

# Determination *Enterococcus faecalis* in Asymptomatic Urinary Tract Infection Associated with Diabetes Type 2 Patients in Suwayrah General Hospital-Iraq

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## Abstract

**Background:** Asymptomatic Urinary Tract Infection is common in diabetes type-2 patient and predisposes these patients to more severe urinary tract infections (UTIs). Enterococci have been documented to cause infection of the urinary tract and other sites. Although several species of *Enterococcus* have been recognized, *Enterococcus faecalis* has been considered one of the major agents of urinary tract infections.

**Objectives:** To determine the percentage *Enterococcus faecalis* of ASB , antimicrobial resistance in diabetics and non-diabetics in urine sample and detection of enterococcal surface protein (*esp*) gene .

**Method:** The study involved a total of 611 participants including 197 diabetes mellitus patients and 414 non-diabetics in Suwayrah-Wasit-Iraq. Mid-stream urine was collected from consented subjects and dipstick , general urine examination and bacterial culture were conducted for each sample. Isolates were identified using standard biochemical tests and VITEK-2 system .

**Results:** All collected samples are asymptomatic bacteriuria. There was a significant difference in the percentage of asymptomatic bacteriuria (ASB) between diabetics and non-diabetics ( $P = 0.010$ ). There was no significant difference in resistance between diabetics and non-diabetics ( $P > 0.05$ ). *Enterococcus faecalis* isolates showed (50%) resistance to Erythromycin and Levofloxacin. The *esp* gene was observed in all *Enterococcus faecalis* isolates .

**Conclusion:** The females of age more than 60 years old have the higher percentage of asymptomatic UTI. Erythromycin and Levofloxacin were more resistance in Enterococci isolated .

**Keywords:** Diabetes mellitus, percentage, asymptomatic bacteriuria, antimicrobial resistance.

## Introduction

Diabetes type-2 has been recently associated with urinary tract infections. The mechanism of pathogenesis for this association is not fully elucidated , however, it is suggested that high glucose concentration in urine may favor the growth of pathogenic microorganisms and stimulate urinary tract infection<sup>(1)</sup> . Asymptomatic bacteriuria is a form of UTI characterized by the presence of significant amount ( $>10^5$  cfu/ml) of bacteria in urine <sup>(2)</sup>.

Several studies have documented the association of asymptomatic UTIs with diabetes; however, reports on the prevalence of ASB appear contradictory <sup>(3)</sup>.

Most studies reported the percentage to be higher in people with diabetes than people without diabetes (4). On the contrary, another study reported no significant difference in the percentage of ASB between diabetic and non-diabetic ( $P > 0.05$ ) <sup>(5)</sup>, others report that *Enterococcus spp.* are more often associated with UTI among diabetics and cause 13% of asymptomatic bacteriuria in diabetics compared to 4.9% in non-diabetics <sup>(6)</sup>.

The incidence of urinary tract infections due to *Enterococcus faecalis* has risen steadily over the years and *Enterococcus faecalis* urinary tract infection now outnumbers *Enterococcus faecium* urinary tract infection 5:1 <sup>(7)</sup>.

Recent results indicate that *Enterococcus faecalis* can not only adhere to epithelial cells in the urinary tract, but also invade cells, leading to formation of intracellular bacterial communities in the bladder. The combination of these multiple virulence mechanisms, the ability to survive in harsh conditions, and both intrinsic and acquired resistance to many antibiotics explains the high frequency of infection by *Enterococcus faecalis* <sup>(8)</sup>.

The enterococcal surface protein (Esp) is a high-molecular-weight surface protein of unknown function whose frequency is significantly increased among infection-derived *Enterococcus faecalis* isolates. Esp is another surface protein and has been shown to promote colonization and persistence in bladder (but not kidneys) Esp also influences, at least in some strains, biofilm formation in vitro <sup>(9)</sup>.

