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BACTERIAL DIVERSITY IN ORAL CAVITY THAT ISOLATE FROM DENTAL CARIES CASES IN NAJAF / IRAQ

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ABSTRACT

A total of 100 clinical swab samples were collected from patients with dental caries in Al-Kafeel teaching clinic and dental clinics in Al-Najaf Governorate from November 2023 to January 2024. Study revealed that male were more (64) in compare with female (36). Age groups were between (9-60) years. The results showed that Gram positive bacteria were (77%) while Gram negative were (23%) which diagnosis by VITEK 2 system as the following: *Streptococcus mutans* 35 (35%), *Staphylococcus aureus* 15 (15%), *Lactobacilli* 9 (9%), *Staphylococcus* species 5 (5%), *Streptococcus* species 5 (5%), *Bacillus* species 4 (4%) and *Rothia 2(2%) and Actinomycetes 2(2%).* Gram negative bacteria that isolated from dental caries were only 23. The bacterial species were*: Prevotella* 7(7%), *Haemophilus influenza* 5(5)%, *Veillonella parvula* 5(5%), *Neisseria* 5(5%) and *E.coli* 3(3%) *Acinetobacter baumannii* 2(2%). **Aim of study:** Detection of most common micro flora in oral cavity from patients with dental caries.

KEYWORDS: Dental plaque, Gram positive bacteria, VITEK2, *Streptococcus mutans*, oral flora.

INTRODUCTION

The wide variety of microorganisms in the mouth plays a crucial role in preserving dental health. The oral cavity contains a diverse and intricate microbial community, consisting of

more than 700 known species of bacteria. The presence of diverse microorganisms is crucial for maintaining a well-balanced microbiome, which plays a vital role in processes such as digestion and defense against dangerous infections. Diet, hygienic practices, genetics, and overall health are among the various factors that affect the diversity of oral bacteria. Imbalances in the microbial equilibrium can result in disorders such as tooth caries, periodontal disease, or halitosis.

Dental caries is a highly prevalent chronic condition worldwide.[1] Dental caries result from bacterial activity that erodes the enamel, dentin, or cementum of teeth. Dental caries remains a significant public health concern in the majority of developed countries, impacting 60% to 90% of school-aged children and the vast majority of adults. The primary cause of this is primarily attributed to heightened sugar intake and inadequate exposure to fluoride.[2]

The primary etiological factor of dental caries is the presence of bacterial biofilm on tooth surfaces. Biofilms consist of surface-bound communities of microorganisms composed of cells, water, and extracellular matrix constituents.[3] *Streptococcus mutans*, the main factor responsible for dental decay, lives alongside around 500 other bacterial species in a complex community referred to as the dental biofilm.[4] Dental caries is an oral disease that relies on the presence of biofilm, and its initiation and progression are greatly influenced by the consumption of fermentable dietary carbohydrates. The development of dental caries is the result of the interaction between certain bacteria and food components within a biofilm called "dental plaque".[5] We conducted a microbiological study focusing on the presence or absence of acidogenic bacteria in dental plaque. The plaque was stored in reduced pass on media. Our goal was to gain a detailed understanding of the microflora of dental plaque in individuals of different age groups, with varying food habits and brushing schedules. The formation of biofilms is associated with the occurrence of dental caries, a condition that impacts a significant portion of the global population. *Streptococcus mutans* bacteria are believed to be the primary cause of this severe sickness. *S. mutans* plays a crucial role in the development of intricate and intricate formations on the surface of the mouth and tooth enamel.[6]

The cariogenic features of this organism include its ability to adhere to solid surfaces, establish colonies in the oral cavity, and withstand the acidic conditions present in the oral environment.[7] In addition to oral *streptococci, S. Mutans* is a highly acidogenic bacteria found in biofilm, since it has the ability to produce acids through the fermentation of carbohydrates. Moreover, *Streptococcus mutans* produces mutacins, which are bacteriocins

that play a crucial role in its functioning. The colonization of dental biofilm by mutans bacteria.[8,7] Dental plaque contains numerous acidogenic and aciduric bacteria. The microorganisms involved include *Mutans streptococci* (namely *Streptococcus mutans* and *Streptococcus sobrinus*), *lactobacilli*, and maybe *Actinomyces spp*. Acidogenic bacteria are highly associated with the development of dental caries.

