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EFFECT OF *STREPTOCOCCUS MUTANS* BACTERIA CAUSING DISEASES OF TOOTH DECAY AND GINGIVITIS IN MALE AND FEMALE PATIENTS

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Abstract

This study was conducted to investigate the effect of *Streptococcus mutans* on tooth health in both genders. 189 samples of patients with tooth decay and gingivitis of different ages and from both sexes who visited the specialized dental centers in Diwaniyah for the period from October 2019 to February 2020, were collected. *Streptococcus mutans* was isolated from biofilms, which are the most frequent and common pathogen for tooth decay and gingivitis. The results showed that the Gram-positive isolates are the largest percentage of 76.5% (153) bacterial isolates. Each of *the S. mutan* included 55 isolates (35.94%), *S. pyogens* 36 isolates (23.52%), and *S. salivarus* 10 isolates (6.53%). It was followed by *staphylococcus*, from which 52 isolates belonging to two bacterial species were *Staphylococcus aureus* (33 isolates) and *Staph epidermidis* (19 isolates). As for the Gram-negative bacterial species, it accounted for 18%, with 36 isolates distributed by (19, 8, 3, 5, 1) for *E. coli, Klebseilla pneumoniae, Pseudomonas aerugenosa, Proteus spp, Citrobacter spp*. The results showed a difference in the incidence of dental caries and gingivitis for males and females. The number of injured females was 101, at a rate of (53.5%), which is the largest, compared to the injured males 88 (46.5%), which is the lowest.

Key words : tooth decay, gingivitis, *Streptococcus mutans*, oral diseases.

Introduction

Dental caries and gingivitis are among the most common diseases that cause great harm to humans. It is a public health disease that widely affects various segments of society. It is one of the chronic inflammatory diseases associated with humans. Its effect on the patient begins with the emergence of a series of complex chemical reactions as a result of the bacterial reactions that make up the biofilm on the surfaces of the teeth, after which the formation of black tartar. It has a sticky texture that precipitates without the appearance of any color at the beginning of the formation when it accumulates on the surface of the teeth. In most cases, it begins with a pale yellow balloon and then progresses to brown, and it has been found that this color appears on all sides of the chewing teeth, and sometimes it is found on the surfaces of the gums or at the edge of the neck of the lower teeth. Thus, teeth lose minerals from their calcified tissues and then decompose the components of organic matter, Saraf (2006); Khaeim *et al.,* (2019). Energy is required by humans and it is obtained from carbohydrates by anaerobic glycolysis. Then organic acids are obtained that reduce the pH to about (4.5) on the surfaces of the teeth, which causes the removal of minerals, Cardoso *et al.,* (2011); and Khaeim *et al.,* (2019).

Studies have shown that the main cause of tooth decay and gingivitis is the acid affected by the presence of *S. mutant* bacteria and the sugary compounds that humans ingest, Featherstone *et al.,* (2003); and Featherstone, (2006). There are many different types of bacteria present in the oral cavity of a person, which interact with each other to form bacterial aggregations, including *Oral Streptococci*, Devi (2009). *Streptococcus mutans* is the main and most common cause of tooth decay and gingivitis. It is positive for the chromium pigment and appears in the first days after birth, and after a while, other types appear accompanying the rosaries,

positive and negative for the chromium pigment, and it is an optional anaerobic.

The main reason for its increased pathogenic effect may be its ability to form biofilms, acid production, and carry salts and acids, Taketo *et al.,* (2016); and Jeber, B.A. & Khaeim, H. M. (2019). The ability of bacteria to produce lactic acid from foodstuffs that contain carbohydrates, especially glucose and sucrose, which is responsible for building up Exo-cellular polysaccharide (EPS) such as Glucan, by secreting enzymes known as Glucans Transferase (GTFs) under the control of chromosomes that carry the genes. These enzymes build and regulate types of sugars that are soluble in water and others that are insoluble in water. These types combine to form an insoluble glucan complex with branched water and are characterized by adherent on the surfaces. This is considered the main cause of the bacteria's ability to cause tooth decay, Flemming, and Wingender (2010). The reasons why *S. mutans* form biofilms are to protect themselves from antibiotics, disinfectants and plant extracts, D Aljawasim, B., M Khaeim, H., & A Manshood, M. (2020).

The nature of the surfaces of the teeth is an important factor in the attachment of bacteria and the formation of biofilms to preserve their environment. The first step is to be present on the surfaces by using flagella or filaments or depending on the nature of their surfaces to stick, and upon colonization, the materials are little in the environment so cells begin to change their life form and this is done under the control of a group of genes carried on the different chromosomes that vary from one bacterial type to another. Due to the danger of this bacterium in acute and chronic infections of oral and dental diseases, and if it succeeds in forming biofilms in the mouth, it will be resistant to treatments and antiseptics, except for foci of other infections in different areas of the body, so this study came to include:

1. Isolation and diagnosis of the bacteria that form biofilms on the surfaces of the teeth, is the most pathogenic cause of tooth decay and gingivitis.
2. Explain the effect of *S. mutans* that cause oral infections, especially tooth decay and gingivitis in males and females.

