Bacterial Etiological Agents Associated with Urinary Tract Infection and Their Antibiotic Susceptibility in Diabetic and Non-Diabetic Women[+]

ا لمسببات الجرثومية لالتهاب المجاري البولية وحساسيتها للمضادات الحياتية في النساء المصابات بداء السكري وغير المصابات بة

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Abstract:

Diabetes Mellitus (DM) was a risk factor for urinary tract infection (UTI), so to study if DM have any effect on percentage of infection , type of isolated bacteria and their pattern of antibiotic susceptibility , a total of 200 urine samples were collected from diabetic (100) women and non-diabetic (100) women attending Al-Kadhymia teaching hospital (hospitalized and non- hospitalized). Urine samples were cultured and the results revealed that *Escherichia coli* was the most predominant bacteria associated with UTI, and there was no significant differences for percentages and isolated bacterial species from the two studied groups.

The antibiotic susceptibility for the four predominant species of bacteria (*E.coli ,Enterobacter aerogenes ,Pseudomonas aerugenosa and Klebsiella pneumoniae*) revealed high resistance to ampicillin, trimethoprim and susceptibility to Ciprofloxacin. So ciprofloxacin which may be the drug of choice for the treatment of UTI in both groups . DM had no effect on the type of bacteria isolated or their pattern of antibiotic susceptibility.

المستخلص :

يعد داء السكري أحد العوامل المساعدةعلى التهاب المجاري البولية ولغرض دراسة تأثير داء السكري علىنسبة و نوع الجراثيم المعزولة ونمط الحساسية للمضادات الحيوية, فقد تم جمع٢٠٠ نموذج ادرار من النساء المصابات بالسكري(١٠٠) وغير المصابات به(١٠٠) من الراقدات والمراجعات لمستشفى الكاظمية التعليمي وقد تم زرع نماذج الادرارعلى الاوساط الزرعية وأظهرت النتائج ان *E.coli* هي الجرثومة الأكثر شيوعاً والمسببة لالتهاب المجاري البولية ولم تسجل فروق معنوية في أنواع ونسب الجراثيم المعزولة من النساء المصابات بالسكري وغير المصابات به.كذلك تم دراسة نمط الحساسية للمضادات الحيوية للأنواع الأربعة السائدة من الجراثيم *E.coli*, *Pseudomonas aeronenosa* ,*Klebsiella pneumoniae* (*Enterobacter aerogenes*)والمعزولة من كلا المجموعتين وقد أظهرت هدة الجراثيم مقاومة عالية

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للأمبسلين والترايمثبريم كما أظهرت حساسية للسبروفلوكساسين والدي يمكن اعتبارة العلاج المناسب لالتهاب المجاري البولية في المجموعتين و ظهر بأن داء السكري لم يكن له تأثيرا على نسب و نوع الجراثيم المعزولة ولا على نمط الحساسية للمضادات الحيوية لتلك الجراثيم.

Introduction :

Diabetes Mellitus has long been considered to be a predisposing factor for urinary tract infection . The UTI is the microbial invasion of the urinary tract, excluding from the renal cortex to the urethral meatus [1] .

In females, the urinary tract has an important association with the reproductive organs because of its proximity. In healthy individuals, the freshly voided urine is sterile and free from microorganisms; this is why UTI are more common in females [2] . Women with diabetes have higher risk of UTI because of changes in immune system. Any other disorder that suppresses the immune system raises the risk of urinary infection .The length of female urethra is a design flaw which predispose women to UTI. It is very short and easily contaminated since it is very close proximity to the vulvar and perianal regions [1] . In a study conducted by [3] , they found that UTI was the most frequent cause of infection in diabetic women. A changed in bacterial adhesion [4] , granulocyte dysfunction [5] and impaired antioxidant system involved in bacterial activity [6],are all involved in the pathogenesis of UTI in diabetics.

Materials and methods :

Patients and samples :

Mid stream urine samples were collected from 200 hospitalized and non-hospitalized women suffering from urinary tract infection in sterile screw capped tubes. 100 women were non-diabetic and 100 women were diabetic .

