Thi-Qar Medical Journal (TQMJ):Vol.(27),No.(1),2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Online): 3006-4791

Isolation and Identification of Gram Positive and Gram Negative Bacteria Causing Dental Caries in Children in Thi Qar province

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Abstract:

Background: Dental caries is an infectious disease-causing tooth demineralization and cavities. Dental caries in children is caused by the combination of three major factors carbohydrates, the presence of caries bacteria on dental plaque, and the rapid impact of hard tooth surfaces. The mouth contains a large number of bacteria, including a few species that cause dental caries called cariogenic bacteria. Gram positive bacteria are the main contributors to caries in the teeth

Methods: A study was conducted in Thi-Qar province between August and December 2023 AD, involving 100 swabs and dental plaque samples from (70 patients and 30 controls) aged 3-11 years. The samples were processed and cultivated in a specific culture medium.

Conclusions: The current investigation demonstrated a high rate of Gram-positive bacteria isolated from dental cavities in children aged 3 to 11 years. Gram stain, biochemical tests, and VITEK 2 compact results showed that gram-positive bacteria accounted for 81% (180 isolates) and gram-negative bacteria for 19% (42 isolates). There were 109 isolates with typical Streptococcus colony morphologies.

Keywords: **Dental Caries, *cariogenic bacteria* and vitek 2 compact.**

Introduct: Dental caries is the prevailing oral ailment worldwide. The formation of dental caries is primarily associated with the microorganisms found in the mouth (1). The predominant and significant bacterial species found in the mouth are associated with the genus Streptococcus, in particular the members of Streptococcus viridians. Streptococcus viridians (VS) are symbionts of the oral cavity, but they are also opportunistic pathogens since they can cause a wide range of diseases (2).

The cariogenic bacterial population in dental caries is restricted to a subset of the many species found in the biofilms on the tooth surface, which are mediated via biofilms (3).Streptococcus mutans is a significant contributor to the development of dental caries. It has traditionally been regarded as a pathogenic bacterium for dental cavities due to its ability to create lactic acid and thrive in acidic conditions (4) . Many other bacteria have been isolated from carious sites or observed to appear distinctly during the process of caries developing, and they are thought to be linked to caries as *Lactobacillus species* and other bacterial species (5, 6) . Genetic research data indicate that caries is the result of a widespread alteration in the composition of the oral microbiot (4) . Although these microbes can ferment

food carbohydrates (like sucrose and glucose) that become trapped between teeth, the organic acids they create cause the hard tissues (like cementum, dentin, and enamel) to break down as a result of tooth demineralization. The process of surface demineralization (7). Multiple prior clinical studies have demonstrated that S. mutans is the primary etiological factor responsible for tooth decay (8). Caries results in the deterioration of the calcified structure of the tooth enamel (dental apatite layer) (9). Moreover, accumulating evidence indicates that dental caries may have harmful consequences for cardiovascular disorders (10), such as coronary heart disease (11), hypertension (12) , and atherosclerosis (13). Hence, it is imperative to prioritise preventive dental care.

Hence, the primary objective of this investigation was to isolate and identify Streptococcus mutans bacteria and other bacterial species present in caries lesions and the dental plaque.

Aim: The aim of study was Isolation and identification of the bacterial causes of dental caries in children aged 3–11 years in Thi-Qar province.

Materials and Methods

Samples collection: Dental swabs and plaque samples were collected from 100 children with dental caries and dental plaque, between the ages of (3 - 11) years of both sexes. The samples were collected from specialized dental centres and dental clinics in Thi -Qar province between August 2023 and December 2023 AD. Using transport swab media to take the sample from dental swabs.

While dental plaque samples were collected using sterile curettes (Gracey Curettes), The supragingival plaque was first taken from the crown site, then the subgingival plaque was taken. The plaque samples were suspended in 1 ml of sterile phosphate-buffered saline (PBS), and the two types of samples were transported in an ice box to the laboratory. Dental swabs and plaque samples were collected under standard conditions, according to Hadi (14). Swabs containing transport medium are used to preserve the viability of bacteria and prevent contamination (15).

Isolation and Identification of bacterial species:

Isolation: Samples of cotton swabs taken from dental caries and plaque were cultured after being incubated at a temperature of 37°C for 24 hours in brain heart infusion broth (BHI) for the purpose of activating the bacteria, using the planning method on enriched culture media such as Mitis Salivarius Bacitracin Agar (MSB agar), a selective medium for *streptococci mutans*, blood agar and MacConkey media for other bacteria, in both aerobic and anaerobic culture methods. The plates were incubated for 48 hours at a temperature of 37°C. The incubation process was completed, and phenotypic and biochemical tests were performed

Bacterial identification:

Isolates were initially identified using the following methods:

1. Morphological feature

The diagnosis was based on colony shape on MSB agar plates, and other media features, for streptococcus spp and other bacterial spp the explanation provided by (16, 17). (18).