Methods and Materials

Preparation of compound media

Sectional tools that need to be sterilized are dry by an electric oven at a temperature of 180°C for two hours. The culture media and the solutions that need to be sterilized were sterilized by an automatic split separator apparatus at 121°C and a pressure of 15 pounds / eng2 for 15 minutes. Filter papers of 0.22-micron diameter were used to sterilize materials and solutions that are affected by heat (WHO, 2009), Al-Baldawy, *et al.,* (2019).

Blood Agar Medium

The medium was prepared by dissolving 40 grams of hemocoel base in 950 ml of distilled water and adjusting the pH of (7.4) and the volume was completed to 1000 ml with sterile distilled water, then cooled to 55 ° C. (5)% sterilized human blood was added to it and left to harden. The dishes in the hemocytes were inoculated with the bacteria to be diagnosed by the planning method. It then incubated at 37°C for 18-24 hours. The appearance of a clear area of decomposition around the growing colony is an indication that the degradation is of the type B-haemolytic, and if a green color appears around the colony, the decomposition is of the type ±-hemolytic. In the absence of degradation, it is an m-hemolytic cam. This medium was used for the development of aerobic and anaerobic isolates and for testing their susceptibility to hemolysis, Baron *et al.,* (1994); and Khaeim *et al.,* (2019).

Preparation of Solution, Buffers and Reagent

Gram Stain Solution

These solutions were used to study the phenotypic characteristics of isolated bacterial cells under a microscope, according to what was stated in the method, Macfaddin, (2000); and Luma, A. A., &Khaeim, M. H. (2018).

- Crystal Violet to dye the bacteria in purple color for 1.5 minutes, then wash it with water.
- Iodine to hold the dye on the bacteria for 1.5 minutes, then wash it with water.
- Alcohol to remove the dye for five seconds.
- Safranin Stain Red to dye the bacteria in red color for 1.5 minutes, then wash with water and dry to distinguish between the Gram-positive bacteria for the purple color and Gram-negative for the red color.
- Few drops of oil to the slides that contain the sample was applied, as the oil strengthens the examination process under the microscope and prevents the refraction of light rays under the force of magnification X100.

Sample Collection

189 samples were collected in the form of swabs from the patients' mouths to the Specialized Dental Center in Diwaniyah. The number of infected males is 88 isolates,

and the number of females is 101 isolates for both sexes and of different ages for the period from October 2019 to February 2020 of different ages and from acute and chronic injuries. Samples were taken by a specialist doctor using sterile cotton swabs. The samples included areas of the patients' mouths, which are (the surface of the teeth - the gums). It was brought to the laboratory and cultivated on a medium of food (nutrient media) and incubated at 37°C for 24 hours to activate it. After that, it was transplanted back into different, selective, and differential culture media, Alawsy, W. S. A., Alabadi, L. A. S., & Khaeim, H. M. (2018).

Isolation &Identification

The obtained bacterial isolates were isolated and diagnosed according to what Macfaddin (2000) reported as follows:

Morphological characteristics

The characteristics of bacterial colonies growing on different culture media were studied through color, shape, diameter, edges, transparency, growth, or non-growth on differential culture media, and others. This diagnosis was considered preliminary as it relied on the cultivar characteristics of colonies growing on selective and differential media.

Microscopic Examination

The staining results of the isolates prepared from the developing bacteria cells from the cells stained with gram were shown and examined under the lens for light microscopy. The shapes of cells after staining were studied by overlapping the cells with the dye and observing their grouping, sizes, presence of media, their ability to form capsules and chalkboards, and the nature of materials stored in the cytoplasm, Brook *et al.,* (2007); and Khaeim, H. M. (2013).

Vitek compact 2 system diagnosis

- The VITEC system is one of the fast diagnostic systems in bacterial diagnosis. It gives accurate results up to 99% accuracy. To check bacterial isolates, the above system was used according to the instructions of the company supplying it, according to what was stated in Fritsche and his group (2011):
- Germ isolates were cultured and incubated at (37)°C for 24 hours.
- Prepared from microbial culture stuck; By transferring one colony from each dish to test tubes containing (3) ml of physiological saline at a concentration of 0.85%. Then the turbidity of the growth was reduced to obtain a suspension of density between (0.50 - 0.63) mg. Ml-1, which is equivalent to 1.5 x 810 cells. ML-1 using a 230nm spectrophotometer.
- A card cassette for the diagnosis of microbial species was placed in each of the test tubes containing the diluted bacterial suspension, and then the tubes were placed in a VITEC machine that reads the results automatically and determines the type of germ in the suspension.

