Study of the diagnosis and isolation of bacteria associated with dental caries in pregnant women in Baghdad province

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Abstract
This current study aims to investigate in isolation and identification of all the bacteria types associated with dental caries, at the mouth in pregnant women in Baghdad province. Fifty swaps samples are collected from pregnant women after the third month of gestation, in different locations in Baghdad including medicine city and specialty clinic in Baghdad. The swaps cultured on blood agar for enrichment then stained by gram stain and making some biochemical tests such as catalase, oxidase, urease, and indole test, etc, for identification of the bacteria and determine its type. Our result showed that the total percentage of bacterial infection associated with dental caries was 42/50 (84) %. Our results showed the isolated bacteria from dental caries in pregnant women were Streptococcus mutans, Lactobacilli acidophilus, Actinomyces viscusus, Streptococcus sobrinus, and Bifidobacterium dentium. Its percentage was (66) %, (50) %, (28.5) %, (52.3) % and (16.6) % respectively. The pregnant women are special immunological and hormonal condition, therefore, our report focused on oral medicine in this stage, especially related to the dental caries microbiology that accompanied by tooth decay. Our study found a higher percentage was Streptococcus mutans (66) % while; the lower percentage was Bifidobacterium dentium (16) %.

Keywords: bacteria, dental care, dental caries, pregnant women, Baghdad province

INTRODUCTION

Dental caries is a common health problem related to the mouth in all world countries, affecting 60–90% of the people including men and women, children, and adults (1). The early clinical signs of the caries are started with a small patch in the surface of the tooth enamel; sometimes occur between the fissures of the teeth. Caries spread in the bone body and reach to the bulb of the tooth. The enamel becomes weak then collapses to produce cavity developing to destruction status (2) (3).

Effects of the acids cause dental caries. Production of the acids due to metabolism of the food sugars by bacteria that live in the oral cavity. The acid causes loss of Ca and P from the tooth (demineralization) (4).

The acid is a cause of cavities of the tooth, which produce from bacteria that leading to dissolving the hard tissues of the teeth. The bacteria produce the acids for the destruction of the sugar, the sugars are energy sources for the bacteria, and therefore the sugar is a risk factor for dental caries. When the mineral breakdown is more than build up caries results, a low amount of saliva in diabetes mellitus or take some drugs is a risk factor for dental caries. Some drugs cause decrease saliva such as antidepressants and antihistamines. Generally, tooth decay is associated with inadequate cleaning of the mouth and poverty leading to an increase in the occurrence of dental caries (5) (6).

Many studies found a relationship between the bacteria that multiply in the mouth has negative effects on the tooth and enhances the occurrence of dental caries. (7) Found several types of bacteria from mouths such as *streptococcus mutans* that considered the major etiological agent of dental caries, species of the genera *Atopobium, Propionibacterium,* and *Lactobacillus, Bifidobacterium dentium and Actinomyces spp.*

(8) Found *S. mutans* is most common that isolated from dental caries and detected it by using PCR, Besides, many bacteria cause tooth decay in children is included *Streptococcus, Veillonella, Actinomyces, Leptotrichia, Thiomonas* and *Granulicatella* (9).

There is a positive significant relationship between pocket depth, gingival bleeding, and tooth decay with pregnancy. The probability of tooth decay is increased between the first and second trimesters (10).

The main goal of our study is determination number, type, and percentage of bacteria associated with dental caries in pregnant women.

MATERIALS AND METHODS

Sample collection:

The samples of our study are fifty swaps which taken from fifty pregnant women, each swap taken directly on

the dental caries of pregnant women (during pregnancy period) at different ages ranged (20-40) years old during September 2019 to January 2020. The swaps are collected by the clean and sterile swap from the mouth directly in special dentistry clinics and the specialized hospital for dental medicine in Baghdad. The swaps kept in a clean sterile tube and store at (4) C for until reach the lab.

Note: the pregnant women do not use any medicine (drugs), or toothpaste for more than (24) hours before taking the samples for preventing the interference with the results of our study with effect some the inhibition drugs and substances.

Culture:

The swaps cultured directly on blood agar for enrichment wherever it cultured on blood agar by zigzag method (Z-method), then incubation at (37) C for (24-48) hour in the incubator.

The material and procedures:

A- Blood agar procedure:

1- Adding powder (40) gram to the distal water (1) L then mixing.

2- Heating together for (60) minutes to dissolve

4- Sterilization of the mixture by autoclave for fifteen-minute at 121°C

5- Cooling to (45) °C

6- Adding sheep blood (50) mL then pours in the dishes.

