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INVESTIGATION OF ASYMPTOMATIC BACTERIURIA IN A GROUP OF IRAQI PATIENTS WITH DIABETES MELLITUS TYPE 2 IN BAGHDAD CITY, IRAQ

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Abstract Several studies have indicated that asymptomatic bacteriuria (ASB) is a frequent condition in diabetics and may lead to more severe urinary tract infections (UTIs). This study was conducted to determine the frequency of ASB in patients with type 2 diabetes mellitus as well as the identification of the bacterial etiology of ASB in addition to the antibiotic sensitivity profile in a group of Iraqi patients. A total of 100 individuals were included in this study, 50 diabetic outpatients and 50 non-diabetics as a control group. Mid-stream urine specimens were collected from patients and tested using the urine dipstick test, microscopy and culture tests. It was shown that 90% of diabetic patients were culture positive and 10% were culture negative for bacteria. On the other hand, it was observed that 90% of non-diabetics had no bacterial growth in their urine specimens. The most isolated organisms were Staphylococcus aureus (34%), Enterobacter aerogenes (28%), and Klebsiella spp (10%). It was observed that S. aureus was completely resistant to ampicillin (100%), while only 30% of these isolates were resistant to ciprofloxacin. It was noted that S. aureus was the most isolated in the study, which is a clear change in the spectrum of the causative agent of these cases. Given the high prevalence of ASB in patients with type 2 diabetes, this study recommends that the diagnostic program for diabetic patients should include bacterial culture of their urine specimens to determine the antimicrobial resistance of isolated bacteria.

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Keywords: *Asymptomatic bacteriuria, diabetics, uropathogens, antimicrobial susceptibility*

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) is defined as the presence of significant bacterial growth in urine samples of persons without any signs or symptoms of urinary tract infection (UTI). Previous scientific studies have proven that cases of ASB do not need to be treated with antibiotics except in some critical cases. In addition, cases of ASB have been shown to play a protective role in women with recurrent UTIs, and their treatment was noted to be associated with the possibility of previous symptomatic UTIs and a significant spread of antibiotic-resistant bacteria. **[1].** The definition of asymptomatic bacteriuria (ASB) used by researchers usually refers to a recent midstream voided urine sample showing positive cultures ($\geq 10^5$ CFU/ml) of the same bacteria in a patient without any known symptoms of UTI such as fever, frequent urine, and dysuria, etc. **[2].** It is well-known that *E. coli* is the most common organism, but atypical pathogens with increased antimicrobial resistance are found more often in urine cultures of infected patients. Accordingly, previous studies suggested that bacterial culture of urine samples should be performed before and after treatment when there is a possibility of upper urinary tract infection and the possibility of progression of ASB to symptomatic UTI in this group of patients **[3].** It has been proven through previous studies that the prevalence of asymptomatic bacteriuria is three times higher among women with diabetes compared to women without diabetes. However, this incidence rate is equal between men with and without diabetes. It has also been observed from these studies that the prevalence of asymptomatic bacteriuria is not affected by the type or duration of diabetes or the success of the diabetes control program **[4].** It was noted that the bacteria that cause ASB are usually the same in people with and without diabetes. Also note that about more than half of diabetic patients with ASB also have an upper urinary tract infection. However, the long-term consequences of ASB have not been thoroughly documented in patients with diabetes **[5].** The increase in the frequency of infection in diabetic patients is usually attributed

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to the presence of incompletely defined abnormalities in cellular immunity and phagocytic function associated with increased blood sugar as well as vascular diminution. It is well known that upper and lower urinary tract infections are often caused by common bacterial agents such as *Escherichia coli*, although several types of yeast (Candida and *Torulopsis glabrata*) are commonly noted to cause UTIs in diabetic patients. It has been observed that bacteriuria occurs frequently in individuals with diabetic cystopathy. The inability to control blood sugar is a contributing factor to bacterial infection in individuals with diabetes **[6, 7].** This study was conducted to determine the prevalence of ASB in patients with type 2 diabetes and to identify the bacterial agents causing ASB with their antibiotic sensitivity profile

2. MATERIALS AND METHODS

2.1 Participants

A total of 100 participants were included in this study, 50 diabetics and 50 non-diabetics as a control group. All patients participating in the study were outpatients. The study patients were examined by specialist physicians for the diagnosis of type 1 and 2 diabetes mellitus. Fasting blood sugar was measured using a fully automated biochemistry analyzer (Mindray BS 240). HbA1c was measured in venous blood samples using the Cobas B 101 system. The American Diabetes Association standards in CDC guidelines; was employed to confirm diabetes cases when fasting blood sugar is more than 126 mg/dl. This study was carried out in AL-Imam Ali's Hospital Baghdad/ Al-Rusafa during the period from January 2022 until April 2022.

