ANTIBIOTIC RESISTANCE OF BACTERIA IMPLICATED IN URINARY TRACT INFECTIONS IN DIABETIC WOMEN

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ABSTRACT

Urinary tract infections are amongst the most common pathogenic infections with an increasing resistence to antimicrobials. Isolates from urine samples were identified and their susceptibility to antimicrobial agents were studied , also synergetic and antagonism of the combined antibiotics against bacterial isolates and electron microscope study against multi-drug resistent bacteria were studied . During the study , 211 urine samples from diabetic and non diabetic female patient were analysed, of which 115 had significant bacteriuria (98 from diabetic and 17 from non diabetic). For diabetic patient, Escherichia coli was the most common etiologic agent 27 (27.6%) followed by Serratia 12(12.2%), Citrobacter, Enterobacter and Micrococci each one was 10(10.2%) . Salmonella 7(7.1%), Pseudomonas aeruginosa and Coagulase negative staphylococci each one was 6(6.1%), Proteus 5(5.1%), Staphylococcus aureus and Shigella each one was 2 (2%) and Streptococci 1(1%). also for non diabetic patient E.coli was the most common etiologic agent 10(58.8%) followed by Enterobacter and Proteus each one was 3 (17.6%) and Streptococci 1(5.9%) . Also we studied the relationship between percentage of infection and age of patients. Amikacin, ciprofloxacin and norfloxacin expressed the most effective antibiotics on these isolates but all these isolates were resistent to ampicillin/ sulbactam, erythromycin, amoxicillin flomoxin clavulanic acid and piperacillin except Salmonella was sensitive to amoxicillin flomoxin clavulanic acid and Enterobacter was sensitive to piperacillin. the combination between ciprofloxacin and cefotaxim sodium is the most one which showed synergetic against bacterial isolates followed by ciprofloxacin and amikacin and amikacin and nitrofurantion. Pseudomonas aeruginosa was resistent to all antibiotic which used but showed synergetic against the combination between ciprofloxacin and amikacin, ciprofloxacin and norfloxacin, norfloxacin and amikacin and amikacin and nitrofurantion, also, the cell of Pseudomonas aeruginosa appear normal under electron microscope when not treated with norfloxacin but when treated with norfloxacin the cell become longer .

الملخص العربى

مقارنة البكتريا المسئولة عن إصابات الجهاز البولى التناسلى للمضادات البكتيرية

فى النساء المصابات بمرض السكر

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تعد إصابات الجهاز البولى التناسلى من أكثر الإصابات البائرلوجية شيوعاً والمصحوبة بزيادة المقاومة للمضادات البكتيرية، وقد أجريت هذه الدراسة لعزل البكتيريا من عينات بول تم تجميعها من نساء مصابات بمرض السكر وأخريات غير مصابات وتعريفها وتحديد مدى حساسيتها للمضادات البكتيرية، تم أيضاً دراسة التوافق والتضاد الناتج من الجمع بين المضادات البكتيرية ضد المعزولات البكتيرية.

خلال هذه الدراسة تم لمجموع ٢١١ عينة بول حيث وجد من بينهم ١١٥ حالة تعانى من وجود بكتيريا فى البول ٩٨ مصابات بمرض السكر و١٧ غير مصابات)، بالنسبة لمرضى السكر كانت الأشريكية القولونية هى أكثر الأسباب شيوعاً ٢٧ (٢٧,٦١٪) ثم السراتيا ١٢ (١٢,٢٪) ثم الستروبيكتر والأنتيروبيكتر والميكروكوكى كلاً منهم ١٠ (١٠,٢٪) ثم السلامونيلا ٧ (٧,١٪) ثم السودومونس أيروجينوزا والمكور العنقودى السالب لاختبار الكواجيميوليز كلا منها يمثل ٦ (٦,١٪) والبروتيس ٥ (٥,١٪) والمكور العنقودى أوريس والشيجيلا كلاً منها كان ٢(٢٪) والمكور السبحى ١(١٪). وأيضاً بالنسبة لغير المصابات بمرض السكر كانت الأشريكية القولونية هى أكثر الأسباب شيوعاً ١٠ (٥٨,٨٪) ثم الأنتيروبيكتر والبروتيس كلاً منهما ٣(١٧,٦٪) والمكور السبحى ١(٥,٩٪).