B- Gram stain:

1- Put one drop of water on the slide, then adding the suspected colony and mixing to completing distribution and fixing by Bunsen burner

2- Adding of the crystal violet to the slide for 60 seconds then washed by distal water

3- Adding of iodine for 60 seconds then washed by distal water

4- Adding of alcohol for five seconds and then washed by distal water

5- Adding of safranin to the slide for 60 seconds then washed by distal water

6- If the bacteria are Gram-positive, it will take blue, while if the colony was pink, was gram-negative.

C- Catalase test:

1- Adding (3) % hydrogen peroxide on the slide that contains the suspected bacteria

2- if the bubbles are formed, that means is positive for the test

D- Hemolysis test:

This test is carried out by culture suspect colony on blood agar and incubated at (37) C for one day, then determining the degree of the hemolytic percentage alpha, beta, and gamma.

E- Oxidase test:

This test make on filter paper that included adding of 1% Kovács oxidase reagent on the filter paper then adding of the suspected colony then observe for color changes. The colony is oxidase-positive when the color changes to dark purple. If the color doesn't change that means negative.

F- Coagulase test:

S. aureus was incubated (24) hours in nutrient broth. One milliliter of culture was placed in the tube containing rabbit plasma (0.4) ml. then incubated in a (37) C for one day, the results are included the coagulase-positive organism caused clot and high turbidity while the coagulase-negative bacteria don't cause that.

G- Camp test:

Streak a B-lysin producing strain of S. aureus down the center of a sheep blood agar plate, then Streak suspected organisms across the plate. Then kept at (37) °C (18-24) hours.

H- The indole test:

The culture put in tryptophan or peptone broth for one day then incubate at (37) C, then adding five drops of Kovac's reagent on culture broth. If the red color appears in the broth, that means is positive. The negative colony is yellow.

I- Voges Proskauer test:

1- Inoculate the suspected colony at the medium and incubate at (37) C for one day

2- Adding of the broth (2) ml on the test tube

3- incubate at (37) C again for one day, then adding several drops of alpha-naphthol and mixing, then adding several drops of KOH and mix.

8- If the pink color is formed during the half-hour that means it is positive.

Biochemical tests:

Streptococcus mutans:

Table 1. results of the biochemical test of Streptococcus mutans

The test	The results	The test	The results	The test	The results
Capsule	+	**Oxidase**	Negative	**Lactose**	Positive
Catalase	Negative	**Shape**	Cocci	**(Oxidative-Fermentative)**	Facultative anaerobes
Gram Staining	Positive	**Spore**	Non-sporing	**Sucrose**	Positive
Hemolysis	Alfa Hemolysis	**Urease**	Negative		
Motility	Non-motile	**VP (Voges Proskauer)**	Positive		

Lactobacilli acidophilus

Table 2. results of the biochemical test of *Lactobacilli acidophilus*

The test	The results	The test	The results	The test	The results
Capsule	-	**Gram Staining**	+	**-**	-
Catalase	-	**H2S**	-	**Pigment**	Rare; if present, yellow or orange
Citrate	-	**Indole**	-	**Shape**	long and slender
Flagella	If present, peritrichous	**Motility**	Mostly -	**Spore**	-
Gas	-	**MR (Methyl Red)**	-	**Urease**	-
Gelatin Hydrolysis	-	**Nitrate Reduction**	-	**VP**	-

Actinomyces viscusus:

Table 3. results of the biochemical test of *Actinomyces viscusus*

The test	The results	The test	The results	The test	The results
Gram	+	Catalase	+	Acid fast	None
Shape	Rod	Oxidase	+	Spore	None
Methyl red	+	lactose	+		
VP	-	Oxygen	Required	Camp	-
tryptophanase	-	Motility	None	Pigment	-

Streptococcus sobrinus

Table 4. results of the biochemical test of *Streptococcus sobrinus*

The test	The results	The test	The results
Gram	+	aesculin	hydrolyse
Catalase	-	arginine	hydrolyse
lactic acid	+	mannitol	Fermented
Shape	spherical	sorbitol	Fermented
Spore	None	Motility	None
Glucose fermentation	+		

Bifidobacterium dentium

Table 5. results of the biochemical test of *Bifidobacterium dentium*

The test	The results	The test	The results	The test	The results
Gram	+	Oxidase	-	Lactose	+
Motility	None	Gelatin Hydrolysis	-	Mannitol	-
Spore	None	Catalase	-	Oxygen	anaerobic
Shape	Bacilli	Nitrate Reduction	-	Acetic acid	Produce
Arrangement	chains, V-shape	Pigment	-		
Indole	-	Glucose	+		

Statistical Analysis:

The data were tested using SPSS V. (10) at ($p<0.05$). The values represent a percentage by dividing the infection number on the total number multiply with 100.

