compared with patients without DM. These factors include differences in host responses between DM and non-DM patients, a
difference in the infecting bacterium itself, the presence of glucosuria and impairment of granulocyte function (Geerlings, 2008). Moreover, changes with host disease fighting capability, the reputation of diabetic cystopathy along with of micro vascular disease within the kidneys may be likely involved in the larger incidence involving UTI in diabetics (Ferry et al., 1987).

The urinary tract is the principle site of the infection in diabetics with amplified risk of complications of UTI. Immunological impairments such as impaired migration of neutrophils, intracellular killing, phagocytosis, defects in the local urinary cytokine secretions (IL-8, IL-6), increased adherence of the microorganisms to the uroepithelial cells, partly due to a changed and lowered Tamm Horsfall protein, and chemotaxis of polymorphonuclear leukocytes from diabetic patients and neuropathic complications such as impaired bladder emptying, as a result static pools of urine will remain in the bladder (Valerius et al., 1982). In addition, a higher glucose concentration in the urine acts as a favorable culture medium for pathogenic bacteria and promotes rapid bacterial colonization and growth (Hasan et al., 2007).

Urinary tract infection (UTI) can be caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species, *Pseudomonas* species, *Proteus* species and gram-positive bacteria like *Enterococcus* species, streptococci, *Candida albicans* and *Staphylococcus saprophyticus* (Yadav et al., 2014). *E. coli* is the most common organism causing both community as well as hospital acquired UTI (Obel et al., 2010) as well as in both DM and non-DM patients (Bonadio et al., 2006). The difficulties of UTIs such as emphysematous cystitis, pyelonephritis along with renal papillary necrosis occur more commonly in Type 2 diabetes mellitus (Ribera et al., 2006).

Each year, UTIs accounts for about seven million office visits and another one million emergency department visits, resulting in about 1,00,000 hospitalizations involving women, older people, and patients with spinal-cord injuries and/or catheters, multiple sclerosis, HIV, as well as diabetes (Foxman, 2003). The successful management of UTI in diabetics depends on the proper identification of the bacterium responsible and the selection of effective antibiotics against them. The emergence of resistant bacterial strains in hospitals poses a continued challenge to treat and control the spread of infections. This is a great concern due to the high rates of resistance to antimicrobials used in the treatment of infections caused by uropathogens, particularly in developing countries. The extensive and inappropriate use of antimicrobial agents has continually resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide because infection caused by MDR strains often leads to death (Goldstein, 2000).

UTI due to Multi Drug Resistant (MDR) increases the cost of treatment, morbidity and mortality especially in developing countries like Nepal. In developing countries, there is a dearth of information on the antimicrobial susceptibility of pathogens from DM patients with UTIs to the newly available drugs including the fluoroquinolones and third-generation cephalosporins. Therefore, the present study was aimed to determine uropathogens and its antibiotic sensitivity pattern, also the status of multi-drug resistance organism causing UTI among diabetics. The findings will help in the necessary intervention program to monitor the spread of resistant strains and identify the common bacterial agent involved in UTI.

2. Materials and Methods

2.1 Study Design

This cross-sectional study was conducted in the Department of Microbiology, Clinical Laboratory Sciences of Janaki Medical College Teaching Hospital (JMCTH) in joint collaboration with Medicine department of the Hospital, Janakpur from February 2014 to July 2015.

2.2 Ethical Consideration

Approval was taken from the Institutional Ethical Committee. A paper of information letter and consent form was given to patients before participating in the research and providing urine sample. In case of illiterate participants, information was provided by reading the consent form in presence of witness. The informations of patients were collected such as name, age and sex.

2.3 Study Population

The diabetic patients reported with complaint of nausea, difficult to urinate, burning sensation during urination, pain or pressure in back or lower abdomen, cloudy, dark, bloody, or strange-smelling urine, tiredness and shaky were included whereas non-diabetic patients were excluded from the study.

2.4 Urine Specimen Collection and Processing

The Mid Stream Urine (MSU) samples were collected in the clean, sterile, dry, wide-necked leak-proof container. The distinctive instruction was followed by the patient for the sample collection. When immediate processing was not possible, the specimen was refrigerated at 4-6°C, and when a delay of more...
than 2 hours, boric acid (1.8 % w/v) was added as preservative to the urine.

