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Antibiogram of Urinary Pathogens in Patients with Diabetes Mellitus - Experience from a Tertiary Care Hospital

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A B S T R A C T

Urinary Tract Infections (UTIs) in diabetics tends to remain a major health hazard, which are responsible for significant morbidity. It is Cross-sectional study. The urine samples were processed according to the standard protocol over a period of one year from March 2015 to May 2016. The antimicrobial susceptibility was tested by the modified Kirby-Bauer's disc diffusion method as per the CLSI guidelines. Out of 400 diabetic patients, 185 (46.5%) had symptoms of UTI. Culture positivity was observed in 80.54% (149/185). Most common age group affected was between 46 -55 years (mean age is 53.8). Out of 149 culture positive samples, 112 (60.54%) Gram-negative bacilli and 37 (20%) Gram-positive cocci were isolated. The most common isolated uropathogen was *E. coli* (34.1%) followed by *Klebsiella pneumoniae* (15.1%), *Enterococcus* species (11.4%), Coagulase Negative *Staphylococcus* (cons) (1.6%), *Staphylococcus aureus* (7.02%), *Acinetobacter* species (3.2%), *Citrobacter* species (0.5%), *Pseudomonas aeruginosa* (5.9%), and *Proteus* species (1.6%). Among Gram negative bacilli, Imipenem (94.64%), Tobramycin (87.5%), Pipercillin-Tazobactum (75%), Amikacin (74.7%), Gentamicin (63.39%), Amoxicillin-Clavulinic acid (61.6%), Ceftazidime-Clavulanic acid (58.03%), Ceftriaxone (25.8%), Norfloxacin (25.4%) were found to be the most effective drugs. Among Gram positive pathogens Linezolid (100%), Vancomycin (67.7%), Amikacin (54.05%), Amoxycillin-Clavulanic acid (52%) were found to be more sensitive.

Introduction

Urinary tract infections (UTI) are the most common infections in clinical practice.[1] it has been estimated that globally symptomatic UTIs result in as many as seven million visits to outpatient clinics.[2] the urinary tract is the principal site of infection in diabetes. Changes in host defence mechanisms, the presence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients.[3] the most common pathogenic organisms of UTI are *Escherichia coli*, *Staphylococcus saprophyticus* and less common organisms are *Proteus* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococci* and Candida albicans.[4] Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.[5] In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing in antibiotic resistance in urinary tract pathogens.[6]

The prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide. Accurate bacteriologic records of culture results may provide guidance on empirical therapy before sensitivity patterns are available.[7]

Materials and Methods

The present study has been carried out at the department of Microbiology, Sri Chamarajendra Hospital, HIMS, HASSAN, Karnataka state, India from March 2015 to May 2016. It is a cross-sectional study. Out of 400 diabetic patients, 185 (46.5%) had symptoms of UTI. They were included in the study.

Method of sample collection and processing: A freshly voided midstream urine samples (10-20 ml) were collected from UTI suspected patients.

Blood agar, Mac Conkey agar and Nutrient agar were prepared as per the standard guidelines (HiMEDIA).

A loop full (0.001mL) of each urine sample obtained from patients was streaked on MacConkey agar and Blood agar for the growth of bacteria and the culture plates were incubated in an inverted position at 37^0C under aerobic condition for 24 to 48 hrs. Re-inoculation of mixed cultures was further made on blood agar and nutrient agar medium for each discrete colony presented in specimen to obtain well-isolated pure colonies and the pure colonies were preserved for Gram's staining.[8]

Gram staining was done for all isolates as per the standard procedures and the smears were examined microscopically, for their morphology, and staining reactions. All the isolates were identified initially by colony characters, hemolysis on blood agar, lactose fermentation, morphology in Gram staining, Catalase Test, Oxidase test and motility (hanging drop) test. The preliminary identification of potential pathogens, later confirmed up to species level by standard biochemical tests. The data were analyzed using SPSS software 20 (Statistical tests for social package for windows)

Results and Discussion

Total of four hundred patients of diabetes mellitus were screened for urinary tract infection. Among them 185 patients were found to have urinary tract infection. The prevalence corresponds to 46.5%.

A total of 185 samples were collected in the study period of one to one and half year, of which 110 (59.5%) were from female and 75 (40.5%) were from male (Table 1). Culture positivity was 80.54% (149/185) for significant bacteriuria irrespective of all age groups and sex. In this study, out of 149 culture positive samples, 112 (60.54%) Gram-negative bacilli and 37 (20%) Gram-positive cocci were isolated (Figure 1).

