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Study the effect of diabetes mellitus on diversity and antibiotic-resistance of oral aerobic bacteria in children

A thesis

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بسم الله الرحمن الرحيم

(نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

صدق الله العلي العظيم

سورة يوسف الايه (76)

Dedication

To my Parents,

My husband (Khaled),

My sons(Laith,Ahmed,Hassan),

My brother(Nabeel),

And my sisters(Khawla,Khaleda,Ashwaq,Mayada,Khanssa)

Without whom none of my success would be possible.

The price of success is hard work ,dedication to the job at hand ,and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.

Ibtihaj

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Acknowledgement

Summary

The continuous persistence of diabetic mellitus (DM) conditions can affect the diversity and density of the oral normal flora. Many oral bacteria can be changed into pathogens under the effect of DM. Resistance to antibiotics can also affect by DM when many oral bacteria can activate its resistance to antibiotics or get a new one in the presence of DM.

A case-control study was designed to include 105 children aging (1.5-15) years divided into two main groups. The first group included 50 patients aging with DM who also subdivided into two subgroups; 25 patients with controlled DM and 25 patients with uncontrolled DM ;The second group was 55 non-diabetic children . Oral swabs were collected from all children groups and cultured for identification of the colony counting and species diagnosis. Susceptibility tests to many antibiotics by disk diffusion method were performed for all isolates with determination of antibiotic-resistant.

Females were represented a great number of patients with DM and control groups, but without significant differences from males. Duration of DM was mostly found within less than one year (52%), especially at age 11-15 years, while duration of 7-8 years (6%) was observed in low number of diabetic patients. Heavy growth of isolated bacteria that exceed 360 cfu was found higher in both DM and non-diabetic children. It counted in 64.76% of subjects. Uncontrolled DM had more heavy growth (20 patients) than in controlled DM (9 patients). This was also observed in non-diabetic children (39children). Counting of 100-200 cfu was represented the second great count of bacteria isolated from all subjects (20%).

Total number of isolated bacteria was 23 species of Gram positive and 2 species of Gram negative bacteria. Number of isolated bacteria was equal (47.61%) in DM patients, while it increased in control (52.38%), but without significant differences. *Kocuria kristinae*, *Kytococcus sedentarius*, *Streptococcus oralis* and *Granulicatella adiacens* were the most frequent isolated species (23.75%, 13.33%, 12.38% and 9.52%, respectively). In controlled DM, *K. kristinae* and *G. adiacens* were common (3.80% for each), while *K. kristinae and K. sedentarius* were common among patients with uncontrolled DM (6.66% and 4.76%, respectively). Children in non-diabetic group had common isolates of *K. kristinae* (7.61%), and *S. oralis* (8.57%)

Susceptibility of isolated bacteria to tested antibiotics was found variable even in the same species. Isolate 2 of *S. aureus* and isolate 4 of *K. kristinae* isolated from controlled DM patients and all isolates of *K. kristinae* of uncontrolled DM were revealed resistant to all of antibiotics. Meanwhile, *S. aureus*-1 from children of non-diabetic was shown multidrug resistant to at least 9 antibiotics and 8 isolates of *K. kristinae* were resistant to 8 antibiotics. Resistant to ceftriaxone (CRO), cefixime (CFM) and tetracycline (TE) were the most common in isolates of all participants.

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List of Abbreviations

ADA	**American Diabetes Association**
AK	**Amikacin**
ANOVA	**Analysis of Variance**
ATP	**Adenosine triphosphate**
AZM	**Azithromycin**
C	**Chloramphenicol**
CAZ	**Ceftazidime**
CFM	**Cefixime**
CFU	**Colony Forming Unit**
CLSI	**Clinical and Laboratory Standards Institute**
CN	**Gentamycin**
CRO	**Ceftriaxone**
DA	**Clindamycin**
DM	**Diabetes Mellitus**
DNA	**Deoxy ribonucleic acid**
FDA	**Food Drug Association**

H2O2	**Hydrogen Peroxide**
L	**Lincomycin**
Lev	**Levofloxacin**
MIC	**Minimum inhibitory concentration**
P	**Penicillin**
Spp.	**Species**
SXT	**Trimethoprime-sulphamethoxazole**
T1DM	**Type 1 Diabetes Millitus**
T2DM	**Type 2 Diabetes Millitus**
TE	**Tetracycline**
Van	**Vancomycin**
WHO	**World Health Organization**

Chapter One

Introduction

1. Introduction

The oral cavity contains a huge number of different types of microorganism collecting in a single term of normal flora or microbiota that live in a commensal relationship with the human body (Mujumdar and Singh,2014;Kilian *et al.*, 2016). Some members of this oral flora community can be changed into pathogenic agents causing many oral diseases under the effect of many conditions (Kilian *et al.*, 2016; Mrinalini, 2018). Diabetes mellitus (DM) which is characterized by chronic hyperglycemia due to disorder in the insulin synthesis or function may be considered one of these abnormal conditions to create a pathogenic microorganisms (Kulshrestha *et al.*, 2011; Kosti and Kanakari, 2012; Hsaine *et al.*, 2018; Graves *et al.*, 2019; Kori *et al.*, 2020). DM is very common disease with a wide range of prevalence all over the world (WHO, 2019). It can stimulate the oral flora to cause many infectious diseases in the oral cavity through its effect on disturbance the balance between different types of the oral cavity (Belal, 2020). Thus oral infections in patients with DM are usually higher compared to that in non-diabetes individuals (Hsaine *et al.*, 2018).

Antimicrobial resistance is one of very serious problem facing the public health in the present time (Medernach and Logan, 2018; Belal, 2020). Many factors can be associated with the development of antimicrobial resistance. Treatment with antibiotic for a long time is the most important factor to develop antimicrobial resistant (Ready *et al.*, 2003). Transferring of resistant gene between bacterial populations of the oral cavity is another important factor for increasing the number of antimicrobial resistance bacteria (Fair and Tor, 2014).

The aim of the study

1- Evaluating the effect of DM on the diversity and density of antibiotic-resistance in the bacteria of the oral cavity of children aging (1.5-15)year.

2- Determine the antibiotic resistance in DM patients of oral aerobic bacteria.

Chapter Two

Review of Literatures

2. Review of literature

2.1. Oral normal flora

Microbiota or normal flora is a term with the same meaning which is refers to the organisms live in a symbiosis correlation with the human body (Majumdar and Singh, 2014; Kilian *et al.*, 2016). It could be found in many parts of the human body with a various degree of density and diversity. The oral cavity is the second large part of the human body containing microbial communities of bacteria, fungi, and viruses (Kilian *et al.*, 2016; Xiao *et al.*, 2020). A colonization of about more than 700 species within over 500 bacterial taxa and about 22 identified genera has been identification in various parts of the oral cavity (Majumdar and Singh, 2014; Kilian *et al.*, 2016; Xiao *et al.*, 2020). The initiation of oral microbiota is firstly starting from contact with birth canal during birth, then by breast feeding from mother and later from other sources such as water, food and from surrounding environment (Mrinalini, 2018;Xiao *et al.*, 2020).

Many advanced technology try to get a clear view about the complexity of the oral microflora and its role in diseases (Kilian *et al.*, 2016). Environmental conditions of the site of occurring have an effect on the type of oral organism and can effect later on metabolic activities of such organism (Mrinalini, 2018).

Equilibrium between different types of oral flora is an indicator for healthy state of the oral and any change in such correlation will allow pathogenic bacteria to overgrow causing oral diseases (Kilian *et al.*, 2016; Mrinalini, 2018). There are many types of diseases caused by normal flora of

the oral cavity such as caries, gingivitis and periodontitis (Kilian *et al.*, 2016).

2.2. Diabetes mellitus and oral flora

Diabetes mellitus (DM) is one common of the group of multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from a partial or complete impairment in the metabolism of carbohydrates, proteins and lipids (Kosti and Kanakari, 2012). It can be found in the people of every region of the world whatever they are in developed or developing countries (WHO, 2019). Incidence of DM is always elevating within a time. It was estimated in about 30 million in 1985 and raised to 150 million in 2000 and 246 million in 2007 with expected to reach 380 million by 2025 as predict by the International Diabetes Federation (Riaz, 2009). In the Eastern Mediterranean region, DM represented a nineth leading cause of death, especially from type 2 (WHO, 2019). The epidemic of DM as one of noncommunicable diseases makes it a challenge to the public health that currently facing the world (WHO, 2006). The main reasons for the development of DM are consisted from the defect in insulin secretion, action, or both (WHO, 2019). Thus, many types of DM can be recognized to include type -1 (T1DM) as a common in children, which results from defect in insulin secretion after destruction of pancreatic beta producing cell; type-2 (T2DM) resulting from insulin resistance due to dysfunction in beta cells and it common in adults; gestational and other types that may result from the effect of genetic defects, environment, infections or from drugs effects (ADA, 2014; Baynest, 2015). Several factors association with the development of DM and determined its type, such as genetic and predisposition or environmental

factors that inducing of autoimmune destruction of beta cells to form T1DMas well as T2DM (WHO, 2019). The development of DM is more related to the age, in which T2DM is more common among adults, while T1DM is common in children (Kosti and Kanakari, 2012). Other factors associated with DM are including obesity, diet, unhealthy lifestyle, physical inactivity, hormone action, drugs, and infections (WHO, 2006; Riaz, 2009; ADA, 2014; WHO, 2019).

The DM can create a suitable environment promotion growing and colonization various bacterial species of the oral normal flora compared with that in the oral healthy individuals (Kulshrestha *et al.*, 2011;Hsaine *et al.*, 2018; Graves *et al.*, 2019; Kori *et al.*, 2020). From the study in human and animals, oral diseases found to be increased due to DM by its role in enhancement inflammatory reaction in periodontium and increase activity of pathogenic oral microflora to cause periodontal diseases, such as species of *Capnocytophaga* spp.*, Porphyromonas* spp., and *Pseudomonas* spp. (Graves *et al.*, 2019). A greater bacterial diversity with a potential cause of periodontal was diagnosed in diabetic patients compared with non-diabetic (Hsaine *et al.*, 2018). Alteration in oral microflora has been recorded in diabetic patients, in which most of bacteria were from Gram-negative, while it was Gram-positive in normal individuals and also diabetes increases the rate of infection with periodontal diseases (Kulshrestha *et al.*, 2011). A significant abundant of phylum Fimicutes and the most predominant genera of acidogenic bacteria (*Prevotella* spp., *Leptotrichia* spp.) and aciduric bacteria (*Veillonella* spp.) were observed in diabetic patients than in healthy individuals (Kori *et al.*, 2020).

2.3. Resistance of oral bacterial flora

Over prescription and misuse of antibiotic for a long period in the treatment of microbial infection enforce bacterial normal flora to develop an evolution resistant to protect itself from antimicrobial action of such antibiotics (Fair and Tor, 2014). Nowadays, antimicrobial resistance is one of the most significant problem to the public health, in which it association with high rate of morbidity and mortality (Medernach and Logan, 2018; Belal, 2020). Destruction of normal flora, as in the oral cavity, will lead to stimulate multiplication of pathogenic microorganisms that causing serious diseases (Belal, 2020). Another factor associated with faster development of antimicrobial resistance is transferring of resistance elements within bacterial population (Fair and Tor, 2014). Resistance gene can easily be horizontal transfer from normal flora to bacterial pathogens after misuse of antibiotics (Rukke, 2017;Belal, 2020). Depending on a recent study, a single orally administration of antibiotic may encourage enrichment with resistance genes (Rukke, 2017). Moreover, Biofilm forming by different species of oral microorganism on the teeth and mucous membrane is another factor association with the development of bacterial-resistance against antibiotics and also facilitate transferring of resistance gene between bacterial compositions of biofilm (Rukke, 2017).