Results

Isolation and Identification

Different bacterial groups present in the oral cavity and on the surfaces of the teeth have serious health damage to human health and safety. It has aneffect that

Laboratory devices and tools used under study and their origin.

Device name	The manufacturing company	Device name
Sensitive Balance	GallenKamp	England
Incubator	Memmert	Germany
Light-Microscope	Olympus	Japan
Distiller	Ogawa/Seiki	Japan
Autoclave	Termite	Germany
Laminar flow cabinet	Philips	Holland
Benzen Flame	MDIC	China
VITEK®2 GP	Merieux	France
Disposable Petri-dishes	Grenier	Germany
Cylinders	Grenier	Germany
Spreader	MDIC	China
VITEK®2 GP	BioMerieux	France

The laboratory chemicals used under study and their origin.

Material	The manufacturing company	Origin
Crystal Violate	Pico	Jordan
Iodine	Pico	Jordan
Alcohol	Pico	Jordan
Sufranin Stain Red	Pico	Jordan
Oil	Pico	Jordan

The ready-made agricultural media used in the study and their sources.

Culture media	Usage purpose	Manufacture company
Amidst the dens of blood	To test the ability of bacteria to produce hemolysin	India
Amid the nourishing crunch	A general medium for bacterial development	India
Central Brain-Heart Infusion Broth	Lyrical center to activate isolates	India

leads to illness in the entire patient's body through its effects on all different body systems. It causes infections of various organs and organs of the body, such as prostatitis and endocarditis, and causes blood poisoning, pneumonia, and other diseases. It also has a direct effect on the mouth and teeth, such as necrosis, blackening, and periorbital inflammation.

This study included 189 isolates of patients with tooth decay and gingivitis. The current study showed that the cause of blackening and necrosis of the teeth is due to the genus *Streptococcus*, which is common on the surfaces of the teeth. The highest percentage was recorded at 76.5% (153) isolates, due to their main role in dental necrosis, and the generating types are called dental necrosis. It also leads to the occurrence of opportunistic diseases caused by other groups of Gram-positive bacteria represented by the genus *Staphylococcus*. The reason for the spread of Gram-positive bacteria in the mouth is since they have fast resistance mechanisms and through the conjugation, process to facilitate the spread of resistance determinants through plasmids as well as the transformation between these species due to their presence in one environment. Table 1 shows the types of Gram-positive bacteria isolated from the mouths of patients with oral and dental diseases. As the bacteria, *S. mutans*, the most frequent and the main cause of tooth decay, constituted 35.94% (55) isolates. *S.pyogens* bacteria that cause inflammation of the upper part of the respiratory tract such as the nose, pharynx, and tonsils, gave a rate of 23.52% (36) isolates, which have a role in recurrent pharyngitis and tonsillitis. *S.aureus* had 21.56% (33) isolates. *S.epidermis* had 12.41% (19) isolates.

Table 2 shows the most common Gram-negative bacteria types and their different ratios according to the types of bacteria isolated from patients with oral and dental diseases. *Coli* accounted for the highest percentage of isolates, 52.7% (19). *Proteus spp*. 13.88% (5) isolates. *Klebsiella pneumoniae* was isolated with 22.22% (8) isolates. The isolates of *Pseudomnasaerugenosa* (3) hada percentage of 8.33%. *Citrobacterspp* was recorded at 2.77 (1) isolates.

Table 1: Gram-positive bacteria isolated from the mouths of patients with oral and dental diseases, distributed according to type.

Isolated bacteria	The number of isolates	Percentage
Streptococcus mutans	55	35.94%
Streptococcus pyogens	36	23.52%
Streptococcus salivarus	10	6.53%
Staphylococcus aureus	33	21.56%
Staphylococcuse epidermis	19	12.41%
Total	153	

Table 3 shows the effect of the sex factor on the rate of isolation and the incidence of pathogens in the mouth. The results of the current study, which included 189 patients, showed that the infection rate in females was more than males. The number of infected females was 101 isolates (53.5%), while the infected males were 88 isolates (46.5%).

Table 2: Types of Cram-negative bacteria isolated and their numbers from the mouths of patients with oral and dental diseases.