2.2 Processing of Specimens

Midstream urine specimens were collected by patients in sterile containers, received and processed immediately without delay. A general urine analysis was performed for each specimen, including the macroscopic and microscopic examination. Each urine specimen was subjected to centrifugation and the precipitate was examined under a light microscope to detect the presence of red and white blood cells. In addition, each sample was subjected to bacterial culture to determine the causative agent of infection. The dip stick test was performed using urine test strips as described by the manufacturer (One Step Dus 12AaC, UK).

2.3 Culture

Urine specimens were inoculated into MacConkey and blood agar plates (Oxoid™ Ltd., UK) using a calibrated wire loop for quantitative culture and incubated aerobically at 37°C for 24 hours. Urine culture > 105 cfu/ml with no apparent symptoms of UTI was undertaken as an indicator of asymptomatic bacteriuria (ASB). Bacterial growth in each culture plate was calculated according to bacteriological criteria for quantitative culture as follows: <10 colonies per plate represented <103 cfu/ml, 10 to 100 colonies per plate represented 103 to 104 cfu/ml, 100 to 1000 colonies per plate represented by 104 to 105 cfu/ml, and > 1000 colonies per plate represented > 105 cfu/ml [8]. The bacterial isolates in this study were identified at the species level according to standard microbiological tests, which include: direct microscopy of Gram-stained preparations, biochemical testing, and antimicrobial susceptibility methods. In this study, Analytical Profile Index (API) systems (bioMérieux Inc., France) were used as rapid methods for the identification of bacterial isolates [9].

2.4 Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was performed by the disk diffusion test using Muller-Hinton agar (Oxoid™ Ltd., UK) with different types of antimicrobial discs (Oxoid™ Ltd., UK). Results were read according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). [10]

2.5 Statistical Analysis

Statistical significance was considered at highly significant level P-value of <0.01, significant level P-value of <0.05 and insignificant level P-value >0.05. The statistical analysis was done by using SPSS computer program version 16 and Excel application.

3. RESULTS

Table (1) *General characteristics of study patients*

Characteristics	No.	Percentage
Gender		
Male	22	44%
Female	28	56%
Age		
< 15 yrs.	1	2%
15–30 yrs.	14	28%
31–40 yrs.	9	18%
41–50 yrs.	16	32%
> 50 year	10	20%
Blood sugar tests		
FBS* 126 mg/dL or higher	37	74%
HbA1c * 6.5 % or higher	13	26%

Table (1) indicates that the female patients were higher than male patients 28 (56%) versus 22 (44%). The age group (41-50 year) comprised the higher percentage within age grouping of the study patients, 16 (32%). Patients with FBS 126 mg/dL or higher were 37 (74%). The result of HbA1c 6.5 % or higher were detected in 13 (26%) of study patients. Results of these blood sugar tests were read according to the

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guidelines of The American Diabetes Association (ADA) standards in Centers for Disease Control and Prevention (CDC) [11]. Only one patient was recorded with age under 15 years old.

Table (2) *Frequency of bacterial isolates in urine specimens from diabetic and non-diabetic patients*

Urine isolates	Diabetics		Non-Diabetics	
	No.	%	No.	%
Staphylococcus aureus	17	34%	0	0
Enterobacter aerogenes	14	28%	0	0
Klebsiella spp	5	10%	2	4%
E.coli	4	8%	2	4%
Pseudomonas spp	4	8%	0	0
Proteus spp	1	2%	1	2%
Total	45	90%	5	10%
No growth	5	10%	45	90%

Frequency of bacterial isolates in urine specimens from diabetic and non-diabetic patients is shown in Table (2). Out of the 50 samples analyzed from diabetic patients, 90% were culture positive and 10% were culture negative for bacteria. In contrast, 90% of non-diabetic patients were detected with no growth in their urine specimens.

There was a significant difference in the rate and type of bacterial species isolated from diabetics and non-diabetics ($P < 0.001$). The most frequent organisms isolated were *Staphylococcus aureus* (34%), followed by *Enterobacter aerogenes* (28%), and *Klebsiella* spp (10%).