### Method

This work has applied on 611 asymptomatic UTI patients (197 of them had diabetes mellitus and 414 were non-diabetic), 208 males and 403 females, their ages ranged from 20 – 70 years old attended to emergency and consulting unite in the Suwayrah general hospital in Wasit province during the period from November 2018 to October 2019. Out of 197 asymptomatic UTI diabetic patients, seven showed *Enterococcus faecalis* culture results. And five out of 414 asymptomatic non-diabetic patients showed *Enterococcus faecalis* culture results which considered as a control group.

The study participants were educated on how to collect a “clean-catch” midstream urine specimen and the importance to avoid contamination. They were advised on washing of hands prior to collection and labia separation, especially in females. Participants who had difficulties collecting their specimens were assisted by trained personnel. Urine samples were collected into sterile containers, placed in a cool box (0 °C) and transported to the laboratory within 2 hours. The samples were then stored in a refrigerator and analysed within 8

hours of collection. Each sample was separated into two parts under sterile conditions; one part for urinalysis and the other for culture. The dip stick test was performed using urine test strips as described by the manufacturer (Condor-Teco, Beijing, China). Wet preparation of centrifuged urine was observed using 40 times objective lens to detect blood, pus and other cells. A calibrated 10µl wire loop was used to inoculate uncentrifuged urine into MacConkey, blood and plates incubated aerobically at 37°C for 24 hours. Significant ASB was defined as urine culture of > 10<sup>5</sup> cfu/ml without symptoms of cystitis. Isolates were identified using standard biochemical techniques and VITEK-2 System .

Antimicrobial susceptibility testing was performed with the automated VITEK-2 compact system by using AST-P580 cards. These included; Levofloxacin ,Erythromycin , Linezolid ,Teicoplanin ,Vancomycin ,Tetracycline ,Tigecycline and Nitrofurantoin (BioMérieux , France).

The PCR technique was also used detection virulence gene of *Enterococcus faecalis* after DNA Extraction from all bacterial isolates.

### Results

Of the 611 participants enrolled into the study 208 were males while 403 were females. Majority of participants were in the age group more than 60 years , while the least age range was less than 40 years as showed in Table (1). The overall percentage of ASB in this study was (1.96 %). There was a significant difference in the percentage of ASB between diabetics and non-diabetics ( P = 0.010 ) .

Table 1 shows the distribution of ASB with respect to age and sex. There was no significant difference in the distribution of ASB with age (P = 0.357) among the study participants. However, women demonstrated a higher percentage than men in both diabetic and non-diabetic groups.

**Table 1: Distribution of *Enterococcus faecalis* asymptomatic UTIS with respect to age and sex .**

Age	No.	Diabetics with ASB No. (%)	Non-diabetics with ASB No. (%)	Total with ASB No. (%)
Sex				
20-30 year	102	0(0 %)	0(0 %)	0(0 %)
30-40 year	130	1(0.77 %)	1(0.77 %)	2(1.54 %)
40-50 year	121	1(0.82 %)	1(0.82 %)	2(1.64 %)
50-60 year	120	2(1.66 %)	1(0.84 %)	3(2.5 %)
60 +	138	4(2.89 %)	1(0.72 %)	5(3.62 %)
X <sup>2</sup> ,P value		4.681 , 0.322 (NS)	0.823, 0.935 (NS)	4.379, 0.357 (NS)
Male	208	2(0.96 %)	1(0.48 %)	3(1.44 %)
Female	403	6(1.48 %)	3(0.74 %)	9(2.23 %)
X <sup>2</sup> ,P value		0.295, 0.587 (NS)	0.147, 0.702 (NS)	0.446, 0.504 (NS)
Overall Percentage	611	8\197 (4.06 %)	4\414 (0.96 %)	12\611 (1.96 %)