RESULTS

According to our results, the total samples in the current study was fifty (50) sample, all the sample cultured on the blood agar, only forty-two (42) was positive, the other is negative eight (8) sample. The forty-two samples are submitted to some of the biochemical tests for the identification of the causative agent as shown in table (6).

Table 6. showed number and percentage of samples on blood agar and Biochemical tests

Total samples	Cultured on blood agar			Biochemical tests	
	cultured	positive	Negative	Examined	Positive
50	50	42	8	42	42
100%	100%	84%	16%	100%	

The used biochemical tests in our study are included catalases test, oxidase test, coagulase test, hemolysis test, camp test, ureases test, esculin test and motility test for identification of the bacteria, in addition to staining by gram stain as shown in the previous table in material and methods section.

Our study isolated several types of bacteria that accompanied with dental caries in pregnant women such as *Streptococcus mutans, Lactobacilli acidophilus, Actinomyces viscusus, Streptococcus sobrinus,* and *Bifidobacterium dentium* were its percentage was 66%, 50%, 28.5%, 52.3% 16.6% respectively as shown in table (7) and figure (1):

Table 7. showed number and percentage of bacteria species that isolated from dental caries in pregnant women

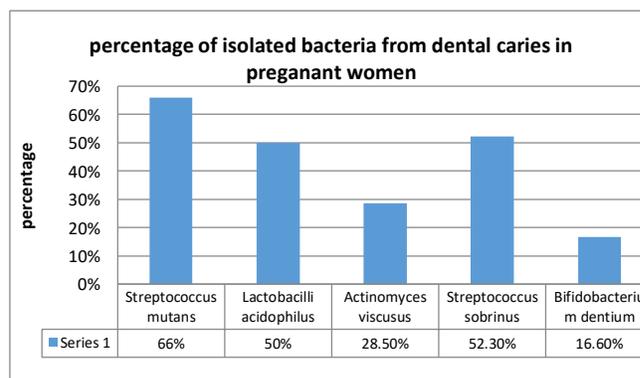| N	Isolated bacteria	Number	Percentage
1	*Streptococcus mutans*	28/42	66%
2	*Lactobacilli acidophilus*	21/42	50%
3	*Actinomyces viscusus*	12/42	28.5%
4	*Streptococcus sobrinus*	22/42	52.3%
5	*Bifidobacterium dentium*	7/42	16.6%

Figure 1. showed the percentage of bacteria spp that isolated from dental caries in pregnant women

Besides that, our study showed some cases have mixed infection (two bacteria spp or more) wherever, percentage of *S. mutans, L. acidophilus,* and *A. viscusus* together was (44) %. Besides that, *S. mutans, S. sobrinus* and *B. dentium* was (38)%, also, *S. mutans, A. viscusus,* and *S. sobrinus* was (52) %, *S. mutans, L. acidophilus,* and *A. viscusus* was *(*32) %, *S. mutans* and *B. dentium* was (10) %, *A. viscusus* and *B. dentium* was 4%, as shown in table (8).

Table 8. showed mixed infection in our study

Mixed species	N.	Percentage
S. mutans + L. acidophilus + A. viscusus	22	44%
S. mutans + S. sobrinus + B. dentium	19	38%
S. mutans + A. viscusus+ S. sobrinus	26	52%
S. mutans + L. acidophilus + A. viscusus	16	32%
S. mutans + B. dentium	5	10%
A. viscusus+ B. dentium	2	4%

DISCUSSION

The pregnancy is a special state that included physiological and emotional changes in the body such as oral health. The Periodontal and Gingival changes are occurring in the pregnancy. The hormonal changes are observed in the pregnancy due to the changes in the concentration of the hormone such as progesterone and estrogen in the serum make the woman more susceptible to gum diseases and oral infections.

Based on our results, the rate of the bacterial infection of dental caries was (84) %, (11) found the percentage of dental caries in males was (78%) and females were (79%), and close to our results. While (12) found the percentage of dental caries was high (92%) and that higher than our results. (13) Recorded a low rate as compared with our results (35) % of the population showed dental caries.

There are some studies found that vary values, wherever the children who have aged (5-19) years with tooth decay were (16.9) %, and the adults which have aged (20-44) with tooth decay were (31.6) % (14).

In addition, (15) found oral disease affects nearly four billion in the world, and the tooth decay has a percentage (44) % of the world's population. Generally, 100% of adults and (75) % of children have tooth decay and results in tooth pain. In another study found the percentage of dental caries was (100) % wherever, (117) bacterial swap were collected from tooth plaques, which included the aerobic genera were (64.96%) and the anaerobic genera were (35.04%) (16).

