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ISOLATION OF BACTERIA CAUSING DENTAL CARIES FROM THE CLINICS OF THE COLLEGE OF BILAD AL-RAFIDAYN UNIVERSITY

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Abstract: This study deals with Isolation of bacteria causing dental caries from the Forty samples (male and female) were collected from patients attending the clinics of the Department of Dentistry at Bilad Al-Rafidayn University College for the period 7/11/2022 to 4/5/2023. The total of (58) isolates before and after cleaning and tooth extraction collected from male and female samples .After diagnosing these isolates, it was found that. Streptococcus (42.5%) and gram-negative (20%) before dental work and cleaning . The Percentage of Streptococcus after cleaning (30%) and the gram-negative rod (7.5%). The percentage of Streptococcus isolated from tooth extraction (20%) and staphylococcus (2.5%) and gram-negative rod(2.5%). The spacemen were collected from the oral cavity and the culture was on mitis salivarius(Himedia/India) agar base, that was determined by biochemical tests by use of blood agar to detect hemolysis, the results revealed the samples were positive.

Keywords: bacteria, dental, caries, College of Bilad AL-Rafidayn University.

Introduction

Streptococcus is a major genus of spherical Gram-positive bacteria which belong to the phylum Firmicutes. Streptococci are classified as alpha-hemolytic, beta-hemolytic or gamma-hemo- lytic according to their appearance on blood agar. Alpha-hemolysis involves the bleaching of heme iron by streptococcal hydrogen peroxide (H_2O_2), resulting in a greenish tinge on blood agar. Alpha-hemolytic streptococci used to be known as the 'Viridans group' for the green- ish color produced by hemolysis. However, alpha-hemolysis is not entirely consistent between different strains of individual Streptococcal species, and therefore the term 'Viridans' is some- what misleading and is no longer used. These organisms are now more commonly known as the oral streptococci.{1}Overall, The human oral streptococci are commensals which often inhabit the gastrointestinal and genitourinary tracts, as well as the oral mucosa and tooth surfaces. In healthy individuals, streptococci can constitute more than 50% of the oral microbiota and these bacteria gener- ally possess low pathogenic potential. However, oral streptococci can invade the bloodstream, and have the potential to cause infective endocarditis (IE) or post-antineoplastic septicaemia in neutropenic patients with haematological disease.{2}Other oral Streptococcus associated condi- tions including odontofacial infections, brain abscesses and abdominal infections have also been reported. Furthermore, recent work has shown that S. mitis group bacteria play a major role in exacerbating influenza infection particularly among immunocompromised indi- viduals; Streptococcus oralis and S. mitis were found to produce neuraminidase (NA), a vital target of anti-influenza drugs. The NA activity exhibited by these oral bacteria stimulates the release of influenza virus, boosts viral M1 protein expression levels and activates the cell signal.{3}

Dental caries is one of the most significant and common infectious diseases in the human oral cavity with bacterial metabolic processes that cause damage in hard tissue of the tooth structure. It is considered as a major public health problem globally due to its high prevalence and significant social impact. Dental caries has plagued human since the dawn of civilization and still constitutes a major public health concern at global scale.{4} This is mostly due to colonization of Streptococcus mutans as a causative agent for dental caries. The bacteria S. mutans has the capacity to metabolize the fermentable carbohydrate into organic acid which causes fall in pH to increase the risk of enamel solubility. The untreated dental caries can lead to pain, tooth loss, infection, inflammation, and death in severe cases. The presence of bacterial flora may be seen in different area of teeth for ample dentin enamel junction beneath white spot lesion, gaps between cavity walls and restoration, areas of penetrated caries, fissures, and other adjacent areas. The cell wall biosynthesis inhibitor is the effective antibiotic against the bacteria or mutans. The Streptococcus mutans is gram positive coccus which is usually susceptible to cell wall biosynthesis thus inhibiting antibiotics . Unfortunately, further difficulties in treating dental caries with conventional antibiotics is observed over time. The recent study on cariogenic S. mutans showed a gradual increase in resistant pattern of bacteria to, Penicillin, Erythromycin, Ciprofloxacin, and other antibiotics. Though antimicrobial resistance is not new in the world, the frequencies, patterns, and distribution of resistant bacteria vary with geographic locations {5}. Streptococcus mitis – Gram-positive bacteria, Small, flat, hard colonies