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Asymptomatic Urinary Tract Infection in Diabetic Patients of District Kohat, Pakistan

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Abstract:

Patients with diabetes mellitus have a higher epidemic involving asymptomatic urinary tract infections (UTIs) as compared with those patients which do not have diabetes mellitus. A total of 60 samples were collected from 20th March 2013 to 20th June, 2013 from diabetic patients with asymptomatic urinary tract infection (UTI) in Kohat, Kohat Development Authority (KDA) teaching Hospital and brought it to Microbiology Laboratory, Department of Microbiology,

Kohat University of Science and Technology, for further process. For examination of E. coli, soon after overnight incubation, the colony forming unit (cfu) was done. All bacterial pathogens were identified on the basis of cultural characteristics, morphology, Gram reactions and biochemical characteristics. The disc diffusion technique was used for antibiotic sensitivity tests. The results were analyzed by using statistical software (Statistic type 9. 0 for home windows 7) and P<0. 005 were considered as significant. A total of Sixty (60) diabetic affected patients with asymptomatic UTI are included. The percentage of males were 22 (36, 67%) and female 38 (63, 33%) of age 30-90 years. A total connected with 23 (38. 3 %) isolates demonstrated significant growth connected with uropathogenic. Out of which twenty 20 (87%) ones showed growth connected with Gram positive in addition to Gram-negative pathogens, two 02 (9%) ones showed fungal development (Candida spp.), One 01 (4.3%) demonstrated yeast. Antibiotic sensitivity examination was performed just for bacterial isolates. In this particular study, the incidence of asymptomatic UTI has been (38. 3%) amid total diabetic members, in women, this prevalence was (73. 9%) in addition to in men this prevalence was (26. 08%). It was seen that growth rate was better in type2 diabetic patients of 40-60 years old as compared in order to other groups. The most common isolates were Escherichia coli (52. 1%). E. coli was most resistant to antibiotic that have been used in the research and among these gram-positive pathogens, the S. aurous has been 100% sensitive in order to FEP, CAZ, CRO while showed 100% resistivity in order to CIP. It was concluded that the incidence of UTI is higher in females as compared to male. E.coli, Klebsiella spp. and Staphylococcus aureus is the most common isolates in female's subjects and in case of male patient E. coli was also a principal ethological agent of UTI. It was also observed that the diseases incidence increase with increasing age. The aims of this study were to determine the prevalence of Asymptomatic Urinary Tract Infection and assess the antimicrobial susceptibility pattern of the isolates in Diabetic Patients.

Key words: *E.coli*, Asymptomatic UTI, Colony forming Unit, Antibiotic Sensitivity.

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Introduction

Patients with diabetes mellitus have a very higher epidemic of asymptomatic urinary tract infections as compared with patients without diabetes mellitus [1].UTIs are one of the most common microbial infections worldwide through an estimate regarding 150 million cases a year and cause a cost in excess of 6 million dollars [2, 3].

Conclusive info regarding the morbidly weight of UTIs with Pakistan are lacking. Uncomplicated local community acquired UTI (CA-UTI) is normally caused by simply *E. coli* [2-4]. Some other pathogens implicated are *Klebsiella* spp, *Proteus* spp, *Pseudomonas* spp, *Enterobacter* spp as well as *Staphylococcus saprophyticus* amongst others [4].

The difficulties of UTIs such as emphysematous cystitis, pyelonephritis along with renal papillary necrosis occur more commonly in themes with Type 2 diabetes mellitus [5]. The urinary tract will be the principal site of contamination in diabetic patients. 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 [6].UTI continues to be an important & some sort of frequent root cause of morbidity & mortality in our community & mostly women are usually predisposed. UTI include the most generally found bacterial infections, accounting for pretty much seven million OPD visits and one million urgent situation department appointments, resulting with 100,000 hospitalizations involving women, older people, and patients with spinal-cord injuries and/or catheters, multiple sclerosis, HIV, as well as diabetes [8].