The difference in the rates of tooth decay occurrence is normal principal, and there are several factors are control on the occurrence of tooth decay, such as the genetic factor, the nature of food, the consumption of sugars, the daily cleaning of teeth, age and the general health condition, in addition to the immune status. In addition, there are several other reasons as well such as the quality of food have an important role in determining whether dental caries occurred (17) (18).

Our results showed that *streptococcus mutans* were (66) % in pregnant women that isolated from dental caries cases, while study (19) found the prevalence of *Streptococcus mutans* was (38.3) % and that represents less than our rates. (20) Recorded rate more than our results, wherever they recorded (91) %, and that considered higher than our percentage and they found that *streptococcus mutans* were the dominant species and most common as compared with another group.

In addition, the children who take penicillin were less than the other groups that mean the immune status and health status have a great role in the rate of these bacteria, and that agrees with our results (21). The study (22) is showing a positive correlation of tooth decay with *S. mutans*. If *Streptococcus mutans* was a high number, that increases the degree of decayed teeth (23).

Lactobacillus acidophilus is growing in oral, gut, and vaginal flora. *Lactobacillus acidophilus* used for the production of cheese and yogurt, and effects on adherence of *streptococci mutans* on the tooth surface (24). The current study found Lactobacilli acidophilus was (50) %, in pregnant women that isolated from dental caries cases

Many studies isolated Lactobacillus acidophilus from dental caries and that agree with our results such as (25) (26) (27) at many different percentages, and that attributed for several causes included taking the milk products or not, taking antibiotics or not, and using an oral antiseptic or not.

Many studies are included examination (65) cases with dental caries by laboratory culture techniques, the lactobacilli spp is one of the numerous bacteria that isolated from an oral cavity that associated with dental caries. Production of the acids by *lactobacilli acidophilus* is causing tooth decay. Wherever lactobacilli acidophilus found in the environment at quantifying the major species (28). *Actinomyces viscusus* is bacteria produce glycogen, derivative activities, and the acid inside dental caries that enhance to development of decay (29)

Our finding found *Actinomyces viscusus* was (28.5) %, in pregnant women that isolated from dental caries cases. 23.3% exhibited carious lesions on root surfaces by (30) and that close to our results. Actinomyces spp. were detected 95% in the dental caries cases and that is a high percentage as compared with our results (31). Many reports isolated *Actinomyces viscusus* from tooth decay (32) (33) (34) at various rates due to several factors.

Administration of NaF with drinking water to rats reduced caries incidence at (P <0.001), despite the high percentage of Actinobacillus sp. NaF is led to inhibition of acid production; therefore taking NaF is one of the factors, which causes the rates to the contrast of Actinobacillus spp (35). No doubt, the immune status has a great role in the prevalence.

Streptococcus sobrinus is causing dental caries in humans and animals, and *Streptococcus sobrinus* is usually associated with S. mutans in dental caries (36). Based on our results, *Streptococcus sobrinus* was (52.3) % in pregnant women that isolated from dental caries cases. (37) found *S. sobrinus* was (50.9) % of dental caries and that close to our results (37). While (38) found the percentage of *S. sobrinus* was (44.5) % and that considered less than our prevalence and *S. sobrinus* was (17.9) % by (39) which considered less than our results.

The prevalence of *S. sobrinus* 18.9% at age (12) years and 8.4% at age (15) years, wherever the age is an

important factor in the epidemiology of *Streptococcus sobrinus* in dental caries (40). One of the important factors of the prevalence of *Streptococcus sobrinus* in dental caries is the amount of saliva, wherever, the persons which have a little amount of produce saliva showed a high percentage of infection of *Streptococcus sobrinus* (41).

According to our study, *Bifidobacterium dentium* was (16.6) % in pregnant women that isolated from dental caries cases, *B. dentium* was found in 30.8% of clinical cases which represent less than our prevalence (42).

Age, gender, and immune status play the main role in the epidemiology of *Bifidobacterium spp* in dental caries (43) (44). Many factors affect spreading rates of tooth decay are included the presence of bacterial spp leading to make pH is low, the absence of mouth hygiene, low immune status, the type of diet, and nature of the teeth structure (45).

Finally, the tooth decay is a common great health problem, especially in pregnancy stages in women, due to changes in hormones titer leading to changes in immune response, therefore we found high rates of bacterial infection of dental caries accompanied with this stage, and should provide super cure to the women in the pregnant stage.

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