وقد تم دراسة العلاقة بين نسبة الإصابة وعمر المرضى، من خلال الدراسة وجد أن الأميكاسين والسيبروفلركساسين والنورفلركساسين كانت هى الأكثر فاعلية ضد هذه المعزولات وكانت جميع المعزولات مقاومة لمضادات الأمبسلين والأوسرومبسن والأمكسيسلين فلرموسين حمض الكلافيولنك والجيبراسلين ماعدا السلامونيلا كانت حساسة للأمكسيسلين فلرموسين حمض الكلافيولنك والأنتيروبكتر كانت حساسة للجيراسلين، وكان الجمع بين السيبروفلركساسين مع السيفاتكسام صوديوم قد أظهر تأثيراً توافقياً ضد المعزورلات البكتيرية يليه الجمع بين السيبروفلركساسين مع الأميكاسين والجمع بين الأميكاسين مع النهتروفليورونيبرن، كانت السودومونس أيروجينوزا مقاومة لجميع المضادات البكتيرية المستخدمة لكنها أظهرت تأثراً عند الجمع بين السيبروفلركساسين مع النورفلركساسين والنورفلركساسين مع الأميكاسين والأميكاسين مع النيتروفيورنتوين.

وقد ظهرت خلية السودومونس أيروجينوزا بشكل طبيعى تحت الميكروسكوب الإلكترونى عندما لم تعالج بالنيتروفيورنتوين لكن عند العلاج بالنيتروفيورنتوين فقد أصبحت الخلية أكثر إستطالة.

INTRODUCTION

Infections of the urinary tract are the second most common type of infection in the body. Urinary tract infections (UTIs) account for about 8.3 million doctor visits each year. Women are especially prone to UTI. One woman is five develops a UTI during her lifetime.

During any given year, 11 percent of women report had a urinary tract infection. and more than half of all women have at least one such infection during their lifetime(Foxman et al 2000).

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract . Normally. urine is sterile and it is usually free of bacteria. viruses, and fungi because it contains a variety of fluids. salts, and

waste products but the infection occurs when any organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply, the most of these organisms is Escherichia coli (E. coli), which normally lives in the colon, in addition to E.coli ,other organisms such as Klebsiella spp, Proteus spp, Enterobacter spp, Citrobacter spp, Salmonella other Enterobacteriaceae, Staphylococcus spp, Streptococci, enterococci and Pseudomonas spp may be involved . In most cases bacteria travel to the urethra and multiply causing urethra infection (urethritis) and if the bacteria move to the bladder and multiply, a bladder infection (cystitis) can occur. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and reach to the kidneys causing kidney infection (pyelonephritis) which is much more serious because it leading to kidneys damaged if a UTI is not treated for months or years. The urinary system is structured in a way that helps ward off infection in which the ureters and bladder normally prevent urine from backing up toward the kidneys and the flow of urine from the bladder helps wash bacteria out of the body (Meyhoff et al 1981, Jepson et al 2000, Liza et al 2003, Bethesda 2005 & David et al 2008) .

Pseudo-monas species which caused UTI are known to be associated with hospital infections (Hilf et al 1989 & Goetz and Yu 1997) . Sexual intercourse is thought to facilitate migration of the organisms from the urethra into the bladder before initiation of infection (Buckely etal 1978 & Nicolle etal 1982).

Urinary tract infections (UTIs) are charac-

terized as being either upper or lower based primarily on the anatomic location of infection : the lower urinary tract encompasses the bladder and urethra , and the upper urinary tract encompasses the ureters and kidneys. The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs as it is relatively short compared with the male urethra and also lies in close proximity to the warm , moist , perirectal regions, which is meeting with microorganisms . Because of the shorter urethra, bacteria can reach the bladder more easily in the female host (Betty et al 1998) .

Urinary tract bacterial infection are common in women. Moreover, they tend to recur through out life especially with frequent sexual activity, pregnancy, stones disease or diabetes (Jeanne and F.Gary 2005) .

Most infections in diabetic patients are located in the urinary tract and in diabetic women, these infections occur frequently, can have a complicated course, are more difficult to treat and often recur than non diabetic patient because of the multiple effects of the disease on the urinary tract and host immune system (Vejlsgaard 1966, I. M. Hoepelman 1994 & Patterson and Andriole 1997) .

