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Bacteriological Study of Type 2 Diabetes

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Annotation: The study aimed to isolate and identify the types of bacteria that cause polycarbonate for patients with type 2 diabetes. Patients samples were collected from Al-Hussein Teaching Hospital in Kerbala for the period From March to May 2021, our results were isolated and diagnosed 30 samples, positive bacteria 80% more than negative bacteria 20 %.Nine isolates were Staphylococcus aureus (30%) .It was the most dominant bacteria in the culture medium, and 4 (13.33%) isolates with Staphylococcus saprophyticus, Hemolytic streptococci, Streptococcus viridians, 2 (6.67%) isolates from Escherichia coli , Klebsiella species, 3 (10%) isolates from Enterococcus, 1 (3.33%) isolate Pseudomonas aeruginosa, Proteus spp. The biochemical study showed an increased increase in the concentration of total cholesterol (PS0.05) in my poly and diabetes groups, as weil as in low-density fatty proteins, where they showed an increase in diabetes inflammation group, Fatty proteins showed The incidence of urinary tract infection varies with gender and age, where the infection occurs in both sexes, the incidence of females is more than the incidence of males, as a study indicated.

The infection in females is three times that of males in the "first ten years of the child's life." The reason for the increase in the incidence of UTI in females is the shortness of the urethra in addition to its proximity to the anus.

Introduction

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity, As of 2015 there were approximately 392 million people diagnosed with the disease compared to around 30 million in1985.Typically it begins in middle or older age, although rates of type 2 diabetes are increasing in young people.[1] Type 2 diabetes is associated with a ten-year-shorter life expectancy.Diabetes was one of the first diseases ever described, dating back to an Egyptian manuscript from c.1500 BCE. The importance of insulin in the disease was determined in the 1920s.[2]

Type 2 diabetes (T2D), formerly known as adult-onset diabetes, is a form of diabetes that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Common symptoms include increased thirst, frequent urination, and unexplained weight loss.Symptoms may also include increased hunger, feeling tired, and sores that do not heal.[3] Often symptoms come on slowly. Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy which can result in blindness,kidney failure, and poor blood flow in the limbs which may lead to amputations.The sudden onset of hyperosmolar hyperglycemic state may occur; however,ketoacidosis is uncommon.[4][5]

Type 2 diabetes primarily occurs as a result of obesity and lack of exercise. Some people are more genetically at risk than others.

Type 2 diabetes makes up about 90% of cases of diabetes, with the other 10% due primarily to type 1 diabetes and gestational diabetes.[1] In type 1 diabetes there is a lower total level of insulin to control blood glucose, due to an autoimmune induced loss of insulin-producing beta cells in the pancreas.Diagnosis of diabetes is by blood tests such as fasting plasma Glucose, oral glucose tolerance test, or glycated hemoglobin (A1C).[3]

Type 2 diabetes is largely preventable by staying a normal weight, exercising regularly, and eating a healthy diet (high in fruits and vegetables and low in sugar and saturated fats).[1] Treatment involves exercise and dietary changes.[1] If blood sugar levels are not adequately lowered, the medication metformin is typically recommended. Many people may eventually also require insulin injections.[6] In those on insulin, routinely checking blood sugar levels is advised; however, this may not be needed in those taking pills. Bariatric surgery often improves diabetes in those who are obese.[7]

The classic symptoms of diabetes are frequent urination (polyuria), increased thirst (polydipsia), increased hunger (polyphagia), and weight loss. Other symptoms that are commonly present at diagnosis include a history of blurred vision, itchiness, peripheral neuropathy, recurrent vaginal infections, and fatigue. Other symptoms may include loss of taste. Many people, however, have no symptoms during the first few years and are diagnosed on routine testing. A small number of people with type 2 diabetes can develop a hyperosmolar hyperglycemic state (a condition of very high blood sugar associated with a decreased level of consciousness And low blood pressure).[8]

Pathophysiology

Type 2 diabetes is due to insufficient insulin production from beta cells in the setting of insulin resistance. Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue.[9] In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood. The proportion of insulin resistance versus beta cell dysfunction differs among individuals, with some having primarily insulin resistance and only a minor defect in insulin secretion and others with slight insulin Resistance and primarily a lack of insulin secretion.[7]

Bacterial Infections Associated with Type 2 Diabetes

Diabetic foot ulcers are a common complication of diabetes frequently associated with the presence of Staphylococcus aureus [10], as are chronic leg ulcers, surgical site infections, and chronic wounds [11]–[12]. Furthermore, invasive staphylococcal infections such as endocarditis or bacteremia are more prevalent in diabetic than in non-diabetic patients and are associated with a poorer outcome in diabetic patients [13] Invasive group B *streptococcal* infections are also more common in diabetics [14]. In East Asia, diabetes is a wellknown risk factor for *Klebsiella pneumoniae* liver abscess, and those with uncontrolled glycemia (HbA1c≥7%) are at higher risk of metastatic complications [8]. Furthermore, there is increased prevalence of urinary tract infection (UTI) and 1.4