Isolation of bacterial species :

The urine samples were cultured on blood agar, MacConkey agar and Cysteine Lactose electrolyte deficient agar and the plates were incubated at 37 °c for 24 hours. A significant growth was obtained as more than ($10^5 \geq$CFU/ml) of pure culture [7] .Bacterial species were diagnosed according to colony morphology and color on CLED media ,the results of confirmatory biochemical tests (kligler media ,indole ,methyl red ,voges-proskauer,simmon's citrate ,semisolid manitol and oxidase test, coagulase ,catalase , novobiocin sensitivity test) according to [8]

Antibiotic susceptibility test : It was done using agar disc diffusion method as described by [9].

The ten types of antibiotic discs used and their concentration were: ampicillin 10 µg, augmentin 30 µg, piperacillin 100 µg, ciprofloxacin 10 µg, trimethoprim 5 µg,

norfloxacin 10 µg, 10 µg, cefotaxime 30 µg, amikacin 30 µg, gentamicin 30 µg and ceftazidime 30 µg.

Statistical analysis: were conducted to describe the relationship of different variables with each other .Chi-Square was used for quantitative and descriptive data to reflect on the level of significance of differerce and / or the association between variables.

Results :

Diabetic females were more likely to present with asymptomatic bacteriuria (presence of at least 10^5 CFU/ml of urine in one culture).

The types and percentage of bacterial species isolated from the two groups of women with their statistical analysis were listed in table 1.

Table (1): Comparison the types of bacterial species isolated from diabetic and non-diabetic women

by Chi-square test

Bacterial type	Diabetic n=100		Non-diabetic n=100		Statistical Significance
	no.	%	no.	%	
Escherichia coli	46	46	42	42	NS
Enterobacter aerogenes	16	16	11	11	NS
Pseudomonas aeruginosa	14	14	13	13	NS
Klebsiella pneumoniae	10	10	14	14	NS
Proteus mirabilis	8	8	2	2	NS
Citrobacter spp	--	--	3	3	NS
Serratia marcescens	--	--	2	2	NS
Enterococcus faecalis	6	6	7	7	NS
Staph. aureus	--	--	3	3	NS
Staph. saprophyticus	--	--	3	3	NS

Staph = staphylococcus , Spp = species ,No. = number , % = percentage

NS= not significant

The results showed that *E.coli* was the most predominant organism caused UTI in both diabetic and non-diabetic groups of patients (46% , 42%) respectively.

In case of diabetic women, *E.coli* was followed by *Enterobacter* aerogenes (16%), *Pseudomonas aeroginosa(14%)*, and *Kllebsiella pneumoniae(*10%), while in non-diabetic group *E.coli* was followed by *Klebsiella pneumoniae*(14%), *Pseudomonas aeroginosa*(13%)and *Enterbacter* aerogenes (11%).

Citrobacter and *Serratia* marcescens were not isolated from diabetic women and *Staphylococcus* spp were isolated from non-diabetic women only while *Proteus mirabilis* and *Enterococcus* faecalis were isolated from both diabetic and non-diabetic group in low numbers.

There was no statistical significant differences between percentage and the bacterial species isolated from the two studied groups .

The pattern of antibiotic susceptibility for the four predominant organisms isolated from both groups was determined and listed in table 2.

Table 2: Pattern of antibiotic susceptibility (percentage of sensitivity) .

Antibiotic disc	***Escherichia coli***		***Enterobacter aerogenes***		***Pseudomonas aeruginosa***		***Klebsiella pneumoniae***	
	D	ND	D	ND	D	ND	D	ND
Ampicillin	9	17	31	55	R	R	R	R
Augmentin	20	40	25	18	R	8	20	36
Amikacin	59	50	44	55	64	69	60	43
Gentamicin	72	60	69	46	43	54	60	57
Ciprofloxacin	80	69	69	46	71	62	70	71
Norfloxacin	50	55	31	9	14	23	40	43
Ceftazidine	55	52	13	36	64	46	20	43
Cefotaxime	61	48	25	27	7	8	60	50
Piperacillin	22	19	44	36	7	15	10	29
Trimethoprim	33	26	25	36	R	R	20	36

D = diabetic , ND = non-diabetic , R= Resistant.