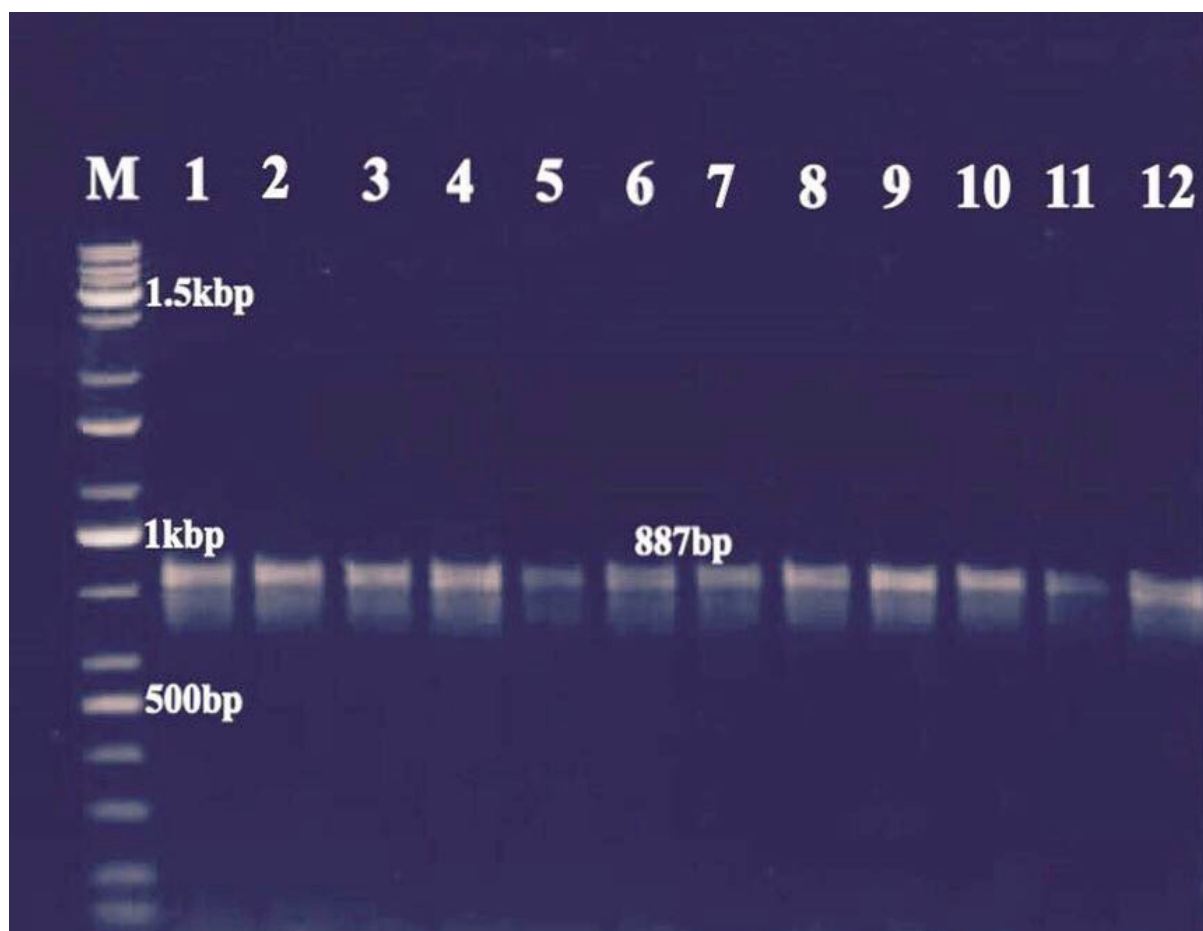
X<sup>2</sup> chi-square value , NS : Non-significant difference ( P >0.05 ) , ASB : Asymptomatic Bacteriuria .

All *Enterococcus faecalis* isolates were (100%) sensitive to Linezolid ,Teicoplanin ,Vancomycin ,Tetracycline ,Tigecycline and Nitrofurantoin antibiotics while they were (50%) resistant to Levofloxacin and Erythromycin in both diabetes and non-diabetes patients as showed in Table (2).