Times higher recurrence rate in women with T2D [15]

The association between tuberculosis (TB) and T2D is still debated. The relative risk estimates of diabetics associated with TB vary considerably from 1.2 to 7.8, with the lowest estimates reported in larger studies and in low–TB-burden countries [10]. Some studies have shown that poor glycemic control is associated with increased TB risk [10]–[11] but not others [12].It could be that the association is evident only in high–TB-burden areas and populations with significant latent TB cases.[10]

In contrast to TB, up to 60% of melioidosis patients have underlying T2D . This makes melioidosis the most highly associated bacterial infection with diabetes. Melioidosis is endemic in Southeast Asia and Northern Australia and caused by the gram-negative bacterium Burkholderia *pseudomallei*[15]

Materials and methods

Collection of samples taken from diabetic patients at Al-Hussein Teaching Hospital during the period from March 2021 to May 2021. Clinical samples were obtained from the urine of patients suspected of having a urinary tract infection and their ages ranged between (35 to 65) years. These patients were separated by residence, age, sex, place of infection, and type of bacteria.

Preparation of blood agar

1. Suspend 28 g of nutrient agar powder in 1 liter of distilled water
2. Heat this mixture while stirring until all ingredients are completely dissolved
3. Autoclave the dissolved mixture at 121 "C for 15 min
4. Once the nutrient agar has been sterilized, allow it to cool but not harden 4 When the agar has cooled to 45-50°C, add 5% (vol/vol) of sterile fibroused blood that
5. has been heated to room temperature and mix gently but well
6. Avoid air bubbles
7. Dispense the liquid into sterile dishes

Staining procedure

1. Heat fixes the smear by passing the slide 2-3 time gently over the flame with the smear side up.
2. Cover the smear with the methyl violet. Allow it to stand for one minute
3. Rinse the smear gently under tap water .
4. Cover the smear with Gram's iodine and allow it to stand for minute
5. Rinse the smear again gently under tap water
6. Decolourise the smear with 95% alcohol for 15 to 20 seconds
7. Rinse the smear again gently under tap water
8. Cover the smear with dilute carbol fuchsin for 30 seconds to 1 minute .
9. Rinse the smear again gently under tap water and air dry it
10. Observe the smear first under low power (10x) objective, and then under oil
11. immersion (100x) objective „Record the observation in the note book

Table1: - Equipment

Equipment	Company	Origin
Petri dishes	Unisonics LTD	England
Flaskes	Unisonics LTD	England
Cotrifuge	Hettch EBA	Germany
Microscope	Olympns	Japan
Incubator	Labtech	Chory
Blance	Sartorins	Germany
Auto clave	Labtech	Chory

Table2:- Materials

Materials	Company	Origin
Blood agar	Hi media	India
Nutriet agar	Hi media	India
Mackoncy agar	Hi media	India
Gram stain	BDH	England
Oxidase	BDH	England
Ureas	BDH	England
KOH	BDH	England

Urine samples were taken and stained, 6 negative bacteria appeared,24positive) (purple) bacteria

Gram stain

of the most important types of pigments used in hospitals to identify the type of bacteria. The attending physician is able to identify the type of bacteria first and begin to treat the patient with the appropriate antibiotic quickly. Its discovery is credited to Hans Christian Gram, a Danish- born physician who worked in the anatomy laboratory of the Berlin Hospital.In the year 1880 AD. Where he developed this method to help him differentiate between the types of bacteria that cause *pneumonia* (Pneumonia), as he noticed that one of the bacteria stained red when placed in a specific solution containing iodine and called it (Gram-negative bacteria) and another type of bacteria was taking the color blue, so he called it (gram-positive bacteria). The color of bacteria in a Gram stain

depends on the chemical composition of the cell wall.[16]

Different bacteria react differently to the staining method. So bacteria are divided into:

*Gram-positive: It is generally dark blue

*Gram-negative: remain across grams without coloring – but color red through fuchsin

Catalase test

It is a quick test to see if the bacteria possess Catalan enzyme.

It is the easiest and faster tests at the Microbiology to contribute to the diagnosis of the disease.

Catalase test method: Take a simple amount of hydrogen peroxide solution and put it on a glass of glass added by Colony from the in bacteria dish.[17]

The appearance of bubbles = Catalase + ve*

Do not appear = Catalase –Ve*

Oxidase test

is used to determine the bacteria that produce cytochrome Sea Oxides, when there is an oxidating detector and transformed intopurplee.

*Positive enzyme reaction: the appearance of dark purple color within 10 seconds, this means that the bacteria contain the enzyme cytochrome c oxidase.