Isolated bacteria	The number of isolates	Percentage
E.Coli	19	52.7%
Klebsiella Pneumoniae	8	22.22%
Pseudomonas aerugenosa	3	8.33%
Proteus spp	5	13.88%
Citrobacterspp	1	2.77%
Total	36	

Discussion

Among the major diseases associated with the oral cavity are dental caries and gingivitis caused by *S. mutans*, which is the main and most common cause, Wallace *et al.,* (1989). Table 1 shows the types of Gram-positive bacteria isolated from the mouths of patients with tooth decay and gingivitis. *S. mutans*, being the most common infection in dental caries, accounted for 35.94% (55) isolates, and this is consistent with the study of Duailibe *et al.,* (2007). *S.pyogens* bacteria that cause inflammation of the upper part of the respiratory tract such as the nose, pharynx, and tonsils, gave a rate of 23.52% (36) isolates. This is what the researchers' mechanism (Brook *et al.,* 2001) indicated to its role in recurrent pharyngitis and tonsillitis, as for the bacteria responsible for the infection of urinary tract infection, which belongs to the widespread staphylococcus, which is considered the main cause of urinary system injuries because it possesses virulent factors such as cilia that enable it from sticking to the host.*S.aureus* bacteria had a rate of 21.56% (33) isolates, and this is confirmed by a study (Bendouah *et al.,* 2006), which stated that staphylococcus may be responsible for inflammation of the urinary tract and the epithelial tissues surrounding the bladder. *S.epidermis* accounted for 12.41% (19) isolates, and the percentage was 6.53% (10) isolates from the share of *S. salivarus* bacteria.

Table 3: The isolation and infection rate for patients with oral and dental diseases, broken down by gender.

Sex	Number of patients	Percentage of infection	Cram-positive isolates	%	Cram-negative isolates	%
Males	88	46.5	72	47	16	44.4
Female	101	53.5	81	53	20	55.6
Total	189	100	153	100	36	100

The calculated X^2 value = 0.08

Tabular X^2 value = 3.84

The results of table 2 also showed the most common types of Gram-negative bacteria and their different proportions according to the types of bacteria isolated from patients with oral and dental diseases. *Coli* resulted in the highest percentage of isolates were 52.7% (19). *Proteus spp* made a rate of 13.88% (5) isolates that return to the intestines and cause urinary tract infection, bladder, and pelvic nephritis and prostatitis. *Klebsiella pneumoniae* was isolated with 22.22% (8) isolates. *Pseudomnas aerugenosa* resulted in 3 isolates of 8.33%. *Citrobacter* spp had a ratio of 2.77 (1) isolates is considered opportunistic bacteria that infect the respiratory system and cause diseases of the bladder and kidney pelvis. This is consistent with a study of Liaw (2001), which indicates that *Citrobacter* spp is opportunism that exploits the weakened immunity of a sick person to cause disease during a bladder catheterization procedure.

The results of table 3 the effect of sex factor on the rate of isolation and infection with pathogens in the mouth. The results of the current study, which included 189 patients, showed that the infection rate in females was more than males .The number of infected females was 101 isolates (53.5%), while the infected males were 88 isolates (46.5%).

As for the relationship of the number of *S. mutans* isolates with sex, the results showed that the number of isolates in females is higher than in males. These results were in agreement with Al-Dulaimi (2011), who showed through his study that the number of female patients is often higher than the number of males with oral and dental diseases. Perhaps the reason for this is that females are more frequently visited by hospitals and clinics. This current study also agreed with the findings of Khamise, (2010) in the province of Najaf, which found that the incidence of males is 25.2% in permanent teeth and is higher in females, reaching 31.2%. As for the injury of the milk teeth, it was higher in males than females, as it represented 31.8% in males and 20.4% in females.

These results did not agree with what was found by AL-Mosawi (2014) in Baghdad, where the infection rate among males was 87 (64%), and thus it is higher than the infection rate among females 34 (36%). The current study does not agree with the results of Ketabi *et al.,* (2006), as it was found that the results of infection in girls are lower than that of boys. They interpreted it to the fact that the level of saliva flow in females is less than what it is in males during life stages according to different years, and this level is a small amount of saliva secretion for females due to the small size of the mammary glands. The reason for this is due to hormonal changes as a result of the exposure of females to many changes during the different stages of life, as is the case during the menstrual cycle, so blackening increases on the surfaces of the teeth, as well as pregnancy in addition to menopause, or the effect of the female reproductive system on the oral cavity due to the activities of different hormones. It increases tooth decay and decay because some bacterial species prefer a high concentration of hormones to form colonies, and also one of the most prominent signs of this during pregnancy is gingivitis frequently noted, Parsek and Singh, (2003).

Conclusions

1. The dominance of *S. mutans* bacteria as a major cause of oral diseases and the formation of biofilms.
2. The vast majority of *S. mutans* bacteria possess the ability to form a biofilm, which is one of the most important virulence factors for oral diseases and dental caries.
3. The incidence of oral diseases varies, especially tooth decay and gingivitis, as the number of female infections was high compared to males.

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