It is strongly recommended that high glucose awareness in urine may favor this growth involving pathogenic germs [9]. Antibiotic opposition of euro-pathogens is usually increasingly becoming reported with high happening of multiple drug resistant strains [10]. Ciprofloxacin has become shown to be effective next to most urinary isolates [11]. The present study was aimed to find out Asymptomatic Urinary Tract Infection in Diabetic Patients of District Kohat and to be used as references in guidelines in the evaluation and management of diabetic patients.

Materials and Methods

Sample Collection & Processing:

A total of 60 samples was collected from 20th March, 2013 to 20th June, 2013 from diabetic patients with asymptomatic urinary tract infection (UTI), in Kohat, KDA teaching Hospital and brought to the Microbiology Laboratory, Department of Microbiology, Kohat University of Science and Technology for further process. The samples, containing 10-20ml volume each sample, were collected in particular labeled sterile, dry, wide mouth and leak proof container from only type 2 diabetic patients, including both genders; aged 20-90 years during OPD through a questionnaire. The specimens were processed following standard guidelines [12]. Urine wets mount and gram stains examination was done in the presence of pus cells and bacteria. Presence of >5 polymorphonuclear leukocytes/ high power field (which correlates with WBC excretion rate of >400,000 WBC/hr) indicated pyuria which is evidence of an inflammatory response in the urinary tract [12,13]. And; one organism per oil-immersion field in gram stain of uncentrifuged urine would suggest bacteriuria with a colony count of $\geq 10^5$ CFU/ml of urine [12]. Specimens were inoculated onto blood agar and MacConkey agar by as standard loop method for semiquantitative culture [14].

Examination of *E. coli* and other bacterial pathogens

For examination of *E. coli*, soon after overnight incubation, the colony forming model (cfu) was done. The samples showing the

number of colonies $> 10^5$ was thought to be pathogenic count pertaining to $E\ coli$. It was also considered as significant bacteria as well as the isolates were afflicted by further antibiogram evaluation. If the colony forming unit (cfu) remained a lot less than 105, it was thought to be nonsignificant growth in case there is $E\ coli$ or maybe negative sample.

The examination for other bacterial pathogens were made judging by cultural characteristics, morphology, Gram allergic reactions and biochemical characteristics. When confirmation ended up being specifically needed in the event of Gram negative microorganism related to Enterobacteriaceae family, the particular API20E (Biomerieux) kit was also used. Once identified, the particular samples positive intended for Gram negative bacteria, and Gram positive bacteria were subsequently further tested intended for antimicrobial susceptibility applying the Kirby Bauer method on Muller Hinton agar [15]. Furthermore, the isolates were identified by biochemical reactions using standard methods [14].

Antibiotic sensitivity test

The antibiotic sensitivity test was performed for all identified organisms. The disc diffusion technique was used for this test as used by [12,15,31]

Statistical analysis

The results were analyzed by using statistical software Statistix type 9. 0 for home windows 7. The results for all variables were presented within percentage (%). Chi Square tests were used to find out positive association among these categorical variables. P<0.005 were considered as significant.

Results and Discussion

A total of (60) diabetic patient with asymptomatic UTI,

including male 22 (36.67%) and females 38 (63.33%) having 30-90 years ages (table. 1). A total of 23 (38.3 %) isolates showed significant growth of uropathogen. Out of which twenty 20 (87%) of them showed growth of Gram positive and Gramnegative pathogens, two 02 (9%) of them showed fungal growth (Candida spp.), One 01(4.3%) showed yeast. An antibiotic sensitivity test was performed only for bacterial isolates. In this study, the prevalence of asymptomatic UTI was (38.3%) among total diabetic participants, in women the prevalence was (73.9%) and in men the prevalence was (26.08%). It was observed that growth rate was much higher in typ2 diabetic patients of 40-60 years of age as compared to other groups (table 1). The commonest isolates were Escherichia coli (52.1%), Klebsiella pneumonia (8.6%), Staphylococcus aureus (4.3%), Candida spp. (8.7%), Proteus mirabilis (8.7%), Pseudomonas aeruginosa (8.7%), S. saprophyticus (4.3%) and yeast (4.3%) were found (table 2). In this study the clinical history was collected from the type 2 diabetic patients, questions such as socioeconomic status like marital status, employment status, income status and literacy level were asked. Table 3 showed that ASB incidence was higher in patients that were unmarried (62.5%), had low literacy lever (53.3%), where employee showed (40%) and having low-income patients showed (88.6%). Table 4 showed that ASB associated risk factors were dissimilar in all patients like the frequency of ASB was higher in patients having lumbar pain (90%), those having dysuria (56.8%) and urgency (56.7%). ASB frequency was also significantly higher in patients having hematuria (46.7%) and nocturia (42.2%).