*Negative enzyme reaction: no purple color (no discoloration), which means that the bacteria do not contain the enzyme cytochrome c oxidase.[18]

Potassium hydroxide test

is a simple procedure used to detect fungi in tissues through the use of potassium hydroxide (KOH), which in turn dissolves tissues and cells. The difference in the composition of tissues and cells on the one hand, and the walls of fungi on the other, makes it possible to separate them using this substance, as potassium hydroxide destroys the protein in the cell walls and the fungi remain in the tissues where they are diagnosed by lightmicroscopyy.[19]

Urease test

that is done to determine the microorganisms capable of decomposing urea with water to produce ammonia and carbon dioxide, and two types of media are used to detect urease activity.

*Positive result: The color of the food center of yellow to purple or pink color, means that the organism is capable of producing enzyme.

*Negative result: The color of the nutrients remains original yellow, meaning that the organism is unable to produce enzyme.[20]

Table 3

The test	Positive test	Negative test
Catalase test	*Staphylococcus* *Klebsiella* *Proteus* *Salmonella* *E.coli*	*Streptococcus* *Enterococcus*
Oxidase test	*Pseudomonas aeruginosa*	*E. coli* *Proteus* *Klebsiella strains*
potassium hydroxide test	*Staphylococcus Sapophyticus* *Staphylococcus aureus*	*E. coli* *Proteus*

	Enterococcus *Streptococcus viridians*	*Klebsiella strains*
Urease test	*Proteus spp.* *Klebsiella spp*	*E. coli* *Salmonella* *. Pseudomonas*

Staphylococcus saprophyticus is a Gram-positive coccus belonging to the genus Staphylococcus. [21] *S. saprophyticus* is a common cause of community-acquired urinary tract infections.

S. saprophyticus was not recognized as a cause of urinary tract infections until the early 1970s, more than 10 years after its original demonstration in urine specimens. Prior to this, the presence of coagulase-negative *staphylococci* (CoNS) in urine specimens was dismissed as contamination *Staphylococcus saprophyticus* infection is spread to the bone from the bloodstream, also cause ulcers in foot because of diabetes [22]

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, facultatively aerobic, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, P. aeruginosa is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated *pneumonia* and various sepsis syndromes.[21]

Escherichia coli or *coli* (scientific name : *Escherichia coli*) is one of the most important types of bacteria that live in the intestines of mammals . It was discovered by Theodore *Escherch* . Also known as the large intestine germ, *Escherichia coli* is a gram-negative bacterium that inhabits the large intestine of humans , and makes up about 80% of its aerobic flora. Note that anaerobes are predominant in his intestines. The presence of this bacterium in the surrounding environment indicates fecal contamination.[22]

These bacteria can also cause prostatitis infection (prostatitis prostatitis infection, gallbladder infection, and infection that occurs after appendicitis appendicitis and diverticulitis, and wound infection (including surgical pieces during surgical work), and amusing ulcers, and foot infections In diabetes.

Proteus is a genus of Gram-negative Proteobacteria. *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial.[23]

Enterococcus is a large genus of lactic acid bacteria of the phylum Firmicutes. *Enterococci* are gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from *streptococci* on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: E. faecalis (90–95%) and E. faecium (5–10%). Rare clusters of infections occur with other species, including E. casseliflavus, *E. gallinarum*, and *E. raffinosus*. Bloody (causing blood or sepsis) Can cause pulmonary inflammation at persons outside health care centers (among society), usually alcohol, older or diabetic patients.[24]

Staphylococcus aureus is a Gram-positive, round-shaped bacterium, a member of the Firmicutes, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.[24] Although *S. aureus* usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine

for *S. aureus* has been approved, *Staphylococcus Aureus* infection is spread to the bone from the bloodstream, or from a sub-fabric nearby, as it may occur when injured with deep compression ulcers, or ulcers in foot because of diabetes.[25]

Viridans streptococci are a large group of commensal *streptococcal* Gram-positive bacteria species that are α-hemolytic, producing a green coloration on blood agar plates[24]

Viridans streptococci can be differentiated from Streptococcus pneumoniae using an optochin test, as *viridans streptococci* are optochin-resistant; they also lack either the polysaccharide-based capsule typical of *S. pneumoniae* or the Lancefield antigens of the pyogenic members of the genus.Her risk to diabetes is diabetic foot injuries Diabetic Foot Infections.[25]

Table 4

Isolate	Number Isolate	% Percentage
Hemolytic streptococci	4	13.33%
Staphylococcus Saprophyticus	4	13.33%
Pseudomonas aeruginosa	1	3.33%
Escherichia coli	2	6.67%
Proteus	1	3.33%
Klebsiella strains	2	6.67%
Staphylococcus aureus	9	30%
Enterococcus	3	10%
Streptococcus viridians	4	13.33%
Total	30	