Several types of urinary tract infections occur more commonly in diabetic patients and these include increasing in clinical severity, asymptomatic bacteriuria, cystitis, emphysematous cystitis, pyelonephritis and emphysematous pyelonephritis and perinephric abscess, emphysematous infections refer to those complicated by gas formation due to

bacterial fermentation and this may occur in the bladder (cystitis) or in the renal pelvis or parenchyma (pyelonephritis) (Ankel et al 1990).

Urinary tract infections (UTI) are very often encounted in patients with diabetes mellitus. They may present themselves as asymptomatic bacteriuria, but may also led to more serious infection. Asymptomatic bacteriuria is more prevalent in women, but no men, with diabetes mellitus compared to controls. The increased prevalence of UTIs in diabetic patients can be the result of differences in the host responses between diabetic and non diabetic patients, or a difference in the infecting bacterium itself, the bacteria causing UTIs in diabetic patients are the same as in complicated UTIs in non diabetic patients. E. coli is the most common causative microorganism. Klebsiella, Entrobacter, Serratia spp. and streptoceous faecalis have been isolated (I. M. Hoepelman 1994).

A common source of infection is catheters, or tubes placed in the urethra and bladder. A person who cannot void or who is unconscious or critically ill often needs a catheter that stays in place for a long time. Some people, especially the elderly or those with nervous system disorders who lose bladder control, may need a catheter for life. Bacteria on the catheter can infect the bladder. People with diabetes have a higher risk of a UTI because of changes in the immune system. Any other disorder that suppresses the immune system raises the risk of a urinary infection. According to several studies, women who use a diaphragm are more likely to develop a UTI than women who use other forms of birth

control (Bethesda 2005).

Antimicrobials such as piperacillin should be considered empiric antibiotic for catheter-associated UTIs and these agent have activity against many nosocomially acquired Gram-negative rods, including P.aeruginosea (Moellering 1998).

Trimethoprim-sulphamethoxazole was recommended as initial therapy of UTI , but only in communities where prevalence of trimethoprim- sulphamethoxazole resistence is less than 20% (J.W.Warren et al 1999).

The antimicrobial agent with the highest levels of activity against Gram-negative bacilli was amikacin which was restricted to hospital use while ciprofloxacin and nitrofurantoin showed acceptable levels of activity . Nitrofurantoin was active against all strains of S.aureus but there is a reduction in the activity of amoxycillin with clavulanate and quinolones to E.coli. (Rosa et al 2001) .

MATERIALS AND METHODS

Two hundred and eleven diabetic and non diabetic cases were subject to this study and admitted public hospital and kidney center at Mansoura. Ages of cases were ranged from 20 to 70 years . This study was done over a period of one year.

Urine samples are most commonly collected by sampling the mid stream flow by the clean - catch technique (Bradbury 1988). Once collected, a specimen of urine must be transported to the laboratory with- out delay (J. A. Porter and J.Brodie 1969) .

DISCUSSION

This study shows the distribution of bacteria isolated from patient with urinary tract infection and their susceptibility pattern to antibiotics.

Escherichia coli was responsible for about 65.1% of urinary tract infection in different parts of Britain where as other coliforms than E. coli were 23.4% , 4.6% Proteus and Morganella spp., 1.8% Pseudomonas spp., 2.4% enterococci, 0.7% group B streptococci, 1.5% coagulase-negative staphylococci and 0.5% Staphylococcus aureus. (S. P. Barrett et al 1999).

The frequency of E.coli in urine samples varies in different studies from 32% (K. okada et al 1994) to intermediate values 40% (F.A.orret and S.M.shurland 1998). to 86%(Gupta et al 1999 & J.c.Nunezsanchez et al 1999), to 65% (J.C.Nunezsanchez et al 1999 & S.P.Barrett et al 1999). A result in Spain was 47% fit with the previous result (Rosa et al 2001).

E.coli was the predominant causative agent 27 (27.6%) and 10(58.8%) for diabetic and non diabetic women respectively followed by Serratia 12(12.2%) , Citrobacter , Enterobacter and Microeocel each one was 10 (10.2%) , Salmonella 7(7.1%) , Pseudomonas aeruginosa and Coagulase negative staphylococci each one was 6(6.1%) , Proteus 5 (5.1%) . Staphylococcus aureus and Shigella each one was 2(2%) and Streptococci 1(1%) for diabetic women but for non diabetic women it was followed by Enterobacter and Proteus each one was 3(17.6%) and Streptococci 1(5.9%) .