Evaluation of Cariogenicity of Isolated Oral Microorganisms

The criteria for assessing pathogenic microorganisms associated with dental caries is not adequately established. The properties of acidogenicity and aciduricity are commonly recognized as being cariogenic in microorganisms.[9] Cariogenic bacteria must possess the ability to both produce acids and flourish in an acidic environment. Bacteria with low pH tolerance can contribute to the progression of dental caries, from the first stages to more advanced forms of the disease. Research has demonstrated that the proliferation and production of acid by acid-tolerant bacteria in the mouth is the primary reason for the demineralization of dental enamel.[10] Moreover, the cariogenicity of bacteria is determined by their ability to adhere to the surface of the tooth. The ability to form biofilms is considered a crucial characteristic. Regular use of sugar can alter the balance between demineralization and remineralization in oral microorganisms, leading to a net loss of minerals and the development of dental caries. The level of enamel demineralization can be used to assess the cariogenic potential of a bacterium.[11]

Oral Flora

The oral cavity consists of mucosal surfaces such as the lips, cheeks, palate, and tongue, as well as teeth. These structures possess unique biological and physical characteristics that facilitate the growth of various bacteria.[1] The typical temperature of the oral cavity is 37°C, creating an optimal environment for bacterial growth. In addition, saliva possesses a pH range of 6.5-7, making it the optimal pH for the majority of bacterial species. Saliva also keeps bacteria moist and delivers nutrients to different microorganisms.[2]

Mouth microbiota refers to the bacteria present in the oral cavity of humans. It is the second-largest microbial community in the human body, following the gut.[13,14] *Streptococcus* and *Actinomyces*, which are both organisms capable of surviving with or without oxygen, are acquired shortly after birth, within the first 15 hours. The mouth cavity has a diverse array of commensal bacteria that serve various functions. Overall, the oral cavity contains roughly 1000 different types of microorganisms, with a single person typically hosting between 50

and 100 species in their mouth. *Streptococci* comprise almost 50% of the microorganisms present in the oral cavity.[16,17]

Streptococci species naturally inhabit the human oral cavity and belong to the Viridans group streptococci (VGS). Viridans Group streptococci are Gram-positive bacteria that have a spherical or ovoid shape. They can survive with or without oxygen and do not have the ability to move or create spores. These bacteria can break down carbohydrates and produce acid, but they do not produce gas. They are a significant factor in the formation of plaque, which is a very intricate ecosystem comprising several bacterial species. The mutans streptococci, specifically *Streptococcus mutans* and *S.sobrinus*, are widely believed to be the main culprits behind tooth decay. The bacteria mentioned are frequently identified as the primary disease-causing agents in dental problems in humans. Their existence has been confirmed through epidemiological studies.[10]

Streptococcus mutans is a Gram-positive coccus bacterium commonly found in the human oral cavity and is a significant contributor to dental caries. It is a mesophilic bacteria that can thrive at temperatures ranging from 18 to 40 degrees Celsius. The oral cavity is colonized by *S. mutans* bacteria, which adhere to the teeth and produce plaque, a malleable and adhesive biofilm that forms on the teeth due to the breakdown of food. The biofilm is formed through bacterial proliferation and abundant growth. *S. mutans* bacteria metabolize carbohydrates and produce lactic acids, resulting in tooth decay. This leads to demineralization, where calcium phosphate is lost from the tooth structure, causing the tooth to weaken and eventually collapse, resulting in the formation of a cavity. The teeth possess an inherent pit and groove structure, facilitating the attachment and colonization of bacteria. Plaque and germs also adhere to the areas where teeth are in close proximity to one other.[12]

MATERIALS AND METHODS

The specimens collection consists of 100 clinical swabs obtained from dental caries patients who were admitted to Al-Kafeel dental clinic and outpatient in AL-Najaf City between November 2023 and January 2024. The collection includes 64 swabs from male patients and 36 swabs from female patients. The patients' age range is between 9 and 60 years. The specimens were promptly labeled and sent to the laboratory using sterile transport swabs for the purpose of Culture and Identification. Medical records for each patient were reviewed, encompassing information such as name, gender, age, and address.

The assembled specimens were primarily introduced onto various culture media, such as Nutrient agar, blood agar, mannitol salt agar (MSA), and MacConkeys agar. These media are widely recognized as essential tools for isolating, purifying, and identifying diverse bacterial strains due to their predominant enrichment, selectivity, and differential properties. The plates were placed in an incubator set at a temperature of 37˚C and left undisturbed for a duration of 24 hours. The Vitek2 automated system was utilized to identify the specimens in the collection. GP and GN identification cards were used to identify the Gram positive and Gram negative bacteria isolated from the oral cavity.

RESULTS

The study comprised of 100 patients with dental caries attending to Al-Kafeel teaching clinic, The swabs taken from patients transported to medical laboratory and have been cultured in media of blood agar for enrichment bacterial growth at 37Cº for 24 hours and cultured later on mannitol salt agar and MacConkeys agar at 37Cº for 24 hours. The identification of culture were depended on colony morphology, microscopy, and (Vitek-2 system) by using GP and GN card.

Figure (1) A: *S. aureus* on mannitol slat agar. **B: *Bacillus* spp on blood agar.**

In this study comprised of 100 patients with dental caries, 64 patients were male while 36 patients were female, figure (2).