2.4. Mechanism of antibiotic resistant in bacteria

Bacteria have ability for adaptation and evolution against the harmful effects of different toxic substances including antibacterial agents through

development many resistant mechanisms (Munita and Arias, 2016; Reygaert, 2018). This type of resistance plays an important role in increasing the treatment complications of bacterial infection and increase health threat to the human worldwide (Tenover, 2006;Munita and Arias, 2016). Generally, antibacterial agents can inhibit or kill several species of bacteria through interfering with different metabolic process in bacterial cell, including the synthesis of nucleic acid (eg. Fluoroquinolones and rifampin), protein (eg. Macrolides and tetracyclines), cell wall (eg. β-lactams and glycopeptide agents), and other metabolic substances (eg. trimethoprim-sulfamethoxazole), and also effects on the structure of bacterial membranes (eg. polymyxins)(Tenover, 2006). Thus, bacteria have been developed several strategies to limit or resist the toxic effects of these antibacterial agents include; activation of drug efflux, reduce antibiotic uptake, change of drug target, and inactivation of drug (Reygaert, 2018).

Acquired antibiotic-resistant by bacteria is usually by two different main sources. The first one is native by the bacteria and considers intrinsically which give a resistance to at least one of antibacterial agents (Tenover, 2006; Reygaert, 2018). This is usually developed by mutation in gene targeting by antibiotic (Munita and Arias, 2016). The second source of resistance is acquired a resistance genes from extrinsic source or from other microorganisms through horizontal gene transfer (Tenover, 2006; Munita and Arias, 2016; Reygaert, 2018). Three process can use by bacteria to acquire external genetic materials, including transduction by phage mediation, transformation by naked DNA, and conjugation by pili connection (Munita and Arias, 2016).

The bacterium *K. kristinae* is facultative anaerobic, cocci, coagulase-negative and catalase negative belonging to the family Micrococcaceae, suborder Micrococcineae, order Actinomycetales (Savini *et al*., 2020). It can found in the environment and on various parts of the human body such as skin and mucous membrane of the oral cavity (Szczerba I, 2003;Savini *et al*., 2020). The predominant of *K. kristinae* was determined at 7.3% from 8 species of the oral cavity of healthy people (*Micrococcus luteus* (26.2%), *Nesterenkonia halobia* (21%) *Kocuria* varians (16.4%), *Micrococcus lylae* (12.2%), *Dermatococcus sedentarius* (9.1%), *Kytococcus nishinomiyaensis* (7.3%), and *Kocuriarosea* (0.3%) with no significant differences between male and females (Szczerba, 2003). *K. kristinae* can cause several types of infections in the children and immunocompromised people (Dunn *et al*., 2011; Chen *et al*., 2015). Seven cases of premature babies and five older patients were diagnosed to have bacteremia by *K. kristinae* due to long-term intravenous catheters (Lai *et al*., 2010; Chen *et al*., 2015). Another cases of catheter-related bacteremia by *K. kristinae* was also mentioned in 51-year women with ovarian cancer (Basaglia *et al*., 2002) and in 29-year pregnant female to add to the 15 cases of infection by the genus of *Kocuria* from 1995 to 2010 that have mean age 54 years, but without gender prefer (Dunn *et al*., 2011). Acute cholecystitis is another infection caused by *K. kristinae* as diagnosed in 56-year old Chinese man (Ma *et al*., 2005).

Kytococcus sedentarius is the second most common bacteria in the oral cavity of individuals of this study. It is Gram positive, strictly aerobic and need amino acid to grow on medium and it is belong to the family *Dermacoccaceae* (Sims *et al*., 2009). The bacterium can found in the environment as a predominant indoor bacteria (above 800 cfu/m^3 of air) and

also in the oral cavity or on the skin of the human body (Sims *et al*., 2009;Folayan *et al*., 2018; AL-Janabi, 2020). Many infections can be resulted from *K. sedentarius* such as valve endocarditis, hemorrhagic pneumonia, and pitted keratolysis due to its ability to produce destruction enzymes of keratinous materials (Longshaw *et al*., 2002;Sims *et al*., 2009). A case of nail infection (onychobacteriosis) by *K. sedentarius* in 54-year old women was diagnosed (Towersey *et al*., 2008).

  Streptococcus oralis and *Granulicatella adiacens* are other most frequently isolated bacteria from the patients of this study. The bacterium *S. oralis* which is belonging to the *S. mitis* group, is common in the oral cavity as a normal flora (Do *et al*., 2009). It can cause many diseases such as meningitis and bacteremia (Patel *et al*., 2019; Watanabe *et al*., 2020). Resistance to antibiotics are mentioned to many isolates of *S. oralis* such as resistant to penicillin, cephem, meropenem and daptomycin, while it susceptible to penicillin, ceftriaxone and vancomycin (Patel *et al*., 2019; watanabe*et al*., 2020). *G. adiacens* is a cocci or polymorphic, facultative anaerobic, catalase and oxidase negative (Collins and Lawson, 2000). It related to the nutritionally variant Streptococci that found as normal flora of the oral cavity and could cause infections such as endocarditis and monomicrobial non-neutrocytic bacterascites (Cargill *et al*., 2012;Cincotta *et al*,2015).

Chapter Three

Materials and Methods

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3. Materials and Methods

3.1. Materials

3.1.1. Apparatuses and Equipments

Apparatus and instruments of this study are included in tables 3-1 and 3-2.

Table (3-1): Apparatuses used in the study

No.	Apparatus	Company	Origin
1.	VITEK2 compact	BioMerieux	France
2.	Autoclave	Hirayama	Japan
3.	Biological safety cabinet	Labtech	Korea
4.	Microscope	Olympus	Phelepin
5.	Micropipette	Slamed	Germany
6.	Digital balance	Denver	German
7.	Incubator	Termaks	Denemark
8.	Oven	Steri-dent	USA
9.	Refrigerator	memmert	Korea
10.	Bunsen Burner	Amal	German
11.	Turbidity meter	BioMerieux	France
12.	Vortex	Stuart	USA

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Table (3-2): Equipment used in the study

No.	Equipment	Company	Origin
1.	Plain tube	ArthAL-Rafidin	China
2.	Glass slides	Supertek	India
3.	Gloves	Top-Glove	Malaysia
4.	Inoculating loop	Ambala	India
5.	Microscope cover glass	Supertek	India
6.	Filter paper	United	India
7.	Cylinders	Marienfeld	Germany
8.	Pyrex conical flask	Marienfeld	Germany
9.	Petridish	PlastLab	Lebanon
10.	Syringe 5 ml	MEDI	China
11.	Sterilized swab	Afco	Jordan
12.	Forceps	Ambala	India
13.	Cotton	Alsalama	Iraq
14.	Mask	PM medical	Turkey
15.	Rack	PlastLab	Lebanon
16.	Disposable loop	Ambala	India
17.	Can tube	Marienfeld	Germany
18.	Yellow and blue tips	PlastLab	Lebanon

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3.1.2. Chemical and biological materials

Different types of chemical and biological materials were used in current study (table 3-3).

Table (3-3): Chemical and biological materials used in the study.

No.	Chemical and biological material	Company	Origin
1.	Ethanol (70%)	Joudtol	Iraq
2.	Gram stain	JouriLabs	Sorachin SwitzerLand
3.	Hydrogen peroxide (3%)	Solvochem	U.K
4.	Mac Farland 0.5	BioMerieux	France
5.	Optochin disc	Sigma	German
6.	Tetramethyl-p-phenylenediaminedihydrochloride	Scharlau	Spain
7.	VITEK R 2 GP Card	BioMerieux	France

Table(3-4) :Antibiotics

No.	Antibiotic classes	Antibiotic	Company	Origin
1.	Beta lactam	Penicillin(5µg)	Bioanalyse	Turkey
2.	Tetracyclines	Tetracycline(10µg)		
3.	Cephalosporins	Ceftazidime(30µg),Ceftriaxone,(10µg),Cefixime(5µg)		
4.	Quinolones	Levofloxacin (10µg)		
5.	Lincomycins	Clindamycin(10µg),Lincomycin(10µg)		
6.	Macrolides	Azithromycin(15µg)		
7.	Sulfonamides	Sulfamethoxazole and trimethoprim(25µg)		
8.	Glycopeptides	Vancomycin(30µg)		
9.	Aminoglycosides	Gentamycin(10µg),Amikacin(10µg)		
10	chloramphenicol	Chloramphenicol(10µg)		

3.1.3 Culture Media

All of cultured media that already prepared we purchased as mention in table (3-5).

Table (3-5): Culture media

No.	Media	Manufacturing company	Origin
1.	Blood Base Agar	Neogen	USA
2.	MacConkey Agar	Neogen	USA
3.	Muller-Hinton Agar	Neogen	USA
4.	Muller-Hinton Broth	Oxoid	England

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3.2. Methods

3.2.1. Material Preparation

3.2.1.1. Preparation of culture Media (ready – made media):

Blood Base Agar, MacConkey Agar, Muller-Hinton Agar and Muller-Hinton Broth media were prepare according to the instructions of the manufacturing company. A suitable amount of each media as described by manufacturing instructions was dissolved in a one liter of distilled water in a conical flask (1 Liter) to obtain a weight of g/L. Sterilization of culture media and solutions are achieved by autoclaving at 121 C /1 Pascal for 15 minutes. At prepare of blood agar after autoclaving allow it to cool but not solidify at 45-50 degree Celsius add 5% sterile defibrinated blood that has been warmed to room temperature and mix gently but well ,avoid air bubbles then dispense in to sterile plates while liquid.At prepare of chocolate agar we heating blood agar ,which in turn ruptures the red blood cell and release nutrients that aid in the growth of fastidious bacteria and to see alfa hemolysis clearly for Streptococcus .The name is derived from the fact that the lysisof RBC gives the medium achocolate brown color.

3.2.1.2. Preparation of oxidase test:

Oxidase solution was prepared by dissolving 1g of substrate tetramethyl-p-phenylenediaminedihydrochloride in 80 ml of distill water in a conical flask (250 ml), followed by completed the volume into 100 ml. Prepared solution was stored in a dark container in the refrigerator until usage.

3.2.2. Isolation of bacteria

3.2.2.1. Patients

A total of 105 children were involved in a case control study. They divided into 2 main groups included 50 patients with diabetic mellitus (DM) and 55 of non-diabetic patients at age range 1.5-13 years. The group of patients with DM included 2 subgroups: 25 patients with controlled DM at age range 1.5-15 years and 25 patients with uncontrolled DM at age range 3-15 years. Subjects of this study were enrolled during admitted as outpatients of diabetic children in the AI- Hassan Endocrine Center of the AI-Emmam AI-Hussein Medical City and in Pediatric Teaching Hospital in Karbala ,Iraq province from 19 November 2020 to 15 January 2021but the non diabetic children collecting them from children of our family and my friends. Diabetes mellitus was primary diagnosed in all involved subjects by physicians of pediatric medicine of the hospital and later by biochemical analysis of blood samples for a total glucose level and HbA1C test. Children with oral infection, chronic diseases, and under antibiotic treatment were excluded.

3.2.2.2. Collection of bacteria

Oral swabs were collected from the up of the oral cavity(hard palate) of all children groups. Samples were immediately cultured on prepared culture media and incubated aerobically at 37 ℃ for 24 hours.For the bacteria that needed CO_2 such *Streptococcus pneumoniae* put it in candle jar for provide 5% CO_2.Grown bacteria were counting as colony forming unit(CFU),then diagnosis.