The most common isolated uropathogen (Figure 2) was E. coli (34.1%) (Figure 3). followed by *Klebsiella pneumoniae* (15.1%), *Enterococcus* species (11.4%), Coagulase Negative *Staphylococcus* (CoNS) (1.6%), *Staphylococcus aureus* (7.02%), *Acinetobacter* species (3.2%), *Citrobacter* species (0.5%), *Pseudomonas aeruginosa* (5.9%), and *Proteus* species (1.6%) (Table 2).

Nearly all the isolates (Gram negative and Gram positive) were found to be resistant against most of the commonly used oral antibiotics.

Overall Gram negative pathogens showed more resistance as compared to Gram positive organisms for empirical antibiotics commonly used to treat UTI. In the present study, antibiotic sensitivity pattern (Table 3) of Gram-negative isolates are Imipenem (94.64%), Tobramycin (87.5%), Pipercillin-Tazobactum (75%), Amikacin (74.7%), Gentamicin (63.39%), Amoxicillin-Clavulinic acid(61.6%), Ceftazidime-Clavulanic acid (58.03%), Ceftriaxone (25.8%), Norfloxacin (25.4%).

In this study, Gram-positive organisms showed the following sensitivity pattern: Linezolid (100%), Vancomycin (67.7%), Amikacin (54.05%), Amoxycillin-Clavulanic acid (52%), Ceftriaxone (51.42%), Norfloxacin (16%), Trimethoprim-Sulfamethoxazole (46%), and Gentamycin (43%).

The susceptibility of diabetic patients to UTI could be explained by diminished neutrophil response, lower urinary cytokines, and leukocyte concentrations, which might facilitate the adhesion of microorganisms to uroepithelial cells.

Total 185 patients 75 were males & 110 were females with mean age of 65years. Commonest age group in the present study was 46-55 years followed by 56-65 years and 36-45 years. Similar age and Sex distribution was observed in majority of the studies. In a study done by Jennifer *et al.*, (2009)[10] shows that UTI is more common in above 45 years age group and in study by Mishra *et al.*, (2015)[9] above 60 years is more common. In post menopausal women this may be due to non-secretory status, less acidic pH of vaginal surface and poor hygienic conditions leads more chance of getting infection and in 36 to 45 years sexually active women poor hygienic condition causes the infection.[97].

In the present study, the prevalence of UTI in female (59.5%) was high as compared to male (40%). A Study by Mishra *et al.*,[9] and Jennifer *et al.*,[10] showed similar prevalence rate of UTI in females as compared to males. The explanation for gender difference is anatomic reason; the short and straight urethra and short distance between the ostium of the urethra and the anus contribute to easy colonization of the peri-urethral region with enteric bacteria.

Burning micturition and dysuria were the most common presenting symptoms in the present study. Whereas only dysuria is the predominant symptom in Mishra *et al.*,[9] and Jennifer *et al.*,[10] study groups.

Presence of pyuria in female patients were more predominant (37%) when compared to males (30%). Study by Hamdan *et al.*, (2015) in Sudan shows that there is no association between pyuria and prevalence of UTI.[14]

Table.1 Age distribution of diabetic UTI cases

AGE GROUP	Number of Patients	Percentage
25-35	12	6.5
36-45	44	23.8
46-55	51	27.6
56-65	48	25.9
66-75	27	14.6
76-85	3	1.6
Total	**185**	**100.0**

Table.2 Isolated uropathogens

Uropathogens	Number of Patients	Percent
E.coli	63	34.1
No growth	36	19.5
Klebsiella	28	15.1
Enterococci	21	11.4
Staph.aureus	13	7.02
Pseudomonas	11	5.9
Acinetobacter	6	3.2
Coagulase negative Staphylococci	3	1.6
Citrobacter	1	0.5
Total	185	100.0

Table.3 Antibiotic Sensitivity Pattern for Gram Negative Isolates

Drug	Sensitive (%)	Resistance (%)
Imp	106(94.64)	6(5.35)
Tob	7(87.5)	1(12.5)
C	4(80)	1(20)
Ak	83(74.77)	28(25.22)
Gen	71(63.39)	41(36)
Amc	69(61.6)	43(38)
Cac	65(58)	47(41)
Caz	61(54)	51(45)
Cpd/cv	51(45)	61(54)
Amp	47(41)	65(58)
Cot	15(39)	23(60)
Ctr	29(25)	83(74)
Nx	28(25)	82(74)
Azm	2(1.7)	110(98)
Cpd	0	51(100)