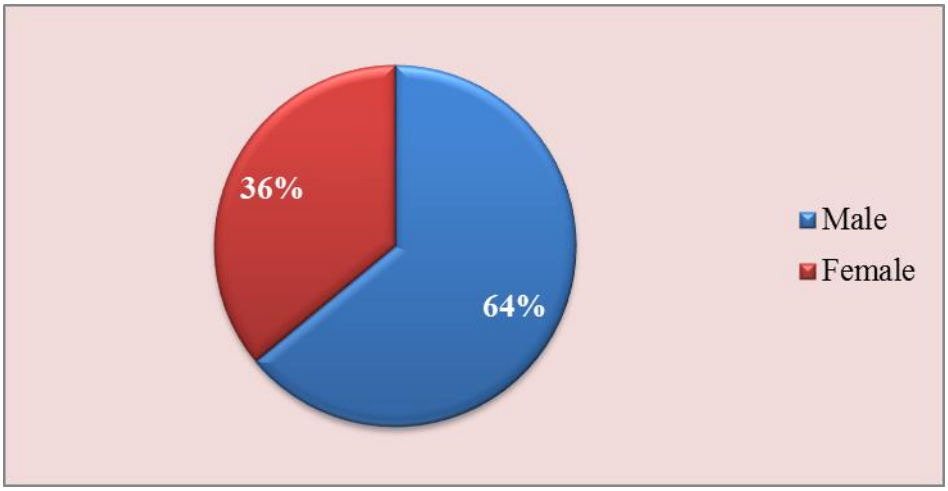

Figure 2: Distribution of patients according to patients gender.

In current study, the average of patients age were (9- 60) years. the results showed higher frequency of dental caries in the age group more than 50 year (42%). Others age group were include: 13% patients (9-20) years, 17% patients (20-30) years, 10% patients (30-40) years and 18% patients were (40-50) years old, figure (3).

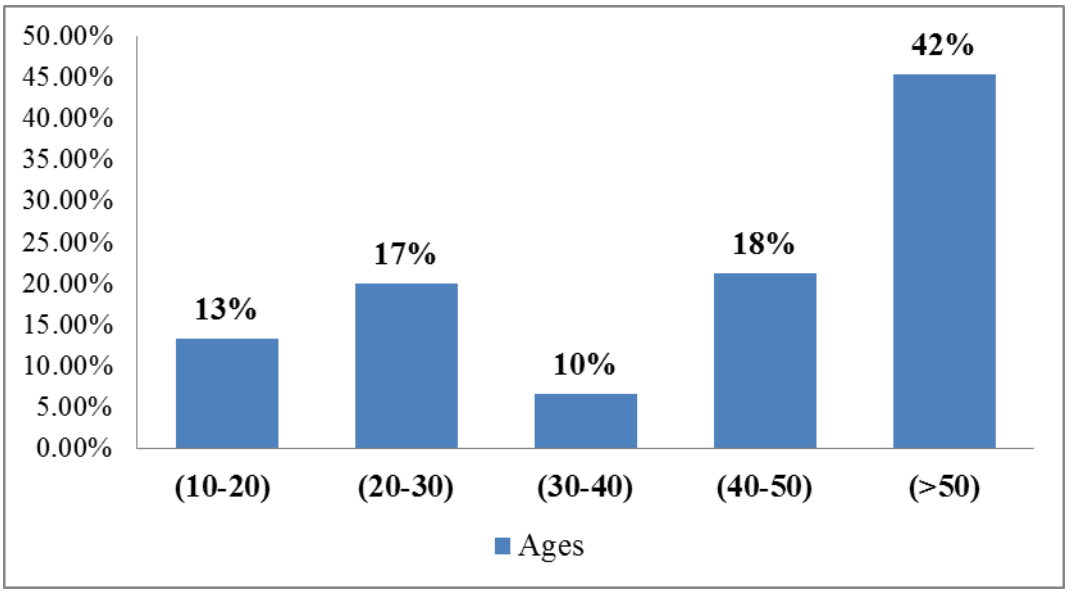

Figure 4: Distribution of Patients according to age.

The swabs taken from dental clinics have been showed that 73% of specimens were gram positive bacteria and 27% specimens were gram negative bacteria, Figure (4).