**Table 2: Antibiotic resistance of urinary *Enterococcus faecalis* in diabetics and non-diabetics**

Antimicrobial agent	Diabetics	Non-Diabetics
Levofloxacin	4(50%)	2(50%)
Erythromycin	4(50%)	2(50%)
Linezolid	0(0 %)	0(0 %)
Teicoplanin	0(0 %)	0(0 %)
Vancomycin	0(0 %)	0(0 %)
Tetracycline	0(0 %)	0(0 %)
Tigecycline	0(0 %)	0(0 %)
Nitrofurantoin	0(0 %)	0(0 %)

enterococcal surface protein (*esp*) virulence gene of *Enterococcus faecalis* was detected in all 12 *Enterococcus faecalis* isolates showed in Figure (1).



**Figure (1) :** Agarose gel electrophoresis (1.5%) showing PCR product of a partial fragment of *esp* gene under U.V. light after staining with ethidium bromide. M: 100 bp marker; lane 1-12: *Enterococcus faecalis* isolates. The size of product is 887 bp.

## Discussion

The association of diabetes mellitus and urinary tract infections is increasingly being reported. Especially, infection by *Enterococcus* bacteria. Asymptomatic bacteriuria is common among diabetic patients and may lead to serious complications if not properly managed<sup>(10)</sup>. ASB has been identified as a risk factor for acquiring symptomatic UTIs especially in diabetic women<sup>(11)</sup>. UTIs are more severe in diabetic patients involving life-threatening complications such as emphysematous pyelonephritis and renal papillary necrosis. The present study revealed an overall percentage of ASB of 1.96%; 4.06% in diabetics and 0.96% in non-diabetics (table 1).

This result is concurrent with that of earlier reports which recorded prevalence of high percentage in diabetics and low percentage in non-diabetics<sup>(13)</sup>. In other earlier reports which recorded percentage low percentage in diabetic and high non-diabetic<sup>(14)</sup>. Consequently, the issue of prevalence of ASB remains debatable. This

inconsistency has been attributed to variations in sample size, geographical location, culture or screening method. In the present study, ASB was significantly higher in diabetics than non-diabetics ( $P = 0.010$ ). This is in line with majority of previous reports<sup>(12)</sup>. However, it is suggested that high glucose concentration in urine may favor the growth of pathogenic microorganisms and stimulate urinary tract infection<sup>(15)</sup>. The females are more susceptible to UTI than males, because their urethra is shorter and closer to anus more than in males, so that the intestinal flora which may ascend to urinary tract and cause UTI<sup>(17)</sup>. The most affected age group in females were these of more than 60 years, it may be due to Estrogen deficiency, diabetes mellitus, gynaecological diseases (cystocele) and operations, urological diseases (incontinence, residual urine, cystopathy), operations, immunological changes and diabetic cystopathy<sup>(18)</sup>.

Results of the antibiotic susceptibility test revealed no significant difference ( $P > 0.05$ ) in the resistant

pattern between diabetics and non-diabetics (Table 2). Resistance to Levofloxacin and Erythromycin may be due its frequent use in our study area to treat UTIs and other infectious diseases <sup>(16)</sup>.

In figure (1) , shows all isolated have *esp* gene , the ability of enterococci to adhere the epithelial cells of urinary tract is a key initial step in UTI pathogenesis due to encoded by the *esp* gene facilities colonization and persistence of *Enterococcus faecalis* in urinary tract infections <sup>(19)</sup>.

### Conclusion

The study revealed diabetic patient have a higher percentage of asymptomatic UTI than non-diabetic patients. Females of age more than 60 years old have the higher percentage of asymptomatic UTI. Erythromycin and Levofloxacin were more resistance in *Enterococcus* isolated . Therefore the need to speed up sensitization against antibiotic abuse in Suwayra -Wasit – Iraq.

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**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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