Culture characteristics of the isolate

MacConkey agar was used for the isolation of Gram negative rods. It inhibits the growth of gram positive cocci due to the presence of salts and crystal violet in its composition. By utilizing the lactose available in the MacConkey agar, *Escherichia coli*, *Enterobacter* and *Klebsiella* produce acid,

which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies. Non lactose fermenting bacteria such, as *Proteus* species use peptone, forming ammonia, which raises the pH of the agar and leads to the formation of white/colorless colonies.

Antibiotic sensitivity

After 24 hours of incubation, the culture plates with antibiotic discs were examined for the presence of growth inhibition, which is indicated by a clear zone surrounding Standards each disc. The susceptibility of organisms was determined, using Clinical and Laboratory Institute (CLSI) recommendations and expressed the results in mm; accordingly, the results were recorded in each investigation as from Sensitive (S) to Resistant (R) respectively. It was found that the leading Gram negative uropathogen E. coli showed highest sensitivity (83%) to ceftriaxone and low susceptibility (50%) to ciftazidine. The highest sensitivity of K. pneumoniae observed was (100%) to meropenem and low susceptibility (0%) to ciprofloxacin. P. mirabilis was (100%) sensitive to augmentin and their low susceptibility was (0%) to cefepime. The highest susceptibility of p. aeruginosa was (100%) to cefepime and low sensitivity against ceftazidine was (0%). During observing the sensitivity of Gram positive bacterial pathogens, it was noted that the highest sensitivity of S. aureus was (100%) to cefemine and their low susceptibility was (0%) against ciprofloxacin. The S.saprophyticus was found to show (100%) sensitivity to augmentin and meropenem and show (100%) resistivity to ceftazidine (table 5 and 6).

Table 1. Distribution of Patients in Relation to their Age Group

Age Group	No of Males	%age	No of Females	%age
31-40 years	3	25.00	9	75.00
41-50 years	4	33.33	8	66.67
51-60 years	8	36.36	14	63.64
61-70 years	7	58.33	5	41.67
71-80 years	0	0.00	2	100.00
Total	22	36.67	38	63.33

P=0.0000<0.05 Significant

Table 2. Distribution of the Uropathogenic isolates from the UTI culture.

Name of Bacteria	No. of isolates	Percentage
Klebsiella spp.	2	8.7
$Proteous\ mirabilis$	2	8.7
$Pseudomonas\ spp.$	2	8.7
Staph. aureus	1	4.3
Staph. saprophyticus	1	4.3
E.coli	12	52.1
$Candida\ spp.$	2	8.7
Yeast	1	4.3
Total	23	99.8%

P=1.0000 >0.05 No significant

Table 3. Distribution of patients based on socioeconomic status.

S.NO	Characteristics	Total .NO (%)	ASB (+) %	ASB (-) %
1	Marital status:			
	Married	52(86.7%)	18(34.6%)	34(65.3%)
	Unmarried	08(13.3%)	5(62.5%)	3(5.8%)
2	Literacy level:			
	Primary	30(50%)	16(53.3%)	14(46.7%)
	Secondary	10(16.7%)	3(30%)	07(70%)
	Graduate	12(20%)	3(25%)	09(75%)
	Postgraduate	08(13.3%)	1(12.5%)	07(87.5%)
3	Employment level:			
	Employment	15(25%)	06(40%)	09(60%)
	Unemployment	45(75%)	17(37.8%)	28(62.2%)
4	Income Status:			
	10,000-20,000	35(58.3%)	13(88.6%)	22(62.9%)
	21,000-30,000	15(25%)	07(46.7%)	08(53.3%)
	31,000-40,000	10(16.7%)	03(30%)	07(70%)

Table 4. Distribution of patients on the basis of ASB associated risk factors.