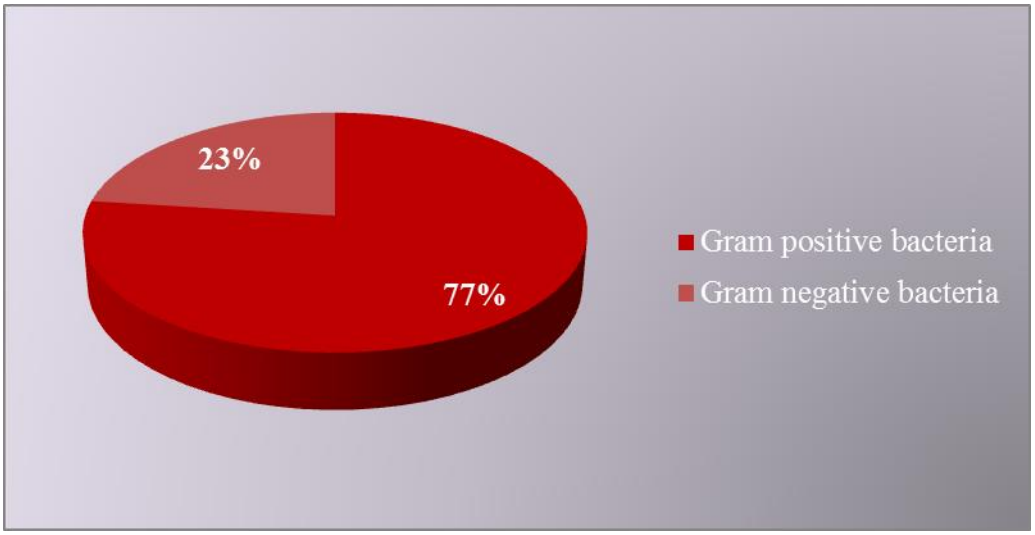

Figure 5: Distribution of Specimens according types of bacteria.

The result showed that about 77 specimens from totally 100 were gram positive and the most common species were: *Streptococcus mutans* 35 (35%), *Staphylococcus aureus* 15 (15%), *Lactobacilli* 9 (9%), *Staphylococcus* species 5 (5%), *Streptococcus* species 5 (5%), *Bacillus* species 4 (4%) and *Rothia 2(2%) and Actinomycetes 2(2%).*

Gram negative bacteria that isolated from dental caries were only 23. The bacterial species were*: Prevotella* 7, *Haemophilus influenza* 5, *Veillonella parvula* 5, *Neisseria* 5 and *E.coli* 3 *Acinetobacter baumannii* 2, Table (1).

Table 1: The percentage of gram positive and gram negative bacteria that isolated from dental caries patients.

Types of bacteria	Name of bacteria	Number of specimens	Percentage %
Gram positive bacteria	*Streptococcus mutans*	35	35%
	Staphylococcus aureus	15	15%
	Lactobacilli	9	9%
	Staphylococcus species	5	5%
	Streptococcus species	5	5%
	Bacillus species	4	4%
	Rothia	2	2%
	Actinomycetes	2	2%
Gram negative bacteria	*Prevotella*	7	7%
	Haemophilus influenza	5	5%
	Veillonella parvula	5	5%
	Neisseria	5	5%
	E.coli	3	3%

	Acinetobacter baumannii	2	2%
Total		100	100%

DISCUSSIONS

Oral bacteria are crucial for preserving the equilibrium of the oral microbiome. Certain bacteria can have a positive impact on oral health, while others might worsen tooth problems. Maintaining consistent dental hygiene habits, such as brushing and flossing, aids in the regulation of the bacterial ecosystem, promoting a state of optimal oral health. Gaining knowledge about the variety and conduct of oral bacteria is crucial for averting dental issues and enhancing general health. The presence of a wide range of microorganisms in the oral cavity is crucial for preserving oral health. An equilibrium of microorganisms in the oral microbiome plays a crucial role in preventing the excessive growth of harmful pathogens, hence ensuring the overall stability of the oral microbiome. Gaining comprehension and advocating for variety can assist in formulating tactics for upholding a robust oral environment.

CONCLUSION

An assay on oral flora entails the examination of the bacterial makeup and abundance within the oral cavity. These methods, such as microbial culture, DNA sequencing, or metagenomic analysis, can be employed to achieve this. These assays aid in the identification of certain bacterial species, their functions in oral health or disease, and provide guidance for the creation of focused therapies to maintain a well-balanced and healthy oral microbiome. Streptococcus mutans is a highly common bacteria found in the mouth.

It is recognized for its involvement in the creation of dental plaque and is a significant factor to the occurrence of dental caries (cavities). Streptococcus mutans flourishes in the presence of sweets, generating acids that have the potential to corrode tooth enamel. In addition to S. mutans, the oral cavity is also home to several other prevalent bacteria, such as different strains of *Streptococcus*, *Actinomyces*, and *Veillonella*. The oral microbiome exhibits a wide range of diversity, with distinct species fulfilling specific functions in the preservation of oral well- being or the promotion of dental ailments. Maintaining regular oral hygiene routines, such as brushing and flossing, assists in regulating the equilibrium of these bacteria and fostering dental well-being.

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