E.coli and *Enterobacte*r spp were susceptible to most antibiotics used in both groups of patients . *Pseudomonas* spp. Were completely resistant to Ampicillin ,Trimethoprim and Augmentin in diabetic patients .*Klebsiella* spp. Were resistant to Ampicillin only .The four bacterial species were susceptible to Ciprofloxacin in high percentage in both groups of patients .

Discussion :

Diabetes and urologic diseases are very common health problems that markedly increase in prevalence and incidence with advancing age.Diabetes is associated with an earlier onset and increased severity of urologic diseases resulting in costly and debilitating urologic complications [10]**.** The colonization of uropathogens in urinary tract of those patients can accelerate the prolonged release of bacteria from urinary tract which may cause bacteriuria [11] .

The most common cause of UTI in women with DM and without DM was *E.coli* and this result was also in agreement with the results observed by [1&2] .Some reports have noted that a lower proportion of UTI is caused by this organisim in diabetic patients as compered with non diabetic patients [11] .

The four predominant types of bacterial species exhibited high percentage of susceptibility to ciprofloxacin. Ciprofloxacin interferes with nucleic acid synthesis by inhibiting the enzyme gyrase, this antibiotic has several binding sites on the enzyme, thus decreases the likehood of resistance. Ciprofloxacin have exellent bioavailability and achieve high urinary concentration [1 2] .

High percentage of *E.coli* isolates were resistant to ampicillin and this was due to the production of β-lactamase by *E.coli* isolates .The resistance of *E.coli* to cefotaxime is attributed also to β-lactamase enzyme production by these bacteria and resistance to trimethoprim is due to dihydrofolate reductase gene produced [13].

Bacterial resistance to aminoglycosides (amikacin and gentamicin) are mediated by enzymatic modification of various sites on the antibiotic, alter the target ribosome ,decrease the drug uptake and due to drug efflux .The resistance is usually transferable, especially among members of the family enterobacteriaceae which are the predominant organisms implicated in UTI [14] .

Pseudomonas was completely resistant to three antibiotics (ampicillin ,trimethoprim and augmentin in diabetic patients which revealed the presence of multidrug resistance and this was in agreement with the results of [14] who found that *Pseudomonas* aerugenosa isolated from urine were multidrug resistance because they were resistant to three or more of the tested antibiotics .

In the seventies MDR was practically not existent and the cause was restricted to mutation of chromosomal genes ,however during the last two decades bacterial resistance mediated by plasmids which carry the resistance genes to a large number of antibiotics which are rapidly transferred has worsen the scenario [14] .Barman et.al. [15] have suggested that MDR may be linked to integrons which are genetic elements capable of recombination ,they report antimicrobial resistance genes clustered in integrons according to them resistance to ampicillin ,trimethoprim ,nalidixic acid ,tertracyclin, gentamycin , are common in isolates with integrons .

Klesiella pneumoniae was an important cause of UTI formed biofilm –like intracellular bacterial communities (IBCs). The IBC pathway may be aconserved mechanism by which many uropathogen sevade the host innate immune response .Uropathogens may form these intracellular biofilmsto multiply unhindeced within the protected niche of the urothelial cells and even escape antibiotic penetration [16] .

The study conducted to focus on the importance of UTI prevention, early detection and eradication of UTI in order to reduce the life threatening consequences of persistent or repetitive infections.

Ciprofloxacin appeared to be the drug of choice for the treatment of UTI in both diabetic and non-diabetic women. Antibiotic therapy should be commenced only after culture and sensitivity tests have been carried out to avoid emerging drug resistance amongst bacteria, this will discourage the indiscriminate use of the antibiotic, and self medication should be avoided in order to prevent spread of multiple drug resistant strains of bacteria.

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