2.5 Culture of Specimen
Medias were prepared as instructed by the manufacturer company (Himedia). The urine sample was streaked on the MacConkey agar (MA) and Blood agar (BA) medium by the Semi-quantitative culture technique using a standard loop. After mixing the urine sample in the container thoroughly, a loopful of sample was touched to the centre of the plate, from which the inoculum was spread in a line across the diameter of the plate. Without flaming the loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies. The plates were incubated aerobically at 37°C overnight. The approximate number of colonies was counted and the number of bacteria i.e. Colony Forming Unit (CFU) per ml urine estimated in accordance to the volume of urine inoculated previously and reported as :- Less than $10^5$ /ml organisms: not significant, $10^5$ - $10^6$ /ml organisms: doubtful significance (suggest repeat specimen) and more than $10^6$ /ml organisms: Significant bacteriuria.

2.6 Identification of the Isolates
Identification of significant isolates were done by using microbiological techniques as described in the Bergy’s manual which involves morphological appearance of the colonies, staining reactions and biochemical properties.

2.6.1 Identification of Gram Positive Isolates
Gram positive organisms were tested by catalase tests, oxidase test and their specific biochemical tests. Catalase, optochin sensitivity, indole, urease bile solubility and specific carbohydrate fermentation tests were done for the identification of *Streptococcus* spp. Similarly, coagulase, urease and mannitol fermentation tests were performed for the identification of *Staphylococcus* spp.

2.6.2 Identification of Gram Negative Isolates
The identification of various Gram negative isolates was done by using standard microbiological techniques described in Bergey’s manual of Systematic Bacteriology (2nd Edition). The isolates were identified on the basis of various biochemical tests such as catalase test, oxidase test, O/F test, MR/VP test, SIM test, citrate test, urease test, TSI test.

2.7 Antibiotic Susceptibility Testing
Antibiotic susceptibility test of the isolated organisms were done by using modified Kirby Bauer disc diffusion method. Bacterial inoculum was prepared by suspending the freshly grown bacteria in 2 ml of sterile nutrient broth for those organisms that were Gram negative and incubated at 37 °C for 3-4 hours. The turbidity of tube was matched with 0.5 Mc Farland turbidity standards. The inoculum was then streaked on entire Muller-Hinton agar (MHA) plate. For *Streptococcus* spp, bacterial inoculum was prepared by suspending the freshly grown bacteria in 2 ml of sterile Brain Heart Infusion broth (BHI) with yeast extract and the turbidity of tube was matched with 0.5 Mc Farland turbidity standards. It was then streaked onto MHA plate with 5% blood. Antibiotic discs were placed around the outer edge of the plate and incubated overnight at 37 °C. Diameter of zone of inhibition was measured and zone diameter criterion was used to interpret the level of susceptibility to each antibiotic (CLSI, 2013).

2.8 Statistical Analysis
The data were analyzed using SPSS 16.0 version and Microsoft excels 2007. The Chi-square test was used to test for the association of culture positive and culture negative among types of diabetic patients. The p-value p<0.05 was considered statistically significant.

2.9 Quality Control
Laboratory equipment like refrigerator, incubator, autoclave and hot air oven were regularly monitored for their efficiency. The temperature of refrigerator and incubator was monitored everyday for their performance and immediately corrected if any deviation occurred. Reagents and media were regularly monitored for their manufacture, expiry date and proper storage. After preparation, they were properly labelled with preparation date. The quality of media prepared was checked by incubating one plate of each lot for sterility and using standard control strains for performance testing. During identification of organisms, for each test *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for culture and sensitivity testing. Strict aseptic conditions were maintained while carrying out all the procedures.

3. Results

3.1 Age and Gender-wise Distribution of Diabetic Patients
Out of total 150 diabetic patients, 64% were female and 36% were male. The highest number of respondents had diabetes in 21-40 years followed by >60 years age group. The results are shown in Table 1.
3.2 Pattern of Culture among Study Population

Altogether 150 urine samples were received for culture from diabetics, among them 113 (75%) samples showed culture positive and 37 (25%) samples showed culture negative. The results are shown in Fig 1.

Table 1: Age and gender-wise distribution of diabetic patients

<table>
<thead>
<tr>
<th>Age groups (yrs)</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>2 (22.22)</td>
<td>7 (77.77)</td>
<td>9</td>
</tr>
<tr>
<td>21-40</td>
<td>36 (35.29)</td>
<td>66 (64.70)</td>
<td>102</td>
</tr>
<tr>
<td>41-60</td>
<td>7 (28)</td>
<td>18 (72)</td>
<td>25</td>
</tr>
<tr>
<td>&gt;60</td>
<td>9 (64.28)</td>
<td>5 (35.71)</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>54 (36)</td>
<td>96 (64)</td>
<td>150</td>
</tr>
</tbody>
</table>

3.3 Significant Growth Pattern of Uropathogens

Out of 150 urine samples, 76 samples showed significant growth and 74 samples showed no significant growth. Majority of females (70.45%) had significant growth than male (39.65%) patients in 21-40 years. The results are shown in Table 2.