3.2.2.3. Counting of bacteria

Grown colonies of each isolate on culture media were visually counting. Number of colonies was recorded in form of colony forming unit (cfu):is aunit used in microbiology to estimate the number of viable bacteria or fungi cells in a sample.Viable is difined as the ability to multiply via binary fission under the control conditions .Counting with colony –forming units requires culturing the microbes and counts only viable cells incontrast with microscopic examination which counts all cells ,living or dead . Three range of counting were used. The first one named low growth (100-200 cfu), moderate (201-360 cfu) and heavy growth (>360 cfu).

3.2.2.4. Diagnosis of isolated bacteria:

3.2.2.3.1. Presumptive diagnosis

Primary diagnosis of isolated bacteria was mainly depending on the culture characters such as colony morphology, color, and blood hemolysis. Type of Gram stain was also performed after staining with Gram stain to differentiate between Gram positive bacteria (taken violet color from crystal violet stain) and Gram negative bacteria (taken a pink color from Safranin stain), also this Gram stain useful to determine the shape, size and arrangement of bacteria cells .

3.2.2.3.2. Biochemical test:

Catalase test:

The catalase test is a biochemical test for aerobic organisms used to

detect production of catalase enzyme that act in breakdown hydrogen peroxide (H_2O_2) to O_2 and H_2O. A small amount of isolated bacteria was taken by awood stick and put on a microscope slide, and then a drop of 3% H_2O_2 was added on the bacterial elements. Bubble observation meaning a positive results (Reiner, 2010).

Coagulase test:

The coagulase test is mainly used to differentiate *Staphylococcus aureus* from *Staphylococcus epidermidis* and other coagulase – negative species. The coagulase test can be performed using two different procedures: slide and tube tests. The slide test is simple and gives results within 10 seconds, but it can give false positive. The tube test is the definitive test and need up to 24 hours to complete. Several colonies from culture of *Staphylococcus* spp. was mixed with 0.5 ml of human plasma in sterile tube. Tube was incubated at 35-37 ℃ in ambient air for 4 hours. The visible clot will indicate the positive result, while negative result indicated by non-clotting appearance. If the result is negative after 4 hours, the tube must be incubated again at room temperature over night and check it for clot formation (Katz, 2010).

Oxidase test:

The oxidase test was used for identifying Gram negative bacteria that has the ability to produce cytochrome oxidase enzyme. A small amount of 24 hours growth of isolated bacteria was picked and streak on a small piece of filter paper, then 1 or 2 drops of 1% oxidase reagent was added on the

organism smear to observe color changes. Positive result was indicated by color changes to dark purple within oxidase negative if the color does not change or it takes longer than 2 minutes (Shields and Cathcart, 2010).

Optochin disk test:

Optochin (ethyl hydrocupreine hydrochloride) is an antibiotic have the ability to react with the ATPase enzyme that effect on production of adenosine triphosphate (ATP) in microorganisms. It used for differentiation of alpha-hemolysis *Streptococcus* species such as *S. pneumoniae* (positive test)from other *Streptococcus* species which are resistant to the optochin. Inoculation of blood agar with 24 hours growth of bacteria by streaking 2-3 colonies on media surface and a disk of optochin was added on the inoculated media by sterilized forceps ;then incubated at 35 ℃ for 18-24 hours in 5% -10% of CO_2. Any inhibition zone around the disk with the diameter more or equal 14 mm is considered positive result (Tille, 2014).

3.2.2.3.3. Confirmatory identification:

Complete diagnosis of isolated bacteria was performed using VITEK2 compact system. The instrument is an automated microbial identification system depending on many biochemical tests using a special Card.

3.2.4. Susceptibility test for isolated bacteria

Disk diffusion method was used to determine susceptibility and resistance of isolated bacteria to antibiotics using of CLSI-M100-S23 (2020).

Ten antibiotic disks were used in this study for Gram-positive bacteria, including Penicillin (P; 5 μg), Vancomycin (VAN, 30 μg), Clindamycin (DA, 10 μg), Lincomycin (L, 10 μg), Azithromycin (AZM, 15 μg), Ceftriaxone (CRO, 10 μg), Cefixime (CFM, 5 μg), Tetracycline (TE, 10 μg), and Chloramphenicol (C, 10 μg). For Gram-negative bacteria, five antibiotic disks were used, including Ceftazidime (CAZ, 30 μg), Gentamycin (GN, 10 μg), Trimethoprime-sulphamethoxazole (SXT, 25 μg), Amikacin (AK, 10 μg), and Levofloxacin (LEV, 5 μg). A suspension of bacterial growth was prepared by culturing each isolate in 2 ml of Muller-Hinton broth and incubated at 37º C for 24 hours. A few milliliters of grown bacteria was re-suspended in 2 ml of normal saline and adjusted with 0.5 McFarland standard to get $1\text{-}2\times10^{8}$cfu/ml. About 100 μl from prepared inoculate of each isolate was spread on Muller-Hinton agar by sterilized swab. Antibiotic disks were added on inoculated plates and incubated at 37º C for 24 hours. The zone of inhibition was measured in mm around effective disk. Resistance to antibiotic was determined based on comparing the diameter of the zone of inhibition with that mentioned in the CLSI-M100-S23 (2020), FDA (2006) and Oxiod (2013).

3.2.5. Statistical Analysis:

Acase control study data of all tests analyzed statistically with one way ANOVA by using Excel application of Window 10. The minimum level of (p) value was ≤ 0.05 concerts as significant level.

Chapter Four

Results

4. Results

4.1. Subjects of the study

Two main groups with two subgroups were included in this case-control study. The 50 diabetic patients were represent the first main group that divided into 25 controlled and 25 uncontrolled DM as two subgroups. Females showed a higher number than male among controlled and uncontrolled patients of DM group (13.33% and 14.28%, respectively), while number of males was less (10.47%% and 9.52%, respectively). This was also found among non-diabetic patients, in which females (29.52%) were in large number than males (22.85%)(Table 4-1). However, there are no significant differences in sex between children of three groups.

Table (4-1): Number of involved subjects

Subject group	Sex	Subject No.	Total No.
Controlled DM	Male	11 (10.47%)	25 (23.80%)
	Female	14 (13.33)	
Uncontrolled DM	Male	10 (9.52%)	25 (23.80%)
	Female	15 (14.28%)	
Non-diabetic patients	Male	24 (22.85%)	55 (52.38%)
	Female	31 (29.52%)	
Total No.			105

4.2. Duration of DM in correlation with age

The duration of DM was determined in all of controlled and uncontrolled patients with DM. Most of involved patients were suffered from DM for less than one year (52%), while very low number had DM for 7 to 8 years (6%). Duration of 3-4 years of DM was also found higher among all involved patients (28%)(Table 4-2).

The large number of either male or female patients with DM was found at age of 11-15 years, except females with controlled DM was significantly higher at 6-10 years (18%).Percentage of females at 11-15 years was 10% in controlled DM and 16% in uncontrolled DM, while it was 10% for males of both groups. The number of females at this age who had less than one year duration of DM was about 2% of controlled group and 8% of uncontrolled group, while males had 6% and 2%, respectively. At age 6-10 years of males in controlled group, there were only 6% with less than one year of DM and 2% with 5-6 years of DM, while no one at the same duration time could find among male with uncontrolled DM. Duration of 7 to 8 years of DM was also found in 2% of each of males and females aged 11-15 years of controlled group with no one in uncontrolled group and in only 2% of females aged 6-10 years of uncontrolled DM (Table 4-2).

Table (4-2): Duration of DM in correlation with age

Patient group	Sex	Age (year)	Patient No.				Total No.
			DM duration (year)				
			<1-2	3-4	5-6	7-8	
Controlled DM	Male	1-5	1 (2%)	1 (2%)	0	0	2 (4%)
		6-10	3 (6%)	0	1 (2%)	0	4 (8%)
		11-15	3 (6%)	0	1 (2%)	1 (2%)	5 (10%)
	Female	1-5	0	0	0	0	0
		6-10	$8^{*,\,**}$ (16%)	1 (2%)	0	0	9 (18%)
		11-15	1 (2%)	2 (4%)	1 (2%)	1 (2%)	5 (10%)
Uncontrolled DM	Male	1-5	2 (4%)	0	0	0	2 (4%)
		6-10	0	3 (6%)	0	0	3 (6%)
		11-15	1 (2%)	2 (4%)	2 (4%)	0	5 (10%)
	Female	1-5	0	0	0	0	0
		6-10	3 (6%)	3 (6%)	0	1 (2%)	7 (14%)
		11-15	4 (8%)	2 (4%)	2 (4%)	0	8 (16%)
Total No.			26 (52%)	14 (28%)	7 (14%)	3 (6%)	50

* Significant differences between ages in the same duration of DM

** Significant differences between duration of DM in the same age group

4.3. Number of Bacteria isolated from the oral cavity

The number of isolated bacteria from the patients with DM and non-diabetic groups was determined. A total of heavy bacterial growth that exceed 360 cfu was represented the highest value (64.76%), followed by the growth of 100-200 cfu (20%). Such heavy growth was mostly found in females of both DM patient subgroups (5 of controlled DM and 12 of uncontrolled DM) compared with males (4 of controlled DM and 8 of uncontrolled DM). The age group that had large number of heavy growth was observed in females at 6 to 10 years (8.57%, 7.61% and 12.38% for controlled, uncontrolled DM and non-diabetic, respectively). Meanwhile, there was no bacterial growth was found among females at 1 to 5 years. Among males of two DM subgroups, age group of 11 to 15 years was the most age with bacterial growth, while it was high among non-diabetic males at age 1-5 years (13.33%). Male with heavy growth was variable between those with controlled DM at age 1 to 5 years (1.90%) and those with uncontrolled DM aged 11 to 15 years (4.76%)(Table 4-3).

Low colony count was noticed highly among patients with controlled DM (4 males and 6 females) compared with uncontrolled DM (2 males only). Most age group with low bacterial count was those at 11 to 15 years in all subjects three groups. The middle range number of colony count (201-360 cfu) was observed among controlled DM at two age groups (6-10 and 11-15 years), while it was in only one age group (11-15 years) of female with uncontrolled DM (Table 4-3).