Table.4 Comparison of common organisms isolated from different study

Study	Year	Proportion of predominant organisms in various Studies
Present study	**2016**	***E.coli*(34.1%)>>*Klebsiella pneumoniae*(15.1%) >>*Enterococci*(11.4%)>>staph.aureus(7.02%)**
Mishra *et al.,* [9]	2015	*E.coli*(35.8%)>>*Klebsiella pneumoniae*(18.9%)>>*Enterococci* spp(12.9%)
Khan *et al.,*[11]	2015	*E.coli*(52.4%)>>*Klebsiella pneumoniae*(12.3%)>>*Citrobacter*(9%>>)*Enterococci* spp(12.9%)>>*Proteus*(5.3%)
Choudary *et al.,* [15]	2014	*E.coli*(55%)>>*Klebsiella pneumoniae*(17.5%)>>Staph aureus(12.5%)>> *Enterococci* spp(10%)>> *Pseudomonas* spp.(5%)
Jennifer *et al.,* [10]	2009	*E.coli*(71%)>>*Klebsiella pneumoniae*(13.5%)>>*Pseudomonas* spp.(9%)*Citrobacter*(2%), Among GPC *Enterococci* spp(59%)
Akram *et al.,* [16]	2007	*E.coli*(61%)>>*Klebsiella pneumoniae*(22%)>>Staph aureus(7%)>>*Pseudomonas spp.*(4%)>>*Acinetobacter* spp. (3%)

