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# Isolated Bacteria in Recurrent Bacterial UTI in Diabetic Patients

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**Abstract**
Diabetes Mellitus (DM) is of the world's biggest health problems with dramatic incidences increase worldwide. Since this physiological disorder is a type of secondary immunodeficiency; Urinary tract infections (UTI) is a common consequence post diabetes. The present study was conducted to determine the causative bacterial pathogen and investigate the related innate immunodeficiency in diabetic type II patients. A total of 60 patients and 30 controls were involved, urine and blood samples were collected from diabetic subjects and controls, during the period from November 2017 to March 2018. Data obtained from urine samples culture were performed for the detection of the infective etiology of UTI to determine prevalence of UTI among diabetics. So far; the most common isolated bacterial pathogens were *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, Proteus miriabilis, Staphylococcus aureus*, Enterococcus *fecalis* and *Pseudomonas aeroginosa*.

**Keywords:** UTI, Bacteria, diabetics.

## INTRODUCTION

Urinary tract infections (UTIs) is a common infection observed in diabetic patients. Diabetes mellitus (DM) is an immunodeficiency type, it is secondary immunodeficiency that alters the genitourinary system where UTI can be a cause of severe complications ranging from dysuria (pain or burning sensation during Urination) organ damage and sometimes even death due to complicated UTI (pyeleonephritis). The risk factors for UTI involve colonization with a different uropathogen in cases of recurrent UTI, glucosuria and impaired granulocyte function. Diabetic patients are at a higher risk developing Immunologic impairments such as defective migration, and phagocytic alterations of chemotaxis in polymorphonuclear leukocytes is well marked in diabetic patients [1].

The mechanisms which potentially contribute to UTI in these patients are defects in the local urinary cytokine secretions (IL-8, IL-6), increased adherence of the microorganisms to the uroepithelial cells, and granulocyte dysfunction, possibly as a result of an abnormal intracellular calcium metabolism. On the other hand, hyperglycemia facilitates the colonization and growth of variety of organism [2].

Neutrophils are one of the most important phagocytotic cell types. Neutrophils follow chemotactic cues to locate sites of inflammation, migrating to infection sites in response to signals such as chemoattractants. Phagocytosis is defined as the ingestion of particles by cells, and this process involves the binding of particles to the surface of phagocytic cells, followed by the internalization and destruction of these particles. The coating of a microorganism with molecules that trigger its destruction by phagocytes is known as opsonization. The reduction of nitroblue tetrazolium (NBT) by monocytes and neutrophils can be used as an indirect marker of the phagocytic activity of these cells. NBT is a dye with low reduction potential that produces an intensely stained product formazan when reduced. NBT is easily phagocytosed by cells and is reduced to formazan inside the mitochondria. Therefore, the aim of this study was to evaluate opsonization, phagocytosis, and NBT reduction in healthy males under non-fasting (before Ramadan) and fasting (during Ramadan) conditions [3].

In patients with DM, abnormalities in polymorphonuclear neutrophils (PMNs), monocytes, and lymphocytes related to adherence, chemotaxis, opsonization, ingestion of bacteria, oxidative burst, and intracellular killing .Decreased neutrophil chemotaxis has recently been documented among type with type II DM, confirming previous studies diminished neutrophil bactericidal activity associated with increased levels of glycated hemoglobin has been attributed in part to the degree and duration of hyperglycemia and may be reversed with optimal glycemic control .Hyperglycemia or the presence of advanced glycation end products is believed by some researchers to lead to a state of low-level, persistent activation in PMNs. This observation has been evidenced by an increased concentration of neutrophil elastase, increased activity of neutrophil alkaline phosphatase and luminol-dependent chemiluminescence, and an increased rate of oxygen consumption among unstimulated PMNs in patients with DM. This hyperexcited state leads to spontaneous activation of the oxidative burst and release of myeloperoxidase, elastase, and other neutrophil granule components. This process may, in turn, lead to a "burned-out" or tolerant PMN that responds less vigorously when stimulated by an infectious pathogen, initiating pathologic processes leading to vascular injury. Resting levels of cytokines (e.g., TNF-a, IL-6, and IL-8) are elevated in individuals with DM, but on stimulation, the cells produce less IL-1 and IL-6 than do similar cells in control subjects. In addition, abnormalities in monocyte and macrophage chemotaxis and phagocytosis have been reported. Adaptive cellular immunity does appear to be affected, however, with decreased lymphocyte proliferative response to stimulants (e.g., phytohemagglutinin) and certain pathogens (e.g., *Staphylococcus aureus*) [4].