Table (4-3): Total count of isolated bacteria in the subjects

Subject group	Sex	Age (year)	Total count (CFU)			Total No.
			100-200	201-360	>360	
Controlled DM	Male	1-5	0	0	2 (1.90%)	2 (1.90%)
		6-10	1 (0.95%)	2 (1.90%)	1 (0.95%)	4 (3.80%)
		11-15	3 (2.85%)	1 (0.95%)	1 (0.95%)	5 (4.76%)
	Female	1-5	0	0	0	0
		6-10	3 (2.85%)	2 1.90%)	4 (3.80%)	9 (8.57%)
		11-15	3 (2.85%)	1 (0.95%)	1 (0.95%)	5 (4.76%)
Uncontrolled DM	Male	1-5	0	0	2 (1.90%)	2 (1.90%)
		6-10	1 (0.95%)	0	2 (1.90%)	3 (2.85%)
		11-15	1 (0.95%)	0	4 (3.80%)	5 (4.76%)
	Female	1-5	0	0	0	0
		6-10	0	0	8 (7.61%)	8 (7.61%)
		11-15	0	3 (2.85%)	4 (3.80)	7 (6.66%)
Non-diabetic patients	Male	1-5	3 (2.85%)	1 (0.95%)	10 (9.52%)	14 (13.33)
		6-10	2 (1.90%)	1 (0.95%)	4 (3.80%)	7 (6.66%)
		11-15	1 (0.95%)	0	2 (1.90%)	3 (2.85%)
	Female	1-5	1 (0.95%)	1 (0.95%)	10 (9.52%)	12 (11.42%)
		6-10	2 (1.90%)	1 (0.95%)	10 (9.52%)	13 (12.38%)
		11-15	0	3 (2.85%)	3 (2.85%)	6 (5.71%)
Total No.			21 (20%)	16 (15.23%)	68 (64.76%)	105

4.4. Frequency of isolated bacteria

A total of 25 species was isolated from all subjects of this study. They distributed between 23 species Gram-positive and two species of Gram-negative. Although a diverse distribution of species within each group was found, the total isolates was in equal number in two DM subgroups (23.80%), while it was highly found in and non-diabetic group (52.38%), but without significant differences. *Kocuria kristinae*, *Kytococcus sedentarius*, *Streptococcus oralis* and *Granulicatella adiacens* were the most frequent isolated species (23.75%, 13.33%, 12.38% and 9.52%, respectively). Gram-negative bacteria were included a single species of *E. coli* and *Pseudomonas aeruginosa* (0.95% for each) which isolated only from patient with uncontrolled DM. Some species were found in all subject groups with a variable number such as *K. sedentarius*, *S. oralis* and *G. adiacens.* Among patient with controlled DM, *K. kristinae* and *G. adiacens* were the larger number (3.80% for each), while 11 species of Gram-positive and all of Gram-negative were absented in this patient subgroup. Species of *K. kristinae and K. sedentarius* were common among patients with uncontrolled DM (6.66% and 4.76%, respectively), while 13 species of Gram-positive were absence. The most frequent species in non-diabetic patients was represented by *K. kristinae* (7.61%), followed by *S.oralis* (8.57%)(Table 4-4).

Table (4-4): Frequency of isolated bacteria in subjects

Isolated species	Total count DM patient		Non-diabetic patients	Total No.
	Controlled DM	Uncontrolled DM		
Staphylococcus vitulinus	1 (0.95%)	0	0	1 (0.95%)
Staphylococcus hominis	2 (1.90%)	0	0	2 (1.90%)
Staphylococcus aureus	2 (1.90%)	0	3 (2.85%)	5 (4.76%)
Staphylococcus epidermidis	0	0	1(0.95%)	1(0.95%)
Staphylococcus haemolyticus	1 (0.95%)	0	0	1 (0.95%)
Micrococcus leuteus	0	0	1(0.95%)	1(0.95%)
Rothia dentocariosa	2 (1.90%)	1 (0.95%)	0	3 (2.85%)
Rothia mucilaginosa	2 (1.90%)	1 (0.95%)	1 (0.95%)	4 (3.80%)
Kytococcus sedentarius	3 (2.85%)	5 (4.76%)	6 (5.71%)	14 (13.33%)
Kocuria kristinae	4 (3.80%)	7[*,**] (6.66%)	8 (7.61%)	19 (18.09%)
Kocuria rhizophila	0	1 (0.95%)	0	1 (0.95%)
Kocuria rosea	0	0	2 (1.90%)	2 (1.90%)
Streptococcus pluranimalium	0	0	1 (0.95%)	1 (0.95%)
Streptococcus oralis	2 (1.90%)	2 (1.90%)	9 (8.57%)	13 (12.38%)
Streptococcus sanguinis	0	2 (1.90%)	3 (2.85%)	5 (4.76%)
Streptococcus salivarius	0	1 (0.95%)	4 (3.80%)	5 (4.76%)
Streptococcus pneumoniae	0	1 (0.95%)	0	1 (0.95%)
Streptococcus pseudoporcinus	0	0	2(1.90%)	2(1.90%)
Streptococcus alactolyticus	0	0	3 (2.85%)	3 (2.85%)

Granulicatella adiacens	4 (3.80%)	2 (1.90%)	4 (3.80%)	10 (9.52%)
Granulicatella elegans	1 (0.95%)	0	5 (4.76%)	6 (5.71%)
Gemella morbilorum	1 (0.95%)	0	1 (0.95%)	2 (1.90%)
Leuconostoc mesenteroides	0	0	1(0.95%)	1(0.95%)
E. coli	0	1 (0.95%)	0	1 (0.95%)
Pseudomonas aeruginosa	0	1 (0.95%)	0	1 (0.95%)
Total No.	25 (23.80%)	25 (23.80%)	55 (52.38%)	105

* Significant differences between species frequency and subject groups

** Significant differences between species frequency in the same subject group

4.5. Susceptibility of isolated bacteria from patients with controlled DM to antibiotics

Isolated bacteria showed a variable susceptibility to different antibiotics. Such observation is also found with the isolates of the same species. All of bacterial species showed resistance to at least one antibiotic. Some species isolated from controlled patients with DM revealed resistant to great number of tested antibiotics such *S. aureus* and *K. kristinae*. Isolate 2 of *S. aureus* and No. 4 of *K. kristinae* of these two species were revealed resistant to all of antibiotics. *S. vitulinus*, isolate 3 of *K. kristinae*, isolate 3 of *K. sedentarius* and *G. morbillorum* were also resistant to 8,8, 7 and 6 antibiotics, respectively. Isolate 2 of *R. mucilaginosa* was found resistant to less number of antibiotics represented by CFM only, followed by four stains of four species, including *S. hominis*-2, *R. dentocariosa*-2, *S. oralis*-1and *G. elegans* that were resistant to two antibiotics (Table 4-5 and table 4-1 index).

The most antibiotic types that resisted by a large number of isolated bacteria from controlled DM patients were cefixime (CFM)(24 isolates) and ceftriaxone (CRO)(20 isolates), followed by tetracycline (TE) (14 isolates). Meanwhile, resistant to chloramphenicol was observed in very low number of isolated bacteria (4 isolates)(Table 4-5).

The isolate of the same species was revealed various susceptibility degrees to antibiotics. Isolate 1 of *S. hominis* and *R. dentocariosa* showed multidrug-resistant to 3 and 4 antibiotics, respectively, while isolate 2 of both was resisted to only two. The resistance to 5 antibiotics was showed by the isolates 1 of *S. aureus*, while isolate 2 had resistant to all of 9 antibiotics. Isolate 1 of *R. mucilaginosa* was more resistant than isolate 2 (three to one, respectively). The third isolate of *K. sedentarius* was more resistant to antibiotics than other two. Almost all four isolates of *K. kristinae* had resistant to large number of tested antibiotics. Resistant to 4-5 antibiotics was also shown by the four isolates of *G. adiacens* (Table 4-5 and table 4-1 index).

Table (4-5): Susceptibility of isolated bacteria from controlled DM patients to antibiotic

Isolated species	Antibiotic susceptibility									No. of R	No. of S
	P	AZM	VAN	DA	L	CRO	CFM	TE	C		
S. vitulinus	R	S	R	R	R	R	R	R	R	8	1
S. hominis-1	S	R	S	S	S	R	R	S	S	3	6
S. hominis-2	S	S	S	S	S	R	R	S	S	2	7
S. aureus-1	R	R	S	S	R	R	R	R	S	5	4
S. aureus-2	R	R	R	R	R	R	R	R	R	9	0
S. haemolyticus	R	R	R	S	S	R	R	S	S	5	4
R. dentocariosa-1	R	S	S	S	S	R	R	R	S	4	5
R. dentocariosa-2	S	S	S	S	S	R	R	S	S	2	7
R. mucilaginosa-1	S	S	S	S	S	R	R	R	S	3	6
R. mucilaginosa-2	S	S	S	S	S	S	R	S	S	1	8
K. sedentarius-1	R	S	R	R	R	S	R	S	S	5	4
K. sedentarius-2	S	S	R	R	R	S	S	R	S	4	5
K. sedentarius-3	R	R	R	R	R	S	R	R	S	7	2
K. kristinae-1	S	S	S	S	R	R	R	R	S	4	5
K. kristinae-2	S	R	S	R	S	R	R	R	S	5	4
K. kristinae-3	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-4	R	R	R	R	R	R	R	R	R	9	0
S. oralis-1	S	S	S	S	S	R	R	S	S	2	7
S. oralis-2	S	S	S	R	S	R	S	R	S	4	5
G. adiacens-1	S	R	S	S	S	S	R	R	S	3	6
G. adiacens-2	S	S	R	R	R	R	R	S	S	5	4
G. adiacens-3	S	S	R	R	R	R	R	S	S	5	4
G. adiacens-4	S	S	S	R	R	R	R	S	S	4	5
G. elegans	S	S	S	S	S	R	R	S	S	2	7
G. morbillorum	S	S	R	R	R	R	R	R	S	6	3
Total No.										115	110

S: Sensitive; R: Resistant.

P:penicillin;AZM:Azithromyci;Van:Vancomycin;DA:Clindamycin;L:Lincomycin;CRO:Ciftr iaxone;CFM:Cefixime;TE:TEtracyclin;C:Chloramphenicol.

4.6. Susceptibility of isolated bacteria from patients with uncontrolled DM to antibiotics

Bacteria isolated from uncontrolled patients with DM were shown resistant to at least one antibiotic. All isolated isolates of *K. kristinae* from such patients had resistant to almost all tested antibiotics. This was also observed with the *K. sedentarius* in which all of its isolates were resistant to at least 4 antibiotics and some of them resistant to all antibiotics as with *K. sedentarius*-4or 8 of them as with *K. sedentarius*-1. Some bacterial isolates showed resistant to only one antibiotics such as *K. kristinae*-7, *R. mucilaginosa,* and *S. oralis*-1(Table 4-7 and table 4-2 index). The Gram-negative *P. aeruginosa* was revealed highly resistant to 8 antibiotics, while *E. coli* had resistant to 7 antibiotics (Table 4-7 and table 4-3 index).

The antibiotic resistant was mostly found to the CFM (24 resistant isolates) and TE (20 resistant isolates), followed by CRO (18 resistant isolates). Resistant to other antibiotics were showed by variable isolates ranged 8 to 13 isolates. There were no resistant to less than 8 antibiotics had been noticed (Table 4-6).

Based on isolate level, there was a variable degree of antibiotic resistant among isolates of the same bacterial species. Some isolates showed highly multidrug- resistant for almost all of tested antibiotics such as the six isolates of *K. kristinae*, while the isolate 7 was resistant to only CFM. Isolate 1 and 4 of *K. sedentarius* showed multidrug-resistant to 8 to 9 antibiotics, while isolate 5 was resistant to only 4 antibiotics. *S. oralis*-2 had resistant to 3 antibiotics, while its isolate No. 1 was resistant to CFM only. The two isolates

of *G. adiacens* were revealed similar multidrug-resistant to antibiotics (CFM, CRO, TE) (Table 4-6 and table 4-2 index).

Table (4-6): Susceptibility of isolated bacteria from uncontrolled DM patients to antibiotic

Isolated species	Antibiotic susceptibility									No. of R	No. of S
	P	AZM	VAN	DA	L	CRO	CFM	TE	C		
K. kristinae-1	R	R	R	R	R	R	R	R	R	9	0
K. kristinae-2	R	R	R	R	R	R	R	R	R	9	0
K. kristinae-3	R	R	R	R	R	R	R	R	R	9	0
K. kristinae-4	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-5	R	R	R	R	R	R	R	R	R	9	0
K. kristinae-6	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-7	S	S	S	S	S	S	R	S	S	1	8
K. rhizophila	R	S	R	S	R	R	R	R	S	6	3
R. dentocariosa	S	S	S	S	S	S	R	R	S	2	7
R. mucilaginosa	S	S	S	S	S	S	R	S	S	1	8
K. sedentarius-1	R	R	R	R	R	R	R	R	S	8	1
K. sedentarius-2	R	S	R	R	R	S	R	S	S	5	4
K. sedentarius-3	R	S	R	R	R	R	R	R	S	7	2
K. sedentarius-4	R	R	R	R	R	R	R	R	R	9	0
K. sedentarius-5	S	S	R	R	R	S	S	R	S	4	5
S. oralis-1	S	S	S	S	S	S	R	S	S	1	8
S. oralis-2	S	S	S	S	S	R	R	R	S	3	6
S. sanguinis-1	S	S	S	S	S	R	R	R	R	4	5
S. sanguinis-2	S	S	S	S	S	R	R	S	S	2	7
S. pneumoniae	S	S	S	S	S	R	R	R	S	3	6
S. salivarius	S	S	S	S	S	S	R	R	S	2	7
G. adiacens-1	S	S	S	S	S	R	R	R	S	3	6
G. adiacens-2	S	S	S	S	S	R	R	R	R	4	5
Total No.										117	91

S: Sensitive; R: Resistant .
P:penicillin;AZM:Azithromyci;Van:Vancomycin;DA:Clindamycin;L:Lincomycin;CRO:Ciftriaxone;CFM:Cefixime;TE:TEtracyclin;C:Chloramphenicol.