2. Gram Staining

Under aseptic conditions, one or two colonies were transferred from the surface of Agar and stained using Gram stain according scientific procedures (19).

3. Biochemical tests

biochemicals test according to MacFaddin (20).

4.API 20 strep. System

According to Leboffe and Piercr, (21), this test has been carried out; it is employed in clinical settings for the quick identification of bacterial isolates

5.Diagnosis by VITEK-2

To diagnose of isolates, samples were grown on blood and MacConkey Agar medium and analysed with VITEK-2 according to company instructions (22).

Result:

This chapter presents the statistical analysis of data gathered from 100 patients with dental caries (DC) and plaque, ranging in age from 3 to 11 years.

1. Isolation and diagnosis of bacterial species from dental caries and plaque:

100 swabs were collected from people with dental caries and plaque, and 222 isolates were obtained, which were identified as Gram-positive and Gram-negative bacteria Isolates were identified to the genus level based on Gram stain, microscopic examination and catalase test. Streptococci are spherical or oval Occurring pairs or chains, Gram-positive and catalase-negative bacteria. Identification isolates to species level depending on colonies shape on the surface of MSBA, blood agar and MacConkey. and sugar fermentation test, API 20 strep test and Vitek 2 compact system.

Gram-positive bacteria are found in higher proportions in the mouth compared to gram-negative bacteria.as in figure. showed that gram positive bacteria were 180 (81%) while gram negative were 42 (19%). with high significant differences at ($p < 0.05$).

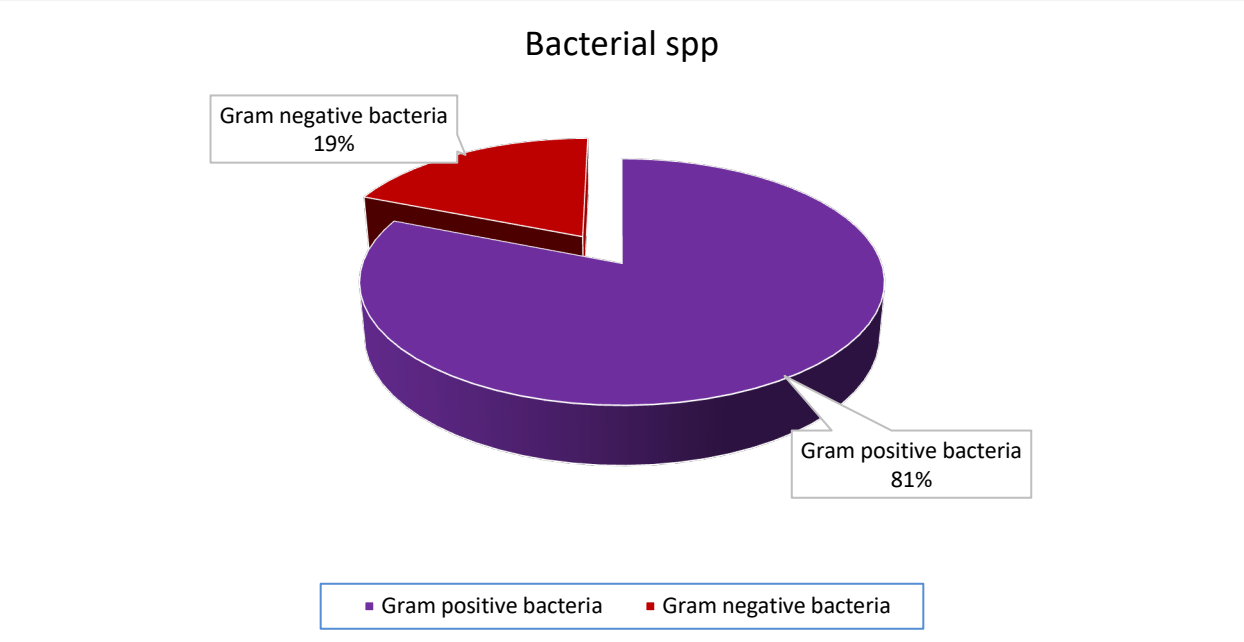

Figure (1): distribution bacterial species that isolated from dental caries according to gram stain.