## Material and Methods

### Sample collection

A total 60 urine and blood samples were collected from 100 DM patients aged from 60 to32 years infected UTI. From Diabetic and Endocrine center in Al-Hussein hospital in Al- Muthanna Governorate during the period from November 2017 to March 2018 patients were clinically diagnosed by the physicians of diabetics. The urine samples were collected antiseptically in sterile tubes. Blood samples were collected in heparin tubes [5].

### Diagnosis Bacteria

All urine samples were cultured on respective selective and differential media to identify bacterial isolates on the basis of colonial, morphological, Gram stain and biochemical tests, Biochemical tests used for identification of bacteria including, catalase, oxidase, indole, Methyl red, Voges Proskauer, Simmons citrate utilization, TSI, confirmation was depended on [6].

### Urine Sample

Midstream urine sample were collected in sterile containers for urinalysis and culture on the following media. Brian heart infusion broth, Blood agar, Nutrient agar, Macconkey agar UTI chrom agar Mannitol Salt agar.

### Isolation and Identification of Pathogenic Bacteria

Each urine samples was cultured on Blood agar, MacConkey agar and Nutrient agar using serial swab and incubated at 37˚C for 24 hours, growing bacteria were isolated and purified by streaking four times on the same media .All the obtained isolates (suspected as pathogenic) were examined by microscope after smear preparation and stained by Gram stain .Also colonies shape ,color, arrangement ,growing style were obserived on petry dishes[6]. Several biochemical tests were used for confirmation according to Baron and Bergey's manual of determinative bacteriology and other references [7].

## Results

Out of the 60 urine specimen sampled for diabetic patients, a total of seven different groups of organisms were isolated and characterized, see the following table and pictures. Isolated bacteria included; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pnemonieae, Proteus miriabilis, Enterobacter aerogenes,and Enterococcus fecalis.*

### Table 1: (most common isolated bacteria)

| NO. | Isolated Bacteria | Frequency |
|---|---|---|
| 1 | *Staphylococcus aureus* | 42 |
| 2 | *Enterococcus fecalis* | 31 |
| 3 | *Escherichia coli* | 10 |
| 4 | *Klebsiella pnemonieae* | 4 |
| 5 | *Enterobacter aerogenes,* | 3 |
| 6 | *Pseudomonas aeruginosa* | 3 |
| 7 | *Proteus miriabilis* | 5 |

## Table 2: Identified Bacterial isolates

| No | Bacteria | Mono infection | | Mixed infection | | Total cases | |
|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % |
| 1 | *Staphylococcus aureus* | 20 | 33.3 | 26 | 43.3 | 46 | 76.6 |
| 2 | *Enterococcus fecalis* | 11 | 18 | 22 | 36.6 | 33 | 55 |
| 3 | *E. Coli* | 1 | 1.6 | 9 | 15 | 10 | 16 |
| 4 | *Klebsiella pneumoniae* | 0 | 0 | 4 | 6.6 | 4 | 6.6 |
| 5 | *Pseudomonas aeruginosae* | 0 | 0 | 3 | 5 | 3 | 5 |
| 6 | *Proteus mirabilis* | 1 | 1.6 | 4 | 6.6 | 5 | 8.3 |
| 7 | *Enterobacter aerogenes* | 0 | 0 | 2 | 3.3 | 2 | 3.3 |