Table (4-7): Zone of inhibition of isolated Gram-negative bacteria in uncontrolled DM

Isolated species	Antibiotic susceptibility									No. of R	No. of S
	CAZ	SXT	LEV	AK	GN	CRO	CFM	TE	C		
E. coli	R	R	S	R	S	R	R	R	R	7	2
P. aeruginosa	R	R	R	R	S	R	R	R	R	8	1
										15	3

S: Sensitive; R: Resistant .

CAZ:Ceftazidim;SXT:Sulfamethoxazol and trimethoprim;LEV:Levofloxacin;AK:Amikacin;GN:Gentamyci; CRO:Ciftriaxone;CFM:Cefixime;TE:TEtracyclin;C:Chloramphenicol.

4.7. Susceptibility of isolated bacteria from non-diabetic children

The bacterium *S. aureus*-1 was the most resistant isolate to all of 9 antibiotics than other isolates from non-diabetic patients. Three isolates of *K. kristinae* were showed multidrug-resistant to 8 antibiotics, while isolate 4 was showed resistant to only one antibiotic. Highly resistant to 6 antibiotics was also observed with 4 other isolates, including *K. rosea, G. elegans*-3, *S. oralis*-3*, and S. alactolyticus*-1. The less number of antibiotics resistant was found in the isolate 1 of *S. oralis* as well as *K. kristinae*-4 (one antibiotic) and *S. alactolyticus*-3 (two antibiotics) (Table 4-8 and table 4-4 index).

The CFM antibiotic was the most type that large number of isolated bacteria were resistant to it (51 isolates), followed by CRO (44 isolates), TE (39 isolates), and L (29 isolates). Meanwhile, less resistant number was found to the antibiotics AZM (16isolates) and C (11isolates) (Table 4-8).

Antibiotic resistant according to the isolate level showed that isolate 4 and 5 of *K. kristinae* were had resistant to a less number of antibiotics than other 6 isolates, while first three isolates were highly resistant to almost all antibiotics. *S. aureus*-2 was also showed resistant to less number of antibiotics (4 antibiotics) than isolate 1 (9 antibiotics). This was also found with isolate 2 of *G. elegans* which had resistant to fewer antibiotics than other two isolates, In addition, three of five isolates of *S. oralis* (isolates 1, 2, and5) showed less resistant to tested antibiotics. *S. alactolyticus*-3 was resistant to two types of antibiotics (CFM and C) and isolate 2 to 3 antibiotics (CRO, CFM and C) compared with resistant to 6 antibiotics by isolate 1 (Table 4-8 and table 4-4 index).

Table (4-8): Susceptibility of isolated bacteria from non-diabetic group

Isolated species	Antibiotic susceptibility									No. of R	No. of S
	P	AZM	VAN	DA	L	CRO	CFM	TE	C		
K. kristinae-1	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-2	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-3	R	S	R	R	R	R	R	R	R	8	1
K. kristinae-4	S	S	S	S	S	S	R	S	S	1	8
K. kristinae-5	S	S	R	R	R	S	S	S	S	3	6
K. kristinae-6	S	R	S	S	R	R	R	S	R	5	4
K. kristinae-7	R	S	S	S	R	R	R	S	S	4	5
K. kristinae-8	R	R	S	S	S	R	R	R	S	5	4
K. rosea	S	S	R	R	R	R	R	R	S	6	3
K. sedentarius	R	S	R	R	R	S	S	R	S	5	4
R. mucilaginosa	S	S	S	S	S	R	R	R	R	4	5
S. aureus-1	R	R	R	R	R	R	R	R	R	9	0
S. aureus-2	R	S	S	S	S	R	R	R	S	4	5
S. aureus-3	R	S	S	S	R	R	R	R	S	5	4
G. elegans-1	R	R	R	R	R	R	R	R	S	8	1
G. elegans-2	S	S	R	S	R	S	S	R	S	3	6
G. elegans-3	R	R	S	R	R	R	R	S	S	6	3

G. adiacens	S	S	R	R	R	S	R	R	S	5	4
S. sanguinis	R	R	S	S	S	R	R	R	S	5	4
S. oralis-1	S	S	S	S	S	S	R	S	S	1	8
S. oralis-2	S	S	S	S	S	R	R	S	R	3	6
S. oralis-3	R	S	R	S	R	R	R	R	S	6	3
S. oralis-4	S	S	R	R	R	S	R	R	S	5	4
S. oralis-5	S	S	S	S	S	R	R	R	S	3	6
S. salivarius-1	S	R	S	S	S	R	R	R	S	4	5
S. salivarius-2	S	S	S	S	S	R	R	R	S	3	6
S. alactolyticus-1	S	R	S	S	R	R	R	R	R	6	3
S. alactolyticus-2	S	S	S	S	S	R	R	S	R	3	6
S. alactolyticus-3	S	S	S	S	S	S	R	S	R	2	7
S. pluranimalium	S	S	S	S	S	R	R	R	S	3	6
M. luteus	S	S	S	S	S	R	R	S	S	2	7
S. epidermidis	R	S	R	S	S	R	R	R	S	5	4
K. sedentarius-1	R	S	R	R	R	R	R	R	S	7	2
K. sedentarius-2	R	S	R	R	R	R	R	R	S	7	2
K. sedentarius-3	R	S	S	R	R	R	R	R	S	6	3
K. sedentarius-4	S	S	R	R	R	S	R	S	S	4	5
K. sedentarius-5	R	S	S	S	S	R	R	S	S	3	6
K. rosea	R	R	S	R	R	R	R	R	S	7	2
S. anguinis-1	S	R	R	S	S	R	R	S	S	4	5
S. anguinis-2	S	S	R	R	R	S	R	R	S	5	4
S. oralis-1	S	S	S	S	S	R	R	R	S	3	6
S. oralis-2	R	S	S	S	S	R	R	R	S	4	5
S. oralis-3	S	S	S	S	S	R	R	R	S	3	6
S. oralis-4	R	R	R	R	R	R	R	R	R	9	0
S.pseudoporuscin-1	S	S	S	S	S	R	R	S	S	2	7
S.pseudoporcinus-2	S	S	S	S	S	R	R	R	S	3	6
S. salivarius-1	R	S	R	R	R	R	R	R	S	7	2
S. salivarius-2	R	R	S	S	S	R	R	R	S	5	4
G. adiacens-1	R	R	R	S	R	R	R	S	S	6	3
G. adiacens-2	S	R	R	S	R	R	S	R	S	5	4
G. adiacens-3	R	R	R	R	R	R	R	R	S	8	1
G. elegans-1	S	S	S	S	S	R	R	R	S	3	6
G. elegans-2	S	S	R	R	R	S	R	R	S	5	4
G. morbillorum	R	S	S	S	S	R	R	R	S	4	5
L. mesenteroides	S	R	S	S	S	R	R	S	S	3	6
Total NO.										261	235

S: Sensitive; R: Resistant;P:Penicillin;AZM:Azithromycin;VAN:Vancomycin;DA:Clindamycin; C RO:Ceftriaxone;CFM:Cefexime;TE:Tetracyclin;C:Cloramphenicol

Chapter Five

Discussion

5. Discussion:

5.1. Duration of DM in correlation with age

In this study, females with DM were found in high number than males, especially at age 11-15 years. This also mentioned by many studies. The prevalence of DM in females of Zuni Indians population was 57% higher than in male (Scavini *et al*., 2003). Majeed and Hassan (2011) were found that T1DM in females of Basrah province (65.6%) is more than in males (34.4%) which was also in similar with that in control group and also they found that age 9.1-13.92 years more frequent for T1DM (43.7%) than in control (40.4%), while they less in age 1.2-4.9 years.In contrast, other studies found no different in DM between male and female. Meta-analysis of 29 reports with 36 studies in 2011 showed no significant differences of the prevalence of DM among males and females (Hilawe *et al*., 2013). Although T2DM is higher among male in middle age, it is reordered that its present is in equal prevalence between male and females in most population (Gale and Gillespie,2001). Moreover, no significant difference in age and gender distribution was found between DM patients and control group (Majeed and Hassan, 2011). The differences of the prevalence of DM in male and female may related to many biological and psychosocial factors associated with each of them, including body composition, genetic, nutrients, culture, lifestyle, environment and socioeconomic status and sex hormones (Willer *et al*.,2016)

The WHO (2019) illustrated that each age group have a specific type of DM with a common frequently. The T1DM is common in age group < 6 months to < 10 years and T2DM at age groups 10 to < 25 years, while both

types can be found in age older than 25 years with respect to the immunity state. Chentli *et al.* (2015) classified DM in elderly into two groups: survivors in younger or middle age due to the effect of autoimmune disease and incident in older age (over 60 years) due to insulin resistance. The T1DM was found constant in female aged 15 months, while it was higher in male at age 15-40 years (Gale and Gillespie, 2001).

The duration of DM in patients of this study was mostly found in less than one year, especially at age groups 6-10 and 11-15 years.The DM duration as well as personal background and co-morbidities are important factors affecting on the consequently, complications and management of the disease (Chentli *et al.*, 2015). It also effect on the quality of life and survival of patients from DM (Manna, 2016). The sex 3:2 male: female ratio of T1DM is usually had constant duration in young adults during 2-3 generation of some population (Gale and Gillespie,2001).

5.2. Correlation of DM with oral normal flora

Community of the microorganisms in the oral cavity is normally living in very complex relationship and it can change constantly within the time (Sweeney *et al*., 2004). A few numbers of oral normal flora, not exceed 10%, can be cultured from a total of approximately 300-500 species or may reach to 1000 species of bacteria, fungi, and protozoa (Sweeney *et al*.,2004; Haque *et al*.,2019). Density and biodiversity of this flora can be influenced by many abnormal conditions such as DM. the presence of DM found to be created a suitable conditions to increase the density and variability of oral flora compared to healthy individuals (Kulshrestha *et al.*, 2011;Hsaine *et al.*, 2018; Graves *et al.*, 2019; Kori *et al.*, 2020). Gram negative bacteria has been

observed dominants in the oral cavity of patients with DM, while Gram positive is higher in healthy individuals with a possibility to increase diseases caused by any of these groups under the effect of DM (Kulshrestha *et al.*, 2011).