3.4 Pattern of Microbial Isolates from the Urine Sample

A total of 77 bacteria isolated from diabetic patients, 74 isolates were Gram negative whereas 3 isolates were found to be Gram positive bacteria. *E. coli* was the most prominent organism 42 (56.75%) followed by *K. pneumoniae* 16 (21.62%). The results are shown in Table 3.

3.5 Antibiotic Susceptibility Pattern of Gram Negative Bacterial Isolates

Among the tested common antibiotic, Gram negative isolates were more susceptible to imipenem (91.9%) followed by meropenem with 85.3% whereas more resistant towards amoxycillin with 90.1%. The results are shown in Table 4.

3.6 Antibiotic Susceptibility Pattern of Gram Positive Bacteria Isolates

Gram positive isolates were more susceptible to tetracycline followed by cotrimoxazole and ciprofloxacin with 75% whereas more resistant towards tobramycin, nalidixic acid and penicillin-G with 100% respectively. The results are shown in Table 5.

3.7 Pattern of Multi Drug Resistance (MDR) Pathogens in Diabetic Patients

Altogether 8 different bacterial isolates were found to be MDR. Among these, *E. coli* showed high MDR followed by *S. aureus*. The incidence of MDR in diabetic patients was found to be 67.2%. The results are shown in Table 6.

3.8 Association of Culture Positive and Culture Negative among Types of Diabetic Patients

Among all the respondents, the higher proportion of culture positive cases was seen among Type1 patients with 51.33%. There is no significant association of culture positive and negative among types of diabetic patients. The results are shown in Table 7.

4. Discussion

Diabetes Mellitus is a common predisposing factor for UTIs caused by fungi, particularly *Escherichia coli*, *Candida* species, *Klebsiella* species, *C. albicans* (Joshu et al., 1999). Boyko et al. (2002) examined the risk of UTIs among postmenopausal women with DM. The present study reveals that the highest number of female (64.70%) had more diabetic than male (35.26%) of 21-40 years followed by 41-60 years ages. A similar finding was also obtained in the study conducted by Ghenghes et al. (2009) which also showed female had more diabetes than male of 71.85% and 28.14% respectively, which is almost similar to this study. This may be due to diabetes and its consequences like hyperglycemia are normally associated with higher age.

A total of 150 urine samples collected from diabetic patients for microbial culture, most of the diabetic patients had culture positive (75%) and rest of them had culture negative. Among all urine samples, 76 samples showed significant growth and 74 samples had no significant growth. Majority of females (70.45%) had significant growth than male (39.65%) patients in 21-40 years followed by others. This may be due to the patients from age group of 21-40 have more chances of exposure to uropathogens, more sexually active, higher cases of pregnancies and high consciousness to health. This indicates that bacteriuria in diabetics are quite common in later stage of life. This finding is compatible with similar type of research carried out by Jha et al. (2014).
The overall prevalence of UTI in diabetics was found to be 50.66% in the present study. Jha et al. (2014) reported the prevalence of UTI in diabetics was 54.76%. This is in agreement with the present study and other reports stating high prevalence of UTI in females (Bonadio et al., 2006; Ramana and Chaudhary 2012; Adeyeba et al., 2007). This may be due to the uterus is close proximity to the anus in female. Increased occurrence of UTI among diabetic patients was due to many reasons but major ones are decreased antibacterial activity due to swine urine, defects in neutrophil function, enough availability of protein and
The present study showed total 77 bacterial isolates from diabetic patients, of which 74 (96.10%) isolates were found to be Gram negative bacteria whereas 3 (3.89%) isolates were Gram positive bacteria. A similar finding was also obtained in the study of Puri et al. (2006). Altogether, nine genuses of bacteria were isolated where 74 isolates was Gram negative and 3 isolates were Gram positive. *E. coli* was chief isolate accounting 56.7% followed by *K. pneumoniae* as 21.62% and others among total isolates in diabetics. This result is supported by similar findings by Puri et al. (2006) and Goswami et al. (2001). Higher prevalence of *E. coli* seen in this study was compatible with the study done by various other workers viz: Yadav et al. (2014); Akbar et al. (2001); Dhakal et al. (2002) and Mendoza et al. (2002). The reason for predominant *E. coli* isolation is that it can bind to the...
glycoconjugate receptor of the epithelial cells of human urinary tract so that it can initiate infection itself. *E. coli* is the most predominant organism to colonize the urethral meatus (Schaeffer and Chmiel, 1983) and perineum (Leigh, 1990) before ascending to the bladder. This ability of *E. coli* is the important reason to be the most frequent organism causing UTI in both sexes all over the world.