In this study we found that the incidance of bacteriuria was higher among diabetic women ages 41 to 50 (70.5%) and this probability decrease by 3.8 % with age 31-40 (66.7%) and the less incidence was among age of 20-30 (20%) which agree with those demonstrated by Nicolle et al who found that both men and women with diabetes have an increased risk of acute pyelonephritis requiring hospital admission. In a recent study, diabetes was estimated to increase this probability 20 to 30 fold under the age of 44 and three to five fold over the age of 44 (Nicolle et al 1996).

But the incidence of bacteriuria was higher among non diabetic women ages 61-70 (71.4) and this probability decrease by 17.6 % with age 20-30 year (53.8%) and the less incidence was among age of 41-50 and 51-60 , both were 0% which disagree with those demonstrated by Betty et al., 1998 and Geo.f. Brooks et al who found that the incidence of bacteriuria among girls age 5 through 14 is 1% to 2% . This incidence increases to 5% in girls over age 10. The prevalence of bacteriuria in females increases gradually with time to as high as 10% to 20% in elderly women. In women between the ages of 20 and 40 whom have UTIs , as many as 50% may become re-infected with 1 year. The association of UTIs with sexual intercourse may also contribute to this increased incidence because sexual activity serves to increase the chances of bacterial contamination of the female urethra (Betty et al 1998).

Pipercillin is effective against more than 80% of Pseudomonas aeruginosa strains (Allan Ronald 1984 and Mocllering 1998) which

disagree with our results which showed that Pipercillin is resistant against Pseudomonas aeruginosa.

Proteus spp , Citrobacter spp and Pseudomonas aeruginosa showed resistance to ciprofloxacin (Hilf et al 1989 & Goetz and Yu 1997) which agree with our results which showed that Proteus , Citrobacter and Pseudomonas aeruginosa were resistant to ciprofloxacin and disagree with other results which showed that ciprofloxacin is the most active against Pseudomonas species (O'Donnell and Gelonc 2000).

Nitrfurantoin showed an acceptable level of activity against Proteus spp and non fermenting Gram-negative bacilli (D.Wolday and W.Erge 1997) which agree with our results which showed that nitrofurantion was sensitive against Protcus. Nitrofurantoin was active against all strains of S.aureus (Rosa ct al 2001) which disagree with our results which showed that Nitrofurantoin was resistant against Staphylococcus aureus .

Nitrofurantoin has no activity against Pseudomonas aeruginosa (Judith el al 2002)

which agree with ours .

The E. coli showed high rates of resistance to amoxicillin, amoxicillin-clavulanate and ciprofloxacin (Kader et al 2000) which agree with our results which showed that amoxicillin-clavulanate is resistant against E.coli but disagree with our as ciprofloxacin was sensitive against E.coli . The E.coli isolates were susceptible to nitrofurantoin and ciprofloxacin but resistant to ampicillin and trimethoprim (Leonid and Vladimir 2006) which agree with ours except that nitrofurantion was resistant .

The combination between ciprofloxacin and cefotaxim sodium show the higher synergetic activity against bacterial isolates followed by the combination between amikacin and nitrofurantion and the combination between amikacin and ciprofloxacin .

Pseudomonas aeruginosa was resistant against all antibiotic tested so we examined it under electron microscope after and before treated with norfloxacin and the cell appear normal when not treated but when treated the cell beeome longer .

Table (1) : The frequency of the bacterial isolates from positive collected samples of diabetic females.

Bacterial isolates	Count	Frequency
E.coli	27	27.6%
Citrobacter	10	10.2%
Enterobacter	10	10.2%
Salmonella	7	7.1%
Serratia	12	12.2%
Shigella	2	2%
Pseudomonas aeruginosa	6	6.1%
Proteus	5	5.1%
Co-agulase negative staphylococci	6	6.1%
Micrococci	10	10.2%
Staphylococcus aureus	2	2%
Streptococci	1	1%
Total	98	100%

Chi-Square Value =70.5   P Value = 0.0001

$$Frequency = \frac{Positive\ samples}{Total\ samples} \times 100$$

Table (2) : The frequency of the bacterial isolates from positive collected samples of non diabetic females :-

Bacterial isolates	Count	Frequency
E.coli	10	58.8%
Enterobacter	3	17.6%
Proteus	3	17.6%
Streptococci	1	5.9%
Total	17	100%

Chi-Square Value = 14.7   P Value = 0.002

Table (3) : Inhibition zone (mm) of different pathogenic bacterial isolates against different tested antibiotic.