From the results of this study, bacterial count was found low among children with DM than in control group. This result also reported by the study of Saeb et al. (2019) who they found that number of oral microbiota was reduced in both type 2 diabetic patients and impaired glucose tolerance compared to that in healthy control. Less abundant of five families and seven genera of phylum Actinobacteria was observed in diabetes patiets compared to normal individuals (Long *et al.*, 2017). Other studies demonstrated no significant difference was found in the count of oral microbiota between diabetes and control individuals (El-Tekeye *et al.*, 2021; Almelda-Santos *et al.*, 2021). Uncontrolled T1DM in younger individuals (6-15 years of age) decreased oral health and increased the risk of oral disease compared to controlled DM and healthy individuals (Babatzia, 2020). This reduction in oral microbial number in diabetic patients may be resulted from three possible reasons. The first is that high glucose level can encourage pathogenic bacteria to limit the abundant of other bacteria species (Almelda-Santos *et al.*, 2021). The second is that dehydration in mouth due to diabetes can reduce the density of oral flora. The third is that acidification in oral condition by the effect of hyperglycemia can reduce microbial density.

On the other hand, diabetic conditions can increase the number of oral flora than in healthy individuals. Number of bacterial genera was found higher in DM patients aged 18 years and above (58.3%) than in non-diabetic individuals (41.7%) (Bissong, 2014). Counting of *Lactobacillus* spp. of the

oral cavity was observed at higher rate in children with T1DM, in addition to low rates of saliva flow and buffer capacity than in control healthy group (Ferizi *et al*., 2018). However, the taxonomic diversity of oral bacteria tries to be constant and not affected by any of oral diseases (Almeida *et al*., 2020).

5.3. Frequency of isolated bacteria

Diversity or frequent of appearance of bacteria in the oral cavity could be shown a variable range in patients with DM. At high taxonomic levels, phylum of Fimicutes with genera of acidogenic and aciduric bacteria are more predominant in DM patients than in healthy individuals (Kori *et al.*, 2020), while phyla Bacteroidetes and Fusobacteria are more predominant in healthy individuals (Almeida *et al*., 2020). The most isolated aerobic bacteria from the oral cavity of DM patients aged 18 years or above are those related to the genera: *Streptococcus* spp. (99.6%), coagulase negative *Staphylococcus* spp. (6.4%), *Serratia* spp. (7.2%) and *Klebsiella* spp. (5.7%) (Bissong, 2014). The levels of *Streptococcus mutans* showed a significantly differences between children with poor controlled DM and the healthy control (Babatzia, 2020). Almelda-Santos *et al.* (2021) found that diversity of the oral microbiota is not significantly different between diabetes and healthy control. Meanwhile, Saeb *et al.* (2019) observed a reduction in oral microbial diversity in diabetic patients compared to that in healthy control.

From the results of this study, Gram positive bacteria in patients with DM were shown great frequency than Gram negative, which represented by two species that found only in uncontrolled DM patients. This result also observed by another study in which a Gram positive bacteria were the most frequently isolated from the oral cavity of children with DM (68.2%) than

Chapter Five……………………………………………..Discussion

Gram negative (16.5%) and the bacterium *E. coli* was singly isolated from only DM patient (Bissong, 2014). *Streptococcus* spp. and *Lactobacillus* spp. as members of Gram positive bacteria were reported higher in supragingival plaque from diabetics than in non-diabeticswithout any significant differences with respect to age range and gender distribution (Kampoo *et al*.,2014). In the present of dental diseases associated with DM, Gram negative bacteria could be showed more frequently than Gram positive group. The oral cavity of DM females with periodontal disease diagnosed to have high prevalent of five species of Gram negative bacteria, including *Porphyromonas gingivalis, Tannerella forsythia, Capnocytophaga ochracea, Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans* compared to healthy individuals and some species such as *Aggregatibacter actinomycetemcomitans* was found only in DM patient (Al-Obaida *et al*., 2020).

From the results of the present study, four species of Gram positive bacteria were common in both controlled and uncontrolled DM patients, including *Kocuria kristinae*, *Kytococcus sedentarius*, *Streptococcus oralis* and *Granulicatella adiacens*. Genera of *Kocuria* and *Kytococcus* as well as *Nesterenkonia*, *Micrococcus* and *Dermacoccus* are dissected from the genus *Micrococcus* by Stackebrandt *et al*. in 1995 (Szczerba, 2003). From 23 isolates of Gram positive bacteria from the oral cavity of healthy individuals, *K. sedentarius, K. kristinae* and *G. adiacens* were diagnosed (AL-Janabi, 2020). The bacterium *K. kristinae* is facultative anaerobic, cocci, coagulase-negative and catalase negative belonging to the family Micrococcaceae, suborder Micrococcineae, order Actinomycetales (Savini *et al*., 2020). It can found in the environment and on various parts of the human body such as skin

and mucous membrane of the oral cavity (Szczerba I, 2003;Savini *et al.*, 2020). The predominant of *K. kristinae* was determined at 7.3% from 8 species of the oral cavity of healthy people (*Micrococcus luteus* (26.2%), *Nesterenkonia halobia* (21%) *Kocuria* varians (16.4%), *Micrococcus lylae* (12.2%), *Dermatococcus sedentarius* (9.1%), *Kytococcus nishinomiyaensis* (7.3%), and *Kocuriarosea* (0.3%) with no significant differences between male and females (Szczerba, 2003). *K. kristinae* can cause several types of infections in the children and immunocompromised people (Dunn *et al.*, 2011; Chen *et al.*, 2015). Seven cases of premature babies and five older patients were diagnosed to have bacteremia by *K. kristinae* due to long-term intravenous catheters (Lai *et al.*, 2010; Chen *et al.*, 2015). Another cases of catheter-related bacteremia by *K. kristinae* was also mentioned in 51-year women with ovarian cancer (Basaglia *et al.*, 2002) and in 29-year pregnant female to add to the 15 cases of infection by the genus of *Kocuria* from 1995 to 2010 that have mean age 54 years, but without gender prefer (Dunn *et al.*, 2011). Acute cholecystitis is another infection caused by *K. kristinae* as diagnosed in 56-year old Chinese man (Ma *et al.*, 2005).

Kytococcus sedentarius is the second most common bacteria in the oral cavity of individuals of this study. It is Gram positive, strictly aerobic and need amino acid to grow on medium and it is belong to the family *Dermacoccaceae* (Sims *et al.*, 2009). The bacterium can found in the environment as a predominant indoor bacteria (above 800 cfu/m^3 of air) and also in the oral cavity or on the skin of the human body (Sims *et al.*, 2009;Folayan *et al.*, 2018; AL-Janabi, 2020). Many infections can be resulted from *K. sedentarius* such as valve endocarditis, hemorrhagic pneumonia, and pitted keratolysis due to its ability to produce destruction enzymes of

keratinous materials (Longshaw *et al*., 2002;Sims *et al*., 2009). A case of nail infection (onychobacteriosis) by *K. sedentarius* in 54-year old women was diagnosed (Towersey *et al*., 2008).

Streptococcus oralis* and *Granulicatella adiacens* are other most frequently isolated bacteria from the patients of this study. The bacterium *S. oralis* which is belonging to the *S. mitis* group, is common in the oral cavity as a normal flora (Do *et al*., 2009). It can cause many diseases such as meningitis and bacteremia (Patel *et al*., 2019; Watanabe *et al*., 2020). Resistance to antibiotics are mentioned to many isolates of *S. oralis* such as resistant to penicillin, cephem, meropenem and daptomycin, while it susceptible to penicillin, ceftriaxone and vancomycin (Patel *et al*., 2019; watanabe*et al*., 2020). *G. adiacens* is a cocci or polymorphic, facultative anaerobic, catalase and oxidase negative (Collins and Lawson, 2000). It related to the nutritionally variant Streptococci that found as normal flora of the oral cavity and could cause infections such as endocarditis and monomicrobial non-neutrocytic bacterascites (Cargill *et al*., 2012;Cincotta *et al*,2015).

5.4. Correlation of DM with antibiotic-resistant oral bacteria

Resistance to antibiotics, natural or synthetic, are considered one of a serious problem in the present days leading to high morbidity and mortality rates (Medernach and Logan, 2018; Haque *et al*., 2019; Belal, 2020). In addition to long-term use of antibiotics (Sweeney *et al*.,2004; Haque*et al*.,2019), transferring of gene with resistance information to many antibiotics among bacterial population is the most causative factor has a role to increase antibiotic-resistance development (Fair and Tor, 2014; Rukke, 2017; Belal,

2020). Development of resistance in bacterial isolates of oral flora need more investigation (Sweeney *et al.*,2004). Resistance genes such as *erm* (58.2%), *bla*$_{TEM}$ (16.4%), *mec*A (2.7%), *pbp*2b and *aac* (6%) were found higher among the flora of the oral cavity (Almeida *et al.*,2020). Bacteria resistance to antibiotics in the oral cavity of healthy children aged 4-5 years were diagnosed from a total of 432 isolates that comprised 18 genera and 47 species (Ready *et al.*, 2003). The DM is found in associated with increase development of antibiotic-resistant in bacteria. The most known drug resistance bacteria associated with DM, including Gram positive such as *Mycobacterium tuberculosis*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, and Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are shown higher prevalence in patients with DM than in those non-diabetic (Boyanova and Mitov, 2013). Multidrug resistant organisms (MDRO) were more prevalent in the DM population (Trivedi *et al.*, 2014).

From the results of this study, most isolated species from all involved subjects were resistance to cefixime, ceftriaxone, and tetracycline antibiotics with not significant differences between subject groups. Many other studies found such none significant differences between the number of antibiotic-resistance bacteria in DM patients and non-DM patients even the rate of these bacteria is higher among DM patients. Isolated *E. coli* from both diabetic and non-diabetic patients showed about the same susceptibility to antibiotics (meropenem; 94% and 94%, imipenem: 92% and 92%, amikacin; 76% and 74%; ampicillin/sulbactam; 68%and 69%, respectively for DM and non-DM)(Chakraborty *et al.*, 2017). Most of isolated *E. coli* and *Enterococcus* spp. was revealed none significant resistant to many antibiotics between DM and

non-diabetic patients (Bonadio *et al*., 2006). Also multidrug resistant bacteria isolated from 63.4% of DM patients revealed insignificant difference from that in 50% of non-diabetic patients (Trivedi *et al*., 2014). Study the effect of DM on the genes of the drug resistance in *E. coli* in compared with that in non-diabetic patients showed that resistance genes to beta lactamase, *AmpC* and *NDM-1* were in approximately an closed percentage between DM and non-diabetic patients (70% and 70.5%, 9.5% and 14.4%, and 7% and 4.5%, respectively)(Chakraborty *et al*., 2017). Several resistant bacteria from the oral cavity of healthy children (7-8 years old) were isolated including 6 isolates of *S. aureus* resistant to chloramphenicol or tetracycline with 4 methicillin resistant; *Haemophilus* spp. resistant to erythromycin (13.3%), ampicillin (17%), and tetracycline (1.9%), and 5.9% of β-hemolytic Streptococci resistant to tetracycline (Millar *et al.*, 2001)

Although there was no different between patient groups of this study in the resistant to a specific type of antibiotic, a differences were found among species or isolate of bacteria resistant to antibiotic when a great variety was found in isolated types between all groups of included subjects. The most common isolated species of bacteria were *S. aureus* and *K. kristinae* from controlled DM patients, *K. kristinae* and *K. sedentarius* as Gram positive and *P. aeruginosa* and *E. coli* as Gram negative bacteria from uncontrolled DM patients, and *S. oralis*-4, *G. adiacens*-3, *K. sedentarius*, and *S. anguinis* from non-diabetic patients. From these results, *K. kristinae* resistance to antibiotic was the most common isolate. Drug resistance in the genus *Kocuria* is poorly investigated for years, but resistant to kanamycin, ampicillin and erythromycin was documented (Szczerba I, 2003;Savini *et al*., 2020). Isolates of *K. kristinae* isolated from central venous catheter-related bacteremia were found

susceptible to many antibiotics, including oxacillin, clindamycin, vancomycin, cefazolin, cefalothin, erythromycin, ciprofloxacin, penicillin, rifampin and trimethoprim/sulfamethoxazole (Basaglia *et al*., 2002;Dunn *et al*., 2011). Another isolate of *K. kristinae* isolated from five patients with the same disease condition (catheter-related bacteremia) showed resistant to oxacillin (Lai *et al*., 2010). Isolated of *K. kristinae* from 56-year old man with acute cholecystitis showed susceptibility to same antibiotics in addition to levofloxacin (Ma *et al*., 2005).