Table 5

Isolate	Number Women	Number Men
Hemolytic streptococci	2	2
Staphylococcus Saprophyticus	3	1
Pseudomonas aeruginosa	1	0
Escherichia coli	1	1
Proteus	1	0
Klebsiella strains	0	2
Staphylococcus aureus	5	4
Enterococcus	2	1
Streptococcus viridians	2	2
Total	18	13

Results

The results of isolation and diagnosis of the sample of urine collected (30) samples from patients with type 2 diabetes that were obtained from patients who attended the Al-Hussein Teaching Hospital in Kerbala, for the period from the beginning of March 2021. The results of the bacterial culture showed a positive result for UTI. At a rate of 66.15%, the highest rate of infection was in the ages between 35-65. And based on the results of the isolates of Table 4, *Staphylococcus aureus* isolates 9 (/r.) It was the most dominant bacteria in the culture medium, And 4 isolates with (13.33%) *Staphylococcus saprophyticus*, and 2 isolates from *Escherichia coli*, *Klebsiella species*, (1) 3.33% isolate from *Pseudomonas aeruginosa*, *proteus sp*. 3(10%) isolates from *Enterococcus*.

Discussion

In (Table no.5) Females had shown are more susceptible to infection than males, and the reason for this may be due to the proximity of the urinary tract opening to the anus in females, which facilitates the ascending infection more than males. These results are consistent with what was found by all Andriole 1987; Millar, 1997; Al Mugeiren et al., 1992; Patterson and Larabi et al., (2003) and also agreed with (Abu Daia etal., 2000) and al-Dabbagh 1998.

The rate of infection of women(60%)number of isolate18, *Staphylococcus aureus* It was noted that was one of the most common causes of UTI , isolates (5) (55%)from(9) Total number of isolates, and 4 isolates 44% from men. Table 5 showed appeared *Klebsiella*(0%) for women to 2 isolate for men 6.6%, and (0.0%)for *Pseudomonas aeruginosa*, *proteus*.

While the results are significantly different with the global study conducted by researcher Chen Wa(2012) obtained 3739 isolates of the bacterial type *s. aureus* from infections of urinary tracts in Thailand Hospitals for the period from 2007-2011 The reason for this difference may be attributed to the geographical location of the isolation area or the difference in circumstances environmental. The results of our current study also did not agree with what was stated in previous studies, as the isolated bacterial species

In this study, similar to what was indicated by (2013., al et Osarimen, who was able to isolate from *P. aeruginosa* and *Klebsiella s*pp. And *Proteus spp*. And *E. coli* and *S. aureus* Diabetic foot ulcers in Nigeria with rates of (38, 24, 20, 10 and 8%) respectively, Also (Patil and Patil 2015) was able to isolate the mentioned species with percentages of (4.25 and 9.6.). and 3.21, 6.8 and 65.10%.

Recommendations

1. **Losing weight and keeping it off**. Weight control is an important part of diabetes prevention. You may be able to prevent or delay diabetes by losing 5 to 10% of your current weight. For example, if you weigh 200 pounds, your goal would be to lose between 10 to 20 pounds. And once you lose the weight, it is important that you don't gain it back.
2. **Following a healthy eating plan**. It is important to reduce the amount of calories you eat and drink each day, so you can lose weight and keep it off. To do that, your diet should include smaller portions and less fat and sugar. You should also eat a variety of foods from each food group, including plenty of whole grains, fruits, and vegetables. It's also a good idea to limit red meat, and avoid processed meats.
3. **Get regular exercise.** Exercise has many health benefits, including helping you to lose weight and lower your blood sugar levels. These both lower your risk of type 2 diabetes. Try to get at least 30 minutes of physical activity 5 days a week. If you have not been active, talk with your health care professional to figure out which types of exercise are best for you. You can start slowly and work up to your goal.
4. **Don't smoke.** Smoking can contribute to insulin resistance, which can lead to type 2 diabetes. If you already smoke, try to quit.

5. **Talk to your health** care provider to see whether there is anything else you can do to delay or to prevent type 2 diabetes. If you are at high risk, your provider may suggest that you take one of a few types of diabetes medicines.
6. **Drink water** Water is one of the best drinks you can drink, and if you drink water most of the time, this will help you avoid drinks that are high in sugar,

Preservatives, and other ingredients that may cause diabetes. In addition, drinking water instead of other beverages helps control blood sugar and insulin levels, and sugary drinks are associated with an increased risk of type 2 diabetes and late adult autoimmune diabetes (LADA), a form of type 1 diabetes that occurs In people over 18 years age.

7. **Improve Vitamin D Levels** Vitamin D is important for controlling blood sugar levels, as it has been found that people who do not get enough vitamin D have a higher risk of developing all types of diabetes Good food sources of vitamin D usually include fatty fish and liver oil .

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