*E .coli* is the most common pathogen usually isolated from UTI in DM patients as stated by many authors[8][9], and even the previous one [10], but proved recently, local isolates of *Staphylococcus aureus* developed a dangerous numerous abilities to resist wide spectrum of antibiotics of this bacteria to invade and colonize by the help of spreading factors naturally produced by these bacteria the authors [11], in their local study investigated about the nosocomial *Staphylococcus aureus* by testing health care workers and patients at al-kadhamia teaching hospital and al-numan hospital in Baghdad/Iraq, they found that 94.3% of *Staphylococcus aureus* isolates were mulity drug resistance *Staphylococcus aureus* MDRSA and considered that as a common health problem in our community which requires attention and support. That can explain the elevated in UTI in DM patients in present study. that also confirmed by [12] in their local research, also the author, [13] and found similar results regarding UTI and *Staphylococcus aureus.*

*Enterococcus feacalis* was the second most common bacteria isolated in this study and recorded 33 cases (55%) from total cases number 60 these isolates classified as mono infection 11 cases (18%) and as mixed infection with other isolates 22 cases (36.6%). *Enterococcus feacalis* is a commensal bacterium which inhabit gastrointestinal tract of healthy people, but usually able to cause serious infections similar to *Staphylococcus aureus* due to wide range of antibiotic resistance and different virulence factors. The explaination of this bacterial invasion of urinary tract can be the suppression of immunity DM patients and because these bacteria are able to transmit from Gastric Tract GT to Urinary Tract UT due to the anatomical factor of excretion. the authors [14] stated that *Enterococcus feacalis* was the second most common bacteria in DM patients suffering from uti and same results was confirmed by [15] (Chatterjee,2016). Other isolates represented *E.* coli*, Klebsiella pneumonia, Pseudomonas aerginosae, Proteus mirabilis* and *Enterobacter aerogenes*, they were less frequent as shown in table (2). The authors [16] explained *E. coli* infections in UTI due to the rectal and vaginal area contain high levels of *E. coli* as the most common bacteria, also the anatomical and functional changes in females and difficulty of maintaining personal hygiene may increase the risk of UTI by *E. coli.*