Resistant *G. adiacens* to various antibiotics was varied between complete resistant as to clindamycin, rifampin, levofloxacin, ofloxacin, quinupristin/dalfopristin, and vancomycin and partial resistant as to penicillin (55%), amoxicillin (81%), ceftriaxone (63%), and meropenem (96%) (Tuohy *et al*., 2000). From 15 healthy children, the prevalence of *G. adiacens* and *G. elegans* that resistance to clindamycin, beta-lactam, macrolides and tetracycline was found higher with diagnosis the presence of *erm* and *mef* genes in these species (Zheng *et al*., 2004).

In the present study, Gram negative bacteria represented by *P. aeruginosa* and *E. coli* isolated from patients with uncontrolled DM showed resistance to most of antibiotics. *P. aeruginosa* isolated from DM patients with UTI showed resistance to 13 antibiotics, including ampicillin, amoxicillin, clavulanic acid, cefepime, cefoperazone, vancomycin, gentamycin, doxycycline, ciprofloxacin, levofloxacin, sulfamethoxazole, nitrofurantion, pipemedic acid and nalidixic acid (Zahra *et al*., 2016). Wound infection with *Pseudomonas* spp. was found higher among patients with DM (61%) compared to non-DM (18.9%) in reverse with *S. aureus* which revealed less infection in DM patients (42.3%) than in non-DM (57.7%)(Trivedi *et al*.,

2014). Although *S. aureus* was found in high prevalence in diabetic patients with foot infection, a non significant increase in incidence of antibiotic resistance was also found (Lebowitz *et al*., 2017). An association between Gram negative and *S. aureus* in patients with DM has also been mentioned by other studies. A high frequency of resistant *S. aureus* to methicillin (63%) and ciprofloxacin (55.5%) was found in association with 43.5% of resistant Gram negative to ciprofloxacin in diabetic foot infections (Pontes *et al*., 2020). The incidence of vancomycin-resistant *Staphylococcus aureus* and polymyxin B-resistant *Pseudomonas* spp. and four other members of *Enterobacteriaceae* family (*Proteus* spp., *Enterobacter* spp., *Escherichia coli*, and *Citrobacter* spp.) has been observed higher in patients with diabetic foot lesions (Perim *et al*., 2015). Antibiotic-resistant *Staphylococcus* spp. in associated with *E. coli* and *Klebsiella* spp. was diagnosed as causative agents of community-onset bloodstream infections in patient with T2DM (61.9%, 18.4% and 10.7% of each, respectively)(Huang *et al*., 2018). Methicillin-resistant *S. aureus* and vancomycin resistant *Enterococcus spp.* were isolated from 0.6% of DM patients with UTI (Zahra *et al*., 2016).

The other Gram negative bacteria found resistant to many antibiotics in this study was *E. coli* that isolated from uncontrolled DM patients. Most of isolated *E. coli* isolated from DM patients with UTI revealed resistant to many antibiotics such as nitrofurantoin, cotrimoxazole and ciprofloxacin (Bonadio *et al*., 2006). Other isolate from DM patients with UTI also showed resistant to many antibiotics (17 antibiotics)(Zahra *et al*., 2016)

Conclusions And Recommendations

Conclusions

1- No significant differences were found in sex between diabetic patients and non diabetic children .

2- DM decreases the counting of oral bacteria based on comparison the heavy growth of isolated bacteria between diabetic patients and non diabetic children.

3- Biodiversity of oral bacteria was differed between diabetic patients and control groups.

4- Uncontrolled DM encourages oral bacteria to grow heavily than controlled DM

5- Antibiotic-resistant bacteria were showed variablity in species and isolates level between subject groups.

6- Multidrug resistance were found higher among isolated bacteria.

7- Resistance to ceftriaxone,cefexime and tetracyclin were the most common in isolates of all participants.

Recommendation

1- Determination of resistance genes from the bacteria of the oral cavity in diabetic patients and make a comparison with that in non-diabetic individuals.

2- Study the antibiotic resistance in facultative and anaerobic bacteria in oral cavity.

3- Determine the genes responsible for virulence factors of oral cavity bacteria.

4- Identification of resistance bacteria in different sites of the oral cavity (teeth,buccal mucosa,gum,submandibular duct opening).

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Table (4-1): Zone of inhibition of isolated bacteria in controlled DM

Isolated species	Zone of inhibition (mm)																	
	P		AZM		VAN		DA		L		CRO		CFM		TE		C	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
S. vitulinus	-	0	20	-	-	0	-	0	-	0	-	0	-	0	-	0	-	0
S. hominis-1	29	-		0	27	-	39	-	38	-	-	0	-	0	26	-	22	-
S. hominis-2	40	-	28	-	31	-	39	-	39	-	-	10	-	0	35	-	40	-
S. aureus-1	-	0	-	20	17	-	25	-	-	23	-	0	-	0	-	19	19	-
S. aureus-2	-	10	-	0	-	16	-	0	-	0	-	0	-	0	-	0	-	0
S. haemolyticus	-	18	-	0	-	15	30	-	25	-	-	0	-	0	20	-	20	-
R. dentocariosa-1	-	24	27	-	20	-	21	-	24	-	-	0	-	0	-	14	25	-
R. dentocariosa-2	40	-	24	-	25	-	45	-	41	-	-	0	-	0	27	-	31	-
R. mucilaginosa-1	18	-	30	-	25	-	36	-	33	-	-	19	-	0	-	0	33	-
R. mucilaginosa-2	42	-	46	-	25	-	43	-	36	-	25	-	-	0	25	-	36	-
K. sedentarius-1	-	25	35	-	-	0	-	15	-	0	21	-	-	22	22	-	35	-
K. sedentarius-2	36	-	29	-	-	14	-	0	-	0	33	-	31	-	-	0	27	-
K. sedentarius-3	-	0	-	0	-	0	-	0	-	0	24	-	-	20	-	12	19	-
K. kristinae-1	34	-	26	-	27	-	29	-	-	17	-	20	-	0	-	0	35	-
K. kristinae-2	34	-	-	15	25	-	-	17	37	-	-	16	-	0	-	0	33	-
K. kristinae-3	-	0	19	-	-	0	-	0	-	0	-	0	-	0	-	0	-	0
K. kristinae-4	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
S. oralis-1	33	-	21	-	30	-	33	-	40	-	-	0	-	0	-	-	40	-
S. oralis-2	20	-	20	-	30	-	-	18	40	-	-	10	-	0	-	20	32	-
G. adiacens-1	26	-	-	15	20	-	43	-	35	-	27	-	-	0	-	0	29	-
G. adiacens-2	26	-	20	-	-	0	-	0	-	0	-	23	-	25	---	-	27	-
G. adiacens-3	29	-	18	-	-	0	-	0	-	0	-	12	-	0	-	-	35	-
G. adiacens-4	30	-	22	-	24	-	-	17	-	0	-	25	-	0	23	-	33	-
G. elegans	45	-	30	-	29	-	25	-	40	-	-	24	-	0	-	-	29	-
G. morbillorum	29	-	24	-	-	11	-	0	-	0	-	22	-	0	-	0	28	-
Total No. of species	**16**	**9**	**17**	**8**	**14**	**11**	**12**	**13**	**12**	**13**	**5**	**20**	**1**	**24**	**11**	**14**	**21**	**4**

S: Sensitive; R: Resistant

A

Table (4-2): Zone of inhibition of isolated Gram-positive bacteria in uncontrolled DM

Isolated species	Zone of inhibition(mm)																	
	P		AZM		VAN		DA		L		CRO		CFM		TE		C	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
K. kristinae-1	-	0	-	15	-	0	-	0	-	0	-	0	-	0	-	0	-	6
K. kristinae-2	-	0	-	17	-	0	-	0	-	0	-	0	-	5	-	0	-	12
K. kristinae-3	-	0	-	17	-	0	-	0	-	0	-	0	-	0	-	0	-	6
K. kristinae-4	-	0	18	-	-	0	-	0	-	0	-	0	-	0	-	0	-	6
K. kristinae-5	-	0	-	15	-	0	-	0	-	0	-	0	-	0	-	0	-	0
K. kristinae-6	-	0	23	-	-	0	-	0	-	0	-	0	-	0	-	0	-	0
K. kristinae-7	40	-	33	-	22	-	29	-	25	-	24	-	-	12	22	-	29	-
K. rhizophila	-	22	39	-	-	12	24	-	-	0	-	0	-	0	-	10	29	-
R. dentocariosa	37	-	29	-	23	-	31	-	31	-	26	-	-	0	-	0	32	-
R. mucilaginosa	35	-	30	-	30	-	35	-	30	-	28	-	-	21	25	-	39	-
K. sedentarius-1	-	11	-	11	-	0	-	18	-	0	-	0	-	0	-	0	27	-
K. sedentarius-2	-	25	25	-	-	0	-	17	-	0	29	-	-	0	19	-	26	-
K. sedentarius-3	-	24	30	-	-	0	-	14	-	0	-	20	-	14	-	17	36	-
K. sedentarius-4	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	14
K. sedentarius-5	30	-	30	-	-	6	-	0	-	0	30	-	32	-	-	17	31	-
S. oralis-1	44	-	37	-	30	-	30	-	34	-	28	-	-	0	30	-	37	-
S. oralis-2	34	-	20	-	20	-	40	-	28	-	-	24	-	0	-	0	32	-
S. sanguinis-1	40	-	30	-	20	-	40	-	30	-	-	20	-	0	-	0	-	20
S. sanguinis-2	37	-	21	-	20	-	43	-	41	-	-	18	-	0	24	-	23	-
S. pneumoniae	34	-	25	-	25	-	42	-	40	-	-	19	-	0	-	26	32	-
S. salivarius	35	-	25	-	19	-	40	-	-	-	27	-	-	0	-	0	30	-
G. adiacens-1	40	-	24	-	30	-	45	-	40	-	-	20	-	0	-	0	31	-
G. adiacens-2	27		19		27		29		29			14		0		19		14
Total No. of species	**12**	**13**	**17**	**8**	**12**	**13**	**12**	**13**	**13**	**12**	**7**	**18**		**24**	**5**	**20**	**15**	**10**

S: Sensitive; R: Resistant

Table (4-3): Zone of inhibition of isolated Gram-negative bacteria in uncontrolled DM

Isolated species	Zone of inhibition(mm)																	
	CAZ		SXT		LEV		AK		GN		CRO		CFM		TE		C	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. coli	-	0	-	6	25	-	-	15	20	-	-	9	-	8	-	0	-	0
P. aeruginosa	-	0	-	7	-	21	-	11	15	-	-	0	-	0	-	0	-	0

S: Sensitive; R: Resistant   **B**

Table (4-4): Zone of inhibition of isolated bacteria in non diabetic group:

Isolated species	Zone of inhibition(mm)																	
	P		AZM		VAN		DA		L		CRO		CFM		TE		C	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
K. kristinae-1	-	0	18	-	-	0	-	0	-	0	-	5	-	5	-	3	-	0
K. kristinae-2	-	0	23	-	-	0	-	0	-	0	-	0	-	0	-	0	-	0
K. kristinae-3	-	0	20	-	-	0	-	0	-	0	-	0	-	0	-	0	-	0
K. kristinae-4	45	-	33	-	32	-	31	-	23	-	28		-	16	27	-	41	-
K. kristinae-5	33	-	39	-	-	14	-	14	-	0	36		35	-	25	-	39	-
K. kristinae-6	44	-		12	30	-	39	-	-	0	-	25	-	0	23	-	-	0
K. kristinae-7	-	26	20	-	27	-	42	-	-	36	-	25	-	0	25	-	35	-
K. kristinae-8	-	0		0	23	-	34	-	26	-	-	0	-	0	-	0	28	-
K. rosea	21	-	25	-	-	0	-	14	-	0	-	21	-	16	-	17	34	-
K. sedentarius	-	26	20	-	-	6	-	0	-	0	26		31	-	-	15	29	-
R. mucilaginosa	20	-	24	-	25	-	29	-	27	-	-	15	-	0	-	0	-	14
S. aureus-1	-	20		0	-	14	-	0	-	0	-	0	-	0	-	0	-	17
S. aureus-2	-	15	24	-	19	-	31	-	27	-	-	11	-	0	-	17	21	-
S. aureus-3	-	12	23	-	17	-	26	-	-	13	-	10	-	0	-	0	22	-
G. elegans-1	-	15		0	-	14	-	0	-	0	-	13	-	0	-	0	25	-
G. elegans-2	31	-	27	-	-	0	20	-	-	0	33		31	-	-	22	39	-
G. elegans-3	-	12		0	26	-	-	0	-	0	-	9	-	0	24	-	34	-
G. adiacens	30	-	29	-	-	15	-	14	-	0	27		-	26	-	15	33	-
S. sanguinis	-	14		12	17	-	26	-	26	-	-	0	-	0	-	0	21	-
S. oralis-1	29	-	18	-	20	-	32	-	26	-	24		-	7	20	-	25	-
S. oralis-2	30	-	23	-	28	-	45	-	36	-	-	25	-	0	26	-	-	16
S. oralis-3	-	0	29	-	-	0	20	-	-	0	-	23	-	30	-	16	36	-
S. oralis-4	28	-	27	-	-	0	-	18	-	0	27		-	26	-	16	34	-
S. oralis-5	31	-	34	-	27	-	40	-	30	-	-	21	-	0	-	22	27	-
S. salivarius-1	24	-	-	17	20	-	34	-	30	-	-	20		0	-	19	28	-
S. salivarius-2	25	-	45	-	28	-	43	-	40	-	-	26	15			0	30	-
S. alactolyticus-1	23	-	-	0	23	-	36	-	-	0	-	16	-	0	-	0	-	7
S. alactolyticus-2	27	-	18	-	25	-	40	-	37	-	-	23	-	0	24	-	-	15
S. alactolyticus-3	35	-	24	-	29	-	45	-	36	-	27	-	-	0	25	-	-	16
S. pluranimalium	24	-	41	-	26	-	39	-	37	-	-	22	-	0	-	0	30	-
Total No. of species	**17**	**13**	**22**	**8**	**18**	**12**	**19**	**11**	**13**	**17**	**8**	**22**	**3**	**27**	**9**	**21**	**20**	**10**

S: Sensitive; R: Resistant

C

Table (4-4): Zone of inhibition of isolated bacteria in non diabetic group:

Isolated species	Zone of inhibition (mm)																	
	P		AZM		VAN		DA		L		CRO		CFM		TE		C	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
M. luteus	35	-	35	-	18	-	35	-	22	-	-	0	-	0	19	-	29	-
S. epidermidis	-	11	21	-	-	16	25	-	26	-	-	0	-	0	-	15	24	-
K. sedentarius-1	-	0	38	-	-	0	-	0	-	0	-	0	-	0	-	0	26	-
K. sedentarius-2	-	12	25	-	-	0	-	0	-	0	-	12	-	20	-	0	27	-
K. sedentarius-3	-	16	21	-	20	-	-	17	-	0	-	11	-	0	-	0	30	-
K. sedentarius-4	29	-	31	-	-	12	-	18	-	0	30		-	29	25	-	32	-
K. sedentarius-5	-	20	28	-	19	-	45	-	36		-	14	-	0	23	-	31	-
K. rosea	-	22	-	15	21	-	-	16	-	15	-	0	-	14	-	0	24	-
S. anguinis-1	26	-	-	15	-	16	32	-	19	-	-	21	-	0	24	-	29	-
S. anguinis-2	28	-	20	-	-	0	-	0	-	0	28	-	-	24	-	19	27	
S. oralis-1	33	-	21	-	28	-	45	-	32	-	-	12	-	0	-	19	25	-
S. oralis-2	-	0	20	-	24	-	40	-	30	-	-	12	-	0	-	0	30	-
S. oralis-3	26	-	19	-	20	-	34	-	32	-	-	26	-	14	-	18	26	-
S. oralis-4	-	0	-	0	-	16	-	0	-	0	-	0	-	0	-	0	-	0
S.pseudoporuscin-1	23	-	20	-	21	-	36	-	34	-	-	20	-	0	28	-	26	-
S.pseudoporcinus-2	26	-	22	-	24	-	40	-	40	-	-	23	-	0	-	0	28	-
S. salivarius-1	-	0	23	-	-	0	-	0	-	0	-	0	-	0	-	0	21	-
S. salivarius-2	-	18	-	17	24	-	40	-	35	-	-	11	-	0	-	21	30	-
G. adiacens-1	-	0	-	0	-	0	19	-	-	0	-	12	-	0	23	-	27	-
G. adiacens-2	30	-	-	0	-	0	25	-	-	0	-	26	31		-	20	32	-
G. adiacens-3	-	9	-	17	-	0	-	0	-	0	-	20	-	20	-	12	24	-
G. elegans-1	35	-	35	-	38	-	35	-	40	-	-	0	-	0	-	0	30	-
G. elegans-2	29	-	29	-	-	11	-	18	-	0	27	-	-	27	-	11	30	-
G. morbillorum	-	15	20	-	19	-	24	-	21	-	-	0	-	0	-	0	25	-
L. mesenteroides	26	-	-	16	25	-	40	-	41	-	-	25	-	0	25	-	32	-
Total No. of species	**12**	**13**	**17**	**8**	**13**	**12**	**15**	**10**	**13**	**12**	**3**	**22**	**1**	**24**	**7**	**18**	**24**	**1**

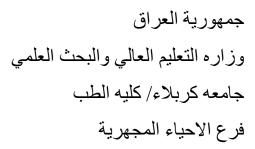S: Sensitive; R: Resistant

D

الخلاصة

ان استمرار تواجد ظروف مرض السكري ممكن ان يؤثر على تنوع وكثافة الاحياء الطبيعية التواجد في الفم, وممكن للعديد من بكتريا الفم ان تتحول الى مرضية بفعل تاثير مرض السكري, كما يمكن لمقاومة المضادات ان تتاثر بفعل السكري اذ يمكن للعديد من بكتريا الفم ان تتنشط لديها المقاومة للمضادات او تكتسب مقاومة جديدة بفعل تواجد السكري.

صممت تجربة الحالة المرضية المتقاطعة لتشمل مائة و خمسة طفل اعمارهم (1.5-15) سنة موزعين الى مجموعتين تتضمن المجموعة الاولى خمسون طفل مصاب بالسكري مقسمين الى مجموعتين صغيره تتضمن خمسة وعشرون مريض لديهم سكري مسيطر علية و خمسة وعشرون مريض لديهم سكري غير مسيطر علية, اما المجموعة الثانية فتشمل خمسة و خمسون طفل بدون مرض السكري. جمعت مسحات من جميع مجاميع الاطفال وزرعت لتحديد اعداد المستعمرات وتشخيص الانواع, وانجزت فحوصات الحساسية ضد العديد من المضادات لجميع العزلات باستخدام طريقة الانتشار من القرص مع تحديد المقاومة للمضادات.

مثلت الاناث الاعداد الاكثر من مرضى السكري ومجاميع السيطرة ولكن بدون فروقات معنوية عن الذكور , وقد وجد بان فترة مرض السكري الاكثر هي للمدة الاقل من سنة واحدة (52%) وخاصة الذين اعمارهم 11-15 سنة, بينما وجد بان فترة مرض السكري 7-8 سنة (6%) كانت الاقل عددا بين مرضى الاسكري, كما وجد بان النمو الكثيف لعزلات البكتريا التي تتجاوز 360 وحدة تكوين المستعمرة نسبة عالية عند كلا من مجاميع المرضى والذين بدون مرض السكري, اذ ان اعدادها كانت عند64.76% عند المشاركين, وكان النمو الكثيف (20 مريض) عند مرضى السكري الغير مسيطر عليه الاكثر مقارنة مع مرضى السكري المسيطر علية (9 مرضى), وهذا ايضا لوحظ عند الاطفال بدون مرض السكري (39 طفل). ان تعداد 100-200 وحدة تكوين المستعمرة مثلت ثاني اكبر مجموعة من البكتريا المعزوله من كل المجاميع(20%).

ان المجموع الكلي لعزلات البكتريا كان ثلاثه وعشرون نوع موجبة صبغة الكرام واثنان سالبة لصبغة كرام, وكان عدد العزلات متساوي (47.61%) عند كلا من مرضى السكري, بينما كانت الاعداد

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متزايدة عند الاطفال بدون مرض السكري (52.38%), ولكن بدون فروقات معنوية. كانت الانواع, *Kocuria kristinae*, *Kytococcus sedentarius* , *Streptococcus oralis* و *Granulicatella adiacens* من اكثر انواع العزلات تكرارا" (23.75%, 13.33%, 12.38% و 9.52%, على التوالي), اما عند مرضى السكري الاالمسيطر عليه فان *K. kristinae* و *G. adiacens* الاكثر شيوعا (3.80% لكل واحدة), بينما *K. kristinae* و *K. sedentarius* الاكثر شيوعا عند مرضى السكري الغير مسيطر عليه (6.66% و 4.76%, على التوالي). كان لدى الاطفال بدون مرض السكري عزلات *K. kristinae* (7.61%) و *S. oralis* (8.57%) هي الاكثر تكرارا".

اظهرت حساسية عزلات البكتريا الى المضادات المفحوصة تباينا" حتى ضمن النوع الواحد, فالعزلة 2 من *S. aureus* والعزلة 4 من *K. kristinae* من مرضى السكري المسيطر عليه وكذلك جميع عزلات *K. kristinae* من مرضى السكري الغير مسيطر عليه كانت مقاومة لجميع المضادات, وفي نفس الوقت فان *S. oralis*-4 و *G. adiacens*-3 و *S. aureus*-1 من الاطفال بدون مرض السكري اظهروا مقاومة متعددة الى 9 مضادات على الاقل, وكذلك 8 عزلات من *K. kristinae* كانت مقاومة الى 8 مضادات. ان المقاومة الى السيفتراكسون والسفيكسين والتتراسايكلين كانت الاكثر شيوعا لجميع العزلات في جميع المشتركين.

جمهورية العراق

وزاره التعليم العالي والبحث العلمي

جامعه كربلاء/ كليه الطب

فرع الاحياء المجهرية

دراسة تاثير مرض السكري على التنوع والمقاومة للمضادات الحيويه عند
بكتريا الفم الهوائية عند الاطفال

رسالة مقدمة الى

مجلس كلية الطب جامعة كربلاء

كجزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية الطبية

من قبل

ابتهاج مالك عبد المحسن

بكالوريوس علوم حياة /جامعة بابل (1996)

بأشراف

الاستاذ الدكتورعلي عبد الحسين صادق الجنابي

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