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2.Bacteria isolated from dental caries and their percentages:

2.1 Gram positive bacteria isolated from dental caries.

The results presented in Table (1) showed the gram-positive bacteria that isolated by the bacteriological culture showed dominance of Streptococcus spp. It recorded the highest percentage of 51% (109 isolates) of the total percentage of bacteria isolated from tooth, followed by Staphylococcus spp. By 11.67% (21 isolates), then Lactobacillus spp. By 11.11% (20 isolates). *Enterococcus faecalis* the isolation rate reached 8.33% (15 isolates), and *Gemella. morbillorum* accounted for 8.33% (15 isolates).

Table (1): Species of Gram-Positive Bacteria that Isolated from Dental Caries.

No	Gram Positive Bacteria	No	%100	Characteristics	No.	%
1	*Streptococcus Spp*	109	60.56	*S. Mutans*	32	29.36
				S. Sobrinus	20	18.35
				S. Salivarius	19	17.43
				S. Parasanguis	14	12.84
				S. Sanguinis	9	8.26
				S. Mitis	9	8.26
				S. Oralis	6	5.50
Total					109	100
2	*Staphylococcus Spp*	21	11.67	*S. Aureus*	11	52.38
				S. Homins	4	19.05
				S. Epidermidis	6	28.57
Total					21	100
3	*Lactobacillus Spp*	20	11.11	*L. Acidophilus*	16	80.00
				L. Casei	4	20.00
Total					20	100
4	*Enterococcus Faecalis*	15	8.33	—		
5	*Gemella Morbillorum*	15	8.33	—		
	Total	180				

It is also noted from the same table (1) that bacteria belonging to the genus Streptococcus spp. It shows the superiority of S. mutans bacteria over other types of streptococci isolated from dental caries by recording the highest percentage of 29.36% (32 isolates), while S. sobrinus came in second place with a percentage of 18.35% (20 isolates), and S. salivarius with a percentage of 17.43% (19 isolates).,

followed by the lowest percentage of isolation was for the species of S. *Parasanguis, S. sanguinis, S. mitis, and S. oralis* by 12.84% (14 isolates), 8.26% (9 isolates),8.26% (9 isolates), and 5.50% (6 isolates), respectively.

The table (1) also shows that Staphylococcus spp. *Staphylococcus aureus,* it gave the highest percentage of 52.38 %(11isolates) when isolated from caries areas compared to S.epidermidis, accounting for 28.57% (6 isolates), and S. homins by19.05(4 isolate).

As for genus of Lactobacillus spp the L. acidophilus bacteria, its percentage reached 80 % (16 isolates), superior to L.casei bacteria of the same genus, which reached 20 % (4 isolates).

2.2 Gram negative bacteria isolated from dental caries.

while the gram-negative bacteria present in lowest percent camper to gram positive bacteria.as in (table 2) all species form 18% (40 isolates) distribution as fellow proteus spp, K. pneumonia, P. aeruginosa and E. coli and Neisseria spp.

The lowest percentages of their presence in dental caries were 14.29% (6 isolates), 19.05% (8 isolates), 2.92% (8 isolates), 23.81% (10 isolates), 23.81% (10 isolates), and 14.29% (6 isolates), respectively.

Table (2): gram negative bacteria isolated from dental caries

No	Characteristic	No	%
1	Pseudomonas. Aeruginosa	10	23.81
2	Escherichia. Coli	10	23.81
3	Klebsiella. Pneumonia	8	19.05
4	Neisseria Spp	6	14.29
5	Proteus Spp	6	14.29
6	Enterobacter Spp	2	4.76
7	Total	42	100

2.3 Percentages of Streptococcus *spp* to other Bacteria isolated from dental caries:

As shown in Figure (2), streptococci are at the top of the list of all bacteria isolated from tooth decay, with a proportion of 49.10%, surpassing all other types of Gram-positive and Gram-negative bacteria. Staphylococcus bacteria (9.46%) were followed by Lactobacillus spp., *Enterococcus faecalis*, and *Gemella morbillorum* (9.01%), 6.76%, and 6.76%, respectively. Gram-negative bacteria were present in very low percentages; Pseudomonas aeruginosa and E. coli each had a percentage of (4.50%), whereas the other species were as shown in the figure (2).