*K. pneumoniae* was second principal isolate among total isolates in this study and similar result also was obtained by Bomjan et al. (2005). *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Citrobacter freundii* and *Staphylococcus aureus* were uropathogens involved in bladder carcinogenesis. The incidence of *S. aureus* in older age reflects pyelonephritis acquired via haemogenous spread.

Antimicrobial resistance among uropathogens causing community and hospital-acquired UTIs is increasing (Bonadio et al., 2001). Few data are available on the role of DM itself as a risk factor for the development of antimicrobial resistance of the uropathogens. The present study found that 67.2% were MDR isolates. Among them, *E. coli* was found to be peak of 78.6% MDR followed by *K. pneumoniae*. A similar study conducted by Niranjan and Malini (2014), reported 76.5% isolates of *E. coli* were MDR which is according to this study. Most of the isolates were resistant to four or more antibiotics which may be due to plasmid carrying drug resistance genes occurring in many bacteria (Shalini et al., 2011; Borderon, 1978). Formation of biofilms inside the bladder causes recurrent infections and also increases the chance of MDR strain causing UTI (Stamm, 2008)

Resistance towards β-lactam drug in Gram negative enteric uropathogens is mediated primarily by β-lactamase, which can hydrolyze the β-lactam ring and thus inactivates the antibiotic. The classical TEM-1, TEM-2 and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of gram negative rods (Livermore, 1995). In addition MDR efflux pump system also contributes the emergence of multi-drug resistance primarily in *E. coli* (Sulavik et al., 2001). Moreover, other newer β-lactamase are now increasing concern towards the emergence of MDR strains in gram negative rods, mainly extended-spectrum β-lactamase, Hyper AmpC β-lactamase producers, Inhibitor resistant β-lactamase, broad spectrum β-lactamase, metallo β-lactamase etc.

Among the tested common antibiotics against all gram negative isolates of diabetic patients, imipenem was the drug of choice followed by meropenem with 91.9% and 85.3% respectively. Similarly, the present study also highlights nitrofurantoin as major drug of choice for Gram negative uro-pathogens. A similar finding was also obtained in the study conducted by Puri et al. (2006). Tetracycline was found to be drug of choice followed by co-trimoxazole and ciprofloxacin with 75.0% each among all gram positive isolates. Cotrimoxazole was one of the drugs of choice which is harmony with the finding of Puri et al. (2006).

This study showed the higher percentage of isolates was isolated from Type1 than Type2 diabetes patients of 55.5% and 40.0% respectively. There is no significant association of culture positive and negative among types of diabetics in the present study. This result showed harmony with the findings from Geerlings et al. (2000).

5. Conclusion

The study concluded that the prevalence of UTI is higher in females as compared to male in diabetics. *E. coli* was the foremost etiological agent of UTI. It was also observed that the diseases incidence increase with increasing age and vice versa. The present study also inferred that antibiotics including amoxicillin, nalidixic acid, tobramicin, and kanamycin are mostly resistant to the urinary pathogens. The most effective antibiotic was found to be imipenem, meropenem and nitrofurantoin for Gram negative bacteria, while tetracycline, cotrimoxazole and ciprofl oxacin for Gram positive bacteria. The high resistance among uropathogenic was *E. coli* which showed MDR. The increased occurrence of UTI due to MDR *E. coli* could be due to increased prevalence of MDR strains in the community. UTI was highly associated with Type1 diabetes which was found statistically insignificant. This study emphasized that the prevalence of UTI among the diabetes was considerably high. Because of the frequency and severity of UTI in diabetes, prompt diagnosis and early treatment is compulsory to prevent consequent complications. The current study recommended, detection of protein in the urine with albuminuric patients should be advised for urine culture for the diagnosis of UTI. Determination of microalbumin in urine in UTI patients should be carried out to prevent the kidneys from further bacterial invasion and damage. Genotypic characterization of MDR strains should be carried out in order to establish the location of drug resistance genes. However, regular monitoring of susceptibility pattern of urinary pathogens is essential to establish reliable information for optimal empirical therapy of diabetic patients with UTI.

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