		E.coli	Citrobacter	Enterobacter	Salmonella	Serratia	Shigella	Pseudomonas aeruginosa	Proteus	Coagulase negative staphylococci	Micrococci	Staphylococcus aureus	Streptococci
Ampicillin Sulbactam (20µg)	IZ	7	0	10	0	0	0	8	0	7	7	8	0
	S	R	R	R	R	R	R	R	R	R	R	R	R
Amikacin (30µg)	IZ	16	18	17	10	9	18	11	19	18	17	7	16
	S	I	S	S	R	R	S	R	S	S	S	R	I
Cefotaxim Sodium (30µg)	IZ	17	17	0	19	7	0	0	0	7	0	0	23
	S	MS	MS	R	MS	R	R	R	R	R	R	R	S
Ciprofloxacin (5µg)	IZ	23	7	25	12	22	21	12	8	21	18	21	8
	S	S	R	S	R	S	S	R	R	S	MS	S	R
Nitrofurantion (300µg)	IZ	0	21	13	0	17	0	0	18	0	19	0	0
	S	R	S	R	R	S	R	R	S	R	S	R	R
Piperacillin (100µg)	IZ	0	0	30	9	0	15	0	0	12	16	0	0
	S	R	R	S	R	R	R	R	R	R	R	R	R
Erythromycin (15µg)	IZ	0	7	0	0	6	0	0	7	0	0	0	0
	S	R	R	R	R	R	R	R	R	R	R	R	R
Norfloxacin (30µg)	IZ	16	16	12	17	17	12	12	18	19	7	20	18
	S	I	I	R	S	S	R	R	S	S	R	S	S
Trimethoprim Sulfamethoxazole (25µg)	IZ	0	0	16	10	14	9	0	0	0	23	0	0
	S	R	R	S	R	MS	R	R	R	R	S	R	R
Amoxicillin/ Clavulanic acid (30µg)	IZ	8	0	0	26	0	0	0	0	8	9	0	9

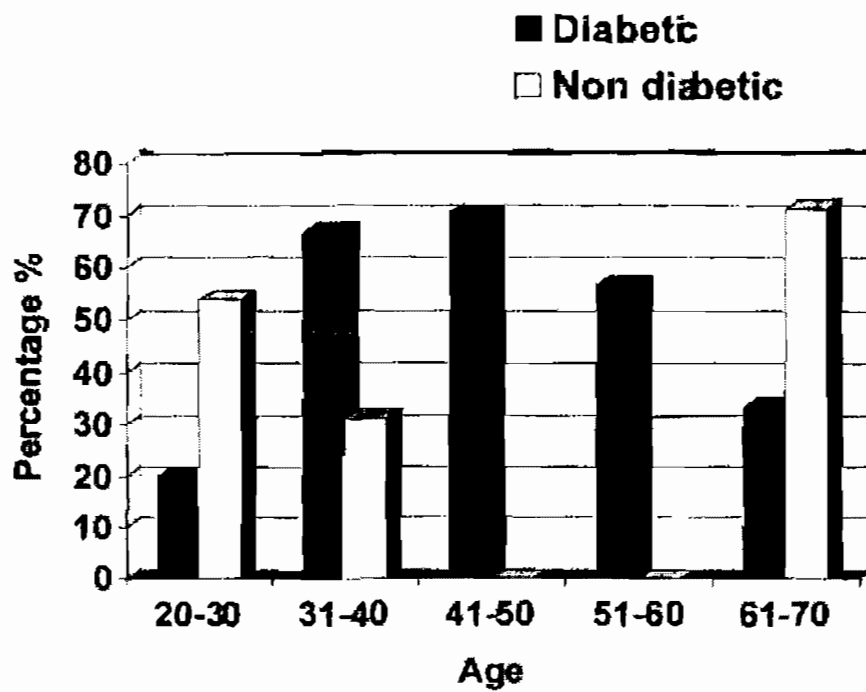IZ = Inhibition zone   R=Resistant   I=Intermediate

MS=Moderately susceptible   S= Susceptible

Fig (1) : Bacterial culture of urine samples collected from
diabetic and non diabetic female patients of
different ages .

■ Diabetic

□ Non diabetic

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