S.NO	Characteristics	Total .NO (%)	ASB (+) %	ASB (-) %
1	Urinary frequency			
	2- times	55(91.7%)	19(34.5%)	36(65.4%)
	Once	05(8.3%)	04(80%)	01(20%)
2	Dysuria			
	Yes	37(61.7%)	21(56.8%)	16(43.2%)
	No	23(38.3%)	02(8.7%)	21(91.3%)
3	Hematuria			
	Yes	15(25%)	07(46.7%)	08(53.3%)
	No	45(75%)	16(35.6%)	39(86.7%)
4	Urgency			
	Yes	30(50%)	17(56.7%)	13(43.3%)
	No	30(50%)	06(20%)	24(80%)
5	Nocturia			
	Yes	45(75%)	19(24.2%)	26(57.8%)
	No	15(25%)	04(26.7%)	11(73.3%)
6	Lumbar pain			
	Yes	10(16.7%)	09(90%)	01(10%)
	No	50(83.3%)	14(28%)	36(72%)
7	Co-morbidity			
	Yes	14(23.3%)	04(28.6%)	10(71.4%)
	No	46(76.7%)	19(41.3%)	27(58.7%)

Table 5. Antimicrobial susceptibility rates (%) of Gram Negative Bacteria $\,$

*Antibiotics	•	E.coli	K.pneumoniae	P.mirabilis	P.aeruginosa
		(12)	(2)	(2)	(2)
MRP	Sensitivity	66.7%	100%	55%	50%
	Resistant	33.3%	0%	45%	50%
TEP	Sensitivity	75%	50%	0%	100%
	Resistant	25%	50%	100%	0%
CAZ	Sensitivity	50%	0%	60%	45%
	Resistant	50%	100%	40%	55%
CRO	Sensitivity	83.3%	50%	50%	60%
	Resistant	16.7%	50%	50%	40%
CIP	Sensitivity	58.3%	55%	55%	50%
	Resistant	41.7%	45%	45%	50%
AUG	Sensitivity	75%	100%	100%	45%
	Resistant	25%	0%	0%	55%
LEV	Sensitivity	33.3%	45%	0%	100%
	Resistant	66.7%	55%	100%	0%

^{*} CAZ, Ceftazidine; FEF, Cefapime; CIP, Ciprofloxacin; LEVO, Leofloxacin; MRP, Meropenem; AMC, Augmentin

Table 6. Antimicrobial susceptibility rates (%) of the Gram positive Uropathogens

*Antibiotic		Organism		
		S.aureus	S.saprophyticus	
SEF	Sensitivity	100%	100%	
	Resistant	0%	0%	
CAZ	Sensitivity	100%	0%	
	Resistant	0%	100%	
CIP	Sensitivity	0%	100%	
	Resistant	100%	0%	
CRO	Sensitivity	100%	NID	
	Resistant	0%	NT	
MRP	Sensitivity	NT	100%	
	Resistant		0%	

*SEF, Cefapime; CAZ, Ceftazidine; CIP, Ciprofloxacin; CRO, Ceftriaxone, MRP, Meropenem, NT, Not Tested

Discussion

Asymptomatic bacteriuria is widespread involving diabetics and can lead to severe troubles otherwise effectively maintained [16]. UTIs are definitely more serious with diabetics concerning life-threatening troubles, including emphysematous pyelonephritis and renal papillary necrosis [16]. Despite the incredible importance of bacteria to diabetes sufferers; there exists a paucity associated with information on the connection in between UTI and diabetes especially with sub-Saharan Cameras. Reviews around the epidemic and etiology associated with ASB seem contradictory especially in developing countries. Therefore, this current analyze seemed to be targeted to look for the epidemic and array associated with germs accountable for asymptomatic bacteriuria with diabetes sufferers also to establish antimicrobial resistance associated with urinary isolates.