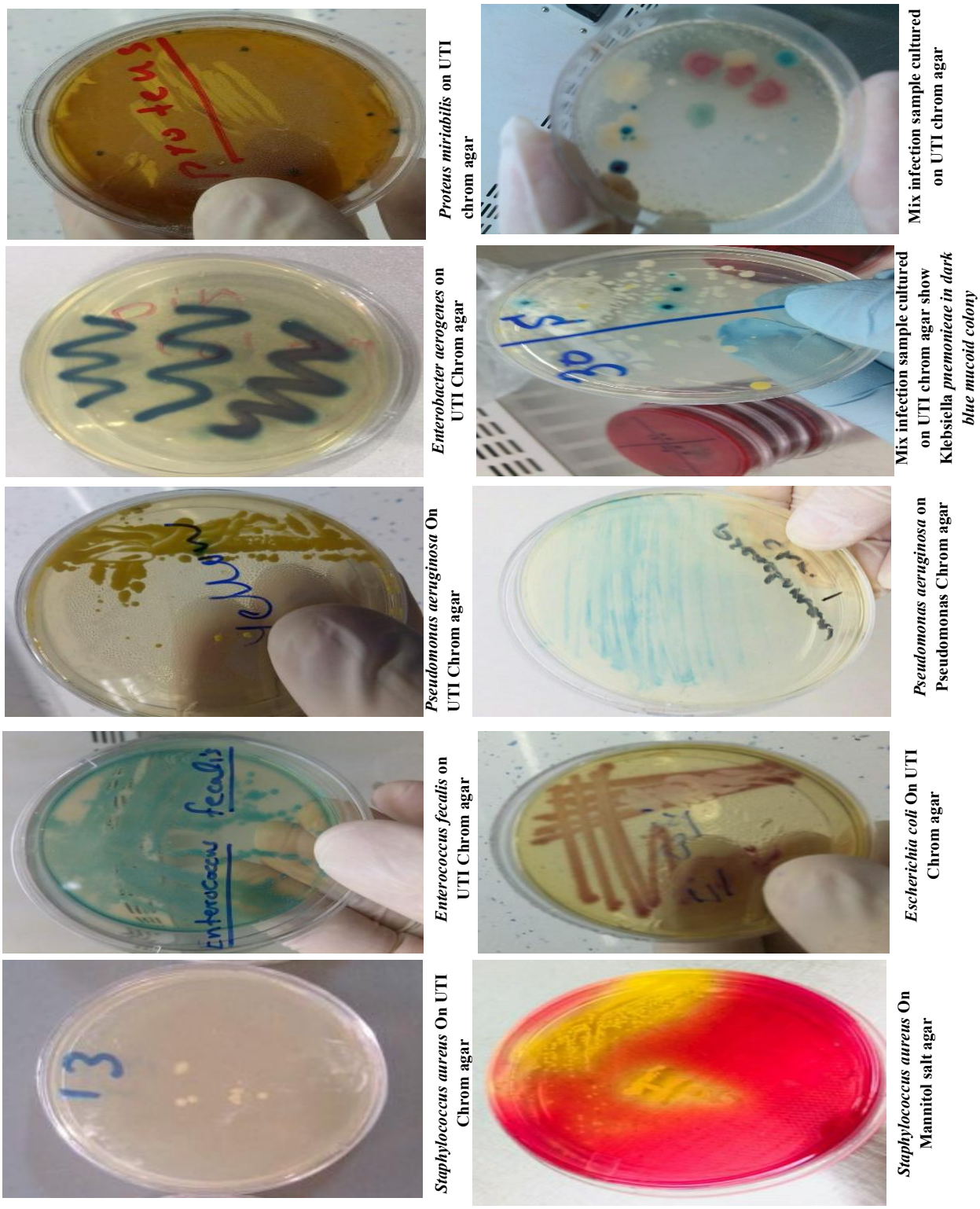
In the study of [17] have attempted to determine the distribution of various bacteria causing UTI among the diabetics, an immunocomprised population, and their antibiotic susceptibility pattern. In the general population, most urinary tract infections are caused by *Escherichia coli* and affect mainly women because of sexual activity and pregnancy. Prevalence in women is also due to decrease of normal vaginal flora (*Lactobacilli*), less acidic pH of vaginal surface, short & wide urethra, proximity of urethra to anus and poor hygienic conditions [18] hospitalized patients gets the nosocomial infections exactly caused by *K. pneumniae* and *P. aeruginosa. P. aeruginosa* is important nosocomial cause of urinary tract infections in with UTI. This organism is able to colonize the surface of catheter, forming a biofilm that interferes with activities of antimicrobial agents and host defense mechanism, that was illustrated by [19] and this result goes slightly with a study on community acquired UT1 which *was E. coli, K. pneumoniae, P.aeruginosa , Proteus spp*. [20].

*E.coli* use adhesions as virulence factor to generate UTI and that is also applicable to other uropathogens like *K. pneumoniae* as stated by the authors [21] in their local study. *Enterobacter aerogenes* considered as opportunistic normal existence in intestine and having many virulence factors like adhesion factors represented by cilia, in which the germs ability to adhere on cell's surfaces is regarded a necessary step to a successful colonization and then causing UTI, in addition to having endotoxins represented by lipopolysaccharide that plays a big role to protect germs from phagocytosis [22].

## Discussion

Diabetic patients have higher risk of UTI particularly in women. In the present study, we investigated the possible impact of the glycemic control on the UTI prevalence. Our main findings until now, since work is still going on; are the following:

(1) Diabetes is a predisposing factor for recurrent UTI.
(2) The most common bacterial pathogens were *Staphylococcus aureus* (70%) and the second common bacteria was *Enterococcus fecalis.* (53%).




## Conclusion

In conclusion the most frequent is *Staphylococcus aureus* and *Enterococcus fecalis*. Many UTIs are asymptomatic, especially in women. Because of the great proportion of asymptomatic UTIs among diabetic patients, urine culture should be performed in all hospitalized diabetic patients. In addition, considering the high prevalence of Asymptomatic Bacteriuria in diabetics, this condition could represent one of the causes leading to an unexplained worsening of the glycosuria in some patients. This study confirms that diabetes predisposes humans to the risk of urinary tract infections due to the changes in bladder function and in circulation. Diabetics infected with UTIs should therefore be promptly treated with the proper antibiotics to prevent development of kidney damage or more serious infections. However further studies with large sample size are highly recommended to authenticate the findings from this study.

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