Table (3) *Antibiotic resistance of S. aureus in urine specimens from study patients*

Antibiotics	No.	%
Ampicillin	17	100%
Tetracycline	16	94%
Chloramphenicol	14	82%
Gentamicin	13	76%
Augmentin	11	65%
Nitrofurantoin	6	35%
Ciprofloxacin	5	30%

Table (3) shows antibiotic resistance of *S. aureus* in urine specimens from study patients. *S. aureus* exhibited total resistance to ampicillin (100%), whereas only 30% of these isolates were resistant to Ciprofloxacin

Antibiotic resistance of *E. coli* in urine specimens from study patients is shown in Table (4). *E. coli* exhibited total resistance to Augmentin and Amikacin (100%), whereas only 25% of these isolates were resistant to Aztreonam.

Table (4) *Antibiotic resistance of E. coli in urine specimens from study patients*

Antibiotics	No.	%
Augmentin	4	100%
Amikacin	4	100%
Ampicillin	2	50%
Oxacillin	3	75%
Ticarcillin	2	50%
Aztreonam	1	25%

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Table (5) *Antibiotic resistance of Klebsiella spp in urine specimens from study patients*

Antibiotics	No.	%
Augmentin	2	40%
Aztreonam	3	60%
Amikacin	2	40%
Chloramphenicol	3	60%
Nitrofurantoin	2	40%

Table (5) shows antibiotic resistance of *Klebsiella* spp in urine specimens from study patients. It was clearly demonstrated that 60% of *Klebsiella* spp exhibited resistance to Aztreonam and Chloramphenicol, whereas 40% of them were resistant to Augmentin, Amikacin, and Nitrofurantoin .

4. DISCUSSION

It was found through this study that the prevalence of ASB cases in patients with diabetes was 90%, while in non-diabetics it was only 5%. This observation is consistent with several previous studies globally [8].

One of those previous studies revealed that the prevalence of ASB was 38% in diabetics and 26% in non-diabetics [12].

Other studies recorded prevalence of 36 % in diabetics and 18 % in non-diabetics. [13]. The current study revealed that Staphylococcus aureus was the most frequently isolated bacteria (34%), followed by Enterobacter aerogenes (28%), and Klebsiella spp (10%). Compared with many previous studies, this is a different pattern of isolation because the results of those studies usually indicate a high prevalence of E. coli in diabetic patients, whether in cases of ASB or clinically apparent urinary tract infections [14].

It was observed through the current study that Escherichia coli is the fourth pathogen isolated in both diabetic and non-diabetic groups. The current study reported that S.

Aureus isolates were detected with complete resistance to ampicillin (100%), while only 30% of these isolates were resistant to ciprofloxacin.

The results of another study for antibiotic sensitivity testing showed no significant difference in the pattern of resistance between diabetics and non-diabetics [15]. Overall, the results of the current study indicated that the rate and spectrum of antibiotic resistance were similar to the results of studies conducted in other developing countries [13].

In Iraq, high rates of antibiotic resistance were recorded among UTI-causing bacteria and the results were similar to the current study. It was observed in one of those previous studies that resistance of all Gram-negative isolates was to amoxicillin (87%), piperacillin (74%) and co-trimoxazole (73%); at the same time, ofloxacine (88%) and imipenem (98%) were the most active antibiotics [16]. It is well known that resistance to Penicillins is usually associated with the production of beta-lactamase by certain pathogenic bacteria. Hence, the high resistance to ampicillin may be due to its frequent use in the area of the current study for the treatment of urinary tract infections in particular and other infectious diseases in general.

5. CONCLUSIONS

The current study confirms that the prevalence of ASB cases is still high in diabetic patients compared to the results of other studies. It was also observed that the most common bacterial isolates in samples of diabetic patients in the current study were S. aureus, a different pattern from what was recorded in previous studies, which obviously confirmed that E. coli is the most common isolate in such cases. Given the high prevalence of ASB in patients with type 2 diabetes, this study recommends that the diagnostic program for diabetic patients should include bacterial culture of their urine specimens to determine the antimicrobial resistance of isolated bacteria.

Further studies are required to examine more samples and for a longer time to detect other types of antimicrobial agents and to determine the extent of resistance of bacterial isolates to them. Since most of isolates were Staphylococcus aureus.

The current study recommends the importance of detecting whether these isolates are methicillin-resistant Staphylococcus aureus (MRSA) or not

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