In the current study, asymptomatic bacteriuria was detected in a total of 60 diabetic patients 38.3%, including 26.08% male and 73.9% females, which were similar to the study overall prevalence rate was 36% and which was also higher in females (43.18%) as compared to males (30.35%)[17]

and 36.15% in Nigeria [10] while less than the study having asymptomatic bacteriuria 59 (47.2%) and urinary tract infections were present in 43 (34.4%) out of the 125 diabetic patients [18]. In the current study the uropathogen most associated with asymptomatic UTI diabetic patients was E. coli (52.1%) which was slightly similar to the study showing 49 (48.0%) [18]. This result was lower than the 59.6% obtained [10] in Nigeria. The majority of reports where *E. coli* prevalence was very high in UTIs [19-22]. Other prevalent uropathogens were isolated by another study in which they Staphylococcus aureus 20 (19.6%), Proteus mirabilis 9 (8.9%), Streptococcus pyogenes 8 (7.8%), fungal isolates 7 (6.9%) Klebsiella pneumoniae 5 (4.9%) and Enterococcus faecalis 3 (2.9%), and Pseudomonas aeruginosa 1 (1.0%) [18]. These pathogens were similar to those observed in other studies [10, 23, 24, 25].

The risk factors for UTI involve colonization with a different uropathogens in instances of recurrent UTI, glucosuria and also impaired granulocyte function [26]. Beside it, a variety of risk factors pertaining to ASB in females with diabetes have been suggested including age, disease duration, renal microangiopathy (proteinuria, albuminuria), UTI in the previous year, sexual task, lower body size index and standing problems (retinopathy, diabetic nephropathy neuropathy) [27]. The bacteria causing UTI in diabetic patients are the same as in complicated UTIs throughout non-diabetic patients. Many of us also found in which Ecoli was main organism. This finding is similar to other previous reports were E-coli staying the predominant organism [28] reported from India.In the current study E. coli was most resistant to antibiotic which were used in the study which also supported by other studies [15]. Among the gram-positive pathogens, the S.aureus was 100% sensitive to FEP, CAZ, and CRO while showing 100% resistant to CIP. Which was slightly dissimilar to different the studies in which the highest sensitivity of K. pneumoniae observed 80% to was cefapime and low susceptibility 13% to ciprofloxacin [15]. The S. saprophyticus showed 100% sensitivity to MRP, CIP, FEP while showed 100% resistivity to CAZ which was similar to the results obtained by other study [15]. Among the gram-negative pathogens, E.coli was 66.7% sensitive to MRP and 33.3% resistant to MRP. K.pneumoniae was 100% sensitive to MRP and 0% resistant to MRP. CRO, CIP and AUG showed higher activity against the isolated pathogens which were supported by the study similar to our results [15]. In other countries Ciprofloxacin resistant strains were found [29] as well as in the Indian study [30].Our results suggest that the following antibiotics, norfloxacin, nalidixic acid, imipenem, oxacillin, erythromycin, nitrofurantoin, vancomycin, Ampicillin, Oxacillin, Vancomycin, Amikacin. Nitrofurantoin. Trimethoprim. Norfloxacin. Cefuroxime, trimethoprim and Methicillin can be chosen in the management of UTIs by the clinicians after having the culture sensitivity results. Further than for prevention involving UTIs implementation involving strict infection manage guidelines. effective hand cleanup and judicious by using antimicrobials is essential which goes a long way to cope upward, with the breakthrough of drug weight among uropathogens [15] as well as should be follow the roles strictly applied for clinic and laboratory methods.

Conclusion and Recommendation

It was concluded that the incidence of UTI is higher in females as compared to male. *E.coli*, Klebsiella spp. and Staphylococcus aureus are the more common isolates in female's subjects and in case of male patient *E.coli* was also principal ethological agent of UTI. It was also observed that the diseases incidence increase with increasing age and vice versa. The Socioeconomically weak patients were more susceptible to UTI than other. The current study suggested that the urine culture might

be recommended in diabetic patients who show no urinary symptoms but who have one or more of the risk factors identified. Prevention of UTI can get through implementation of strict infection control guidelines, effective hand washing and judicious use of antimicrobials.

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