Fig.1

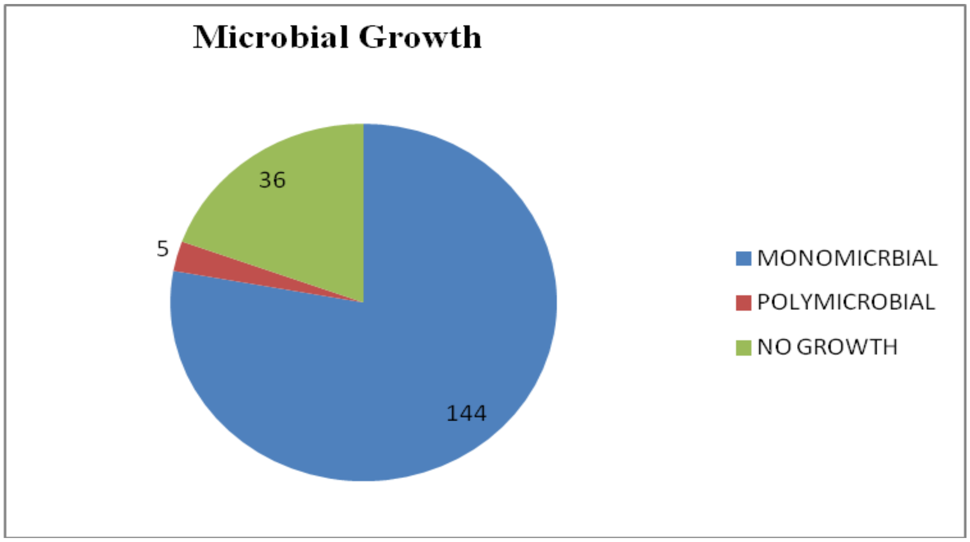

Fig.2 Distribution of uropathogens

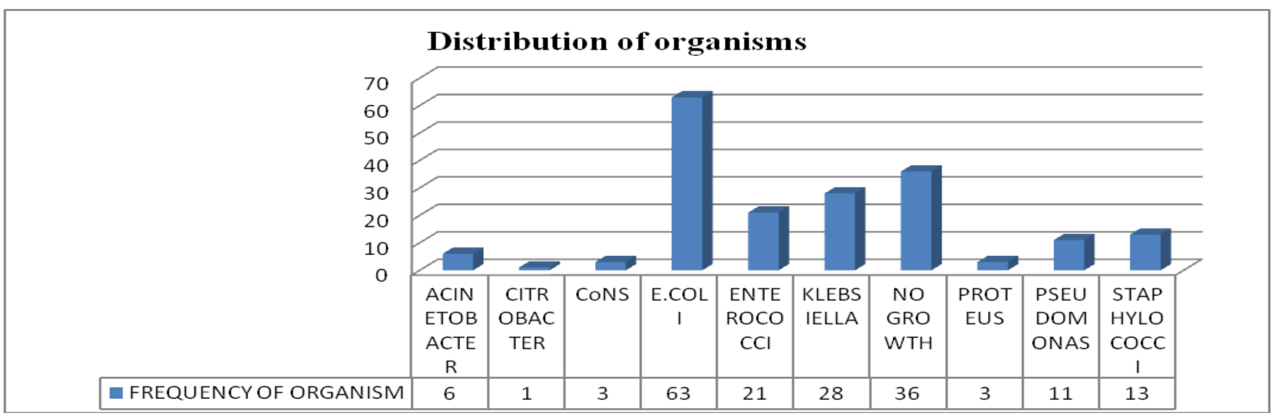

Fig.3 Growth of E. coli on MacConkey agar

Studies by Mishra *et al.,*[9], Akram *et al.,*[16] and Jennifer *et al.,*[10] revealed the involvement of Gram negative enteric organisms that commonly causes urinary tract infections, such as *E. coli,* the *Klebsiella* species, and the *Proteus* species similarly, the predominant number of pathogens isolated in our study were also Gram negative bacilli (Table 4).

In our study proportion of Gram negative isolates was 60.54% almost similar to other studies like Khan *et al.,*[11] (52.4%) and Jennifer *et al.,*[10] (71%).

Proportion of Gram positive isolates in our study was 20%, similar to other studies by Khan *et al.,*[11] (32%) and whereas in a study by Prakash D *et al.,*[17] only (9.68%). The probable reason may be regional variation in prevalence of these organisms.

Among Gram negative uropathogens, *Escherichia coli* was the most common isolated organism (34.1%) followed by *Klebsiella pneumoniae* (15.1%), Pseudomonas spp. (5.9%), *Proteus* (1.6%). Earlier studies by Mishra *et al.,*[9], Khan *et al.,*[11], Choudhary *et al.,*[15] and Jennifer *et al.,*[10] shows *E.coli* was the most predominant isolated organism, followed by Klebsiella spp., These results suggestive of several factors are responsible for attachment of Enterobacteriaceae to the uroepithelium like, they colonize the urogenital mucosa with adhesin and pili.[12,13,14]

Among Gram positive isolates, Enterococcus Species were the most commonly isolated organism 21 (11.4%) followed by *Staphylococcus aureus* 13 (7.02%), in contrast with other study where Enterococcus species were found as the most frequent organism (15%), followed by Coagulase negative *Staphylococcus* (1.6%). Study by Mishra *et al.,* shows *Enterococcus* spp. followed by Coagulase negative *Staphylococcus*, by Akram *et al.,*[16], *Staphylococcus aureus* is the predominant among Gram positive organisms.

In the present study, *E.coli* were highly sensitive to Imepenem(93%), followed by Amikacin (84%), Gentamicin (74%), Amoxy-clav (65%). They were highly resistant to Azithromycin (96%), followed by Ceftriaxone (74%), Norfloxacin (71%).

Klebsiella pneumoniae isolates were sensitive to Imipenem (96%) followed by Ceftazidime with clavulinic acid(75%), Amoxy-clav (71%). They were 100% resistant to Azithromycin followed by Norfloxacin(78%).

Pseudomonas aeruginosa were more sensitive to Imipenem (90%), Tobramycin (85%), Piperacillin with Tazobactum(66%). They were 100% resistant to Azithromycin, followed by Norfloxacin (72%), Ampicillin (90%). Resistance to Fluoroquinolones (Norfloxacin) (74%) were high among Gram negative isolates in this study is similar to other studies by Khan *et al.,*[11] (79%) and by Prakash D *et al.,* [17](90%). This reduced susceptibility is due to irrational use of antibiotics.

Hence among Gram negative isolates, most effective antibiotic was Imipenem followed by Amikacin and Gentamicin and among Gram positive isolates (S. Aureus) most effective antibiotic was Linezolid, Clindamycin, Vancomycin followed by Amikacin.

This difference in percentage of resistance may be because of the number of each isolate in different studies are different and the sample size in each studies were also different.

Another reason may be the geographical variation in the distribution of resistant organisms.

To conclude *E.coli* was the commonest isolate followed by *Klebsiella pneumoniae*, *Enterococci* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter* spp. Most effective antibiotic for Gram negative isolates was Imipenem followed by Amikacin and Gentamicin. These isolates showed highly resistance to flouroquinolones. S. aureus isolates were highly sensitive to Linezolid, followed by Vancomycin, Amikacin. This study would help to make antibiotic policy in our hospital which may helps in better selection of antibiotic therapy to prevent misuse or overuse of antibiotics. Before starting antibiotic therapy to the patients, culture & sensitivity should be done to know the causative organism and its sensitivity pattern in uncomplicated urinary tract infection to avoid the development of multi drug resistance. Before prescribing the antimicrobial therapy, a thorough knowledge of the susceptibility patterns of the uro-pathogens is essential to avoid incongruous and irrational antibacterial usage and to restrain the further development of drug resistance.

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