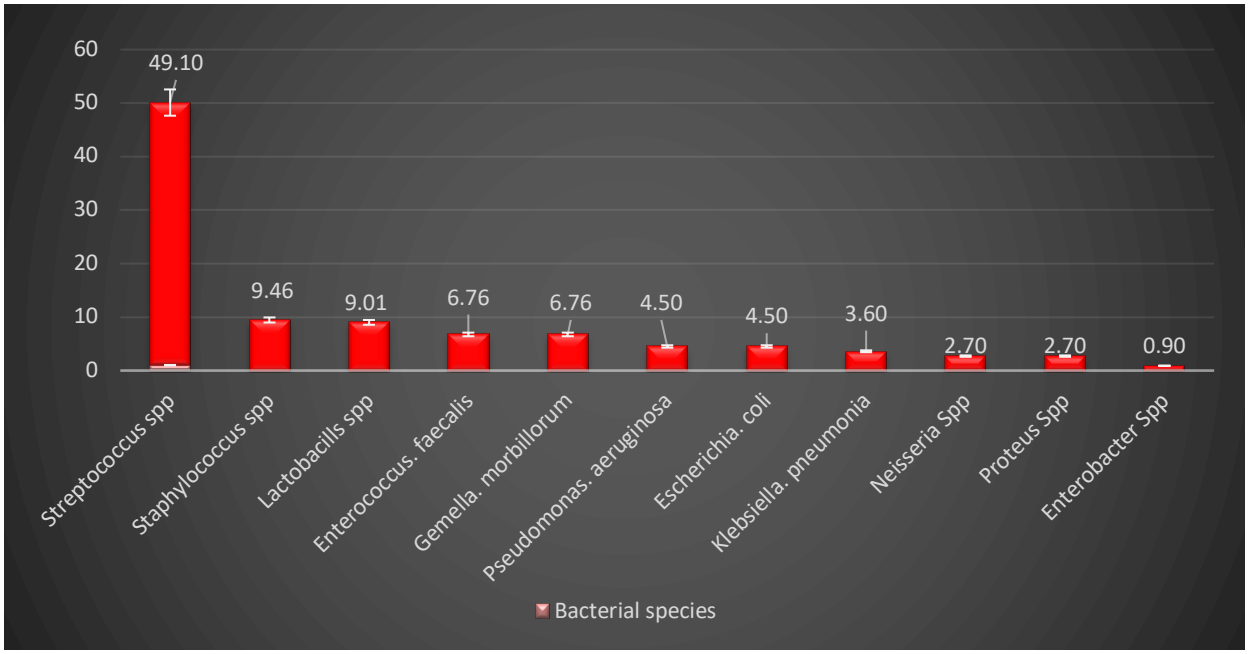

Figure (2): percentage of bacterial species that isolated from dental caries.

Discussion:

The import of the current study in Nasiriyah and throughout Iraq arises from a variety of factors. First, the increased prevalence of dental decay among children is attributable to its decline. Level of education, limited dental services, Most significantly, eating snacks and sweets in between meals while disregarding dental hygiene(23)(24) .

Gram-positive bacteria are found in higher proportions in the dental compared to gram-negative bacteria as in figure (1). These findings indicate that gram-positive bacteria are the main factor contributing to dental caries. This is consistent with a study conducted in the city of Diwaniyah (25).

The dominance of Streptococcus spp bacteria over the rest of the bacteria isolated in the current study as in (table 1)is consistent with the results of (26) that it is the main cause of caries lesions. Also, the dominance of the S. mutans species over the rest of the other species is consistent with what was found (27) regarding the dominance of the S. mutans bacteria over the rest of the bacteria that cause dental caries. The high rate of SVG infection is consistent with findings from India (28) and Mediterranean and North African nations (29).

Also, the appearance of Staphylococcus bacteria, especially the *Staphylococcus aureus*. in the vicinity of the mouth agrees with what I found (30) that the reason for the spread of this bacteria in the vicinity of the mouth may be due to it being one of the important pathogens with the ability to cause opportunistic infections due to its natural presence on the bodies of carriers, on the skin and on the upper part of the mouth. Nose, digestive and reproductive tract (31) . Or because it possesses many surface antigens and enzymes that help it penetrate the body's tissues (32).

Concerning the acidophilic lactobacilli L. acidophilus, the results of its isolation were consistent with Results (33, 34) in the isolation ratio.

Also, the difference in isolation rates for species belonging to the Enterobacteriaceae family (gram-negative) is due to their scarcity in the vicinity of the mouth, and this is confirmed by studies (30, 35) that most Gram-negative bacteria come from respiratory system infections or from the digestive duct. and appears in the mouth.

(36) explained that the difference in isolation rates changes according to the change in infection, and (37) found that bacterial isolation rates increase with the increase in the number of decayed teeth. It is also believed that the quality of the culture medium used, the isolation method, and other unknown factors have a major impact on the difference in the isolation rates of germs, in addition to the difference in the level of health awareness, continuous care for dental cleaning, and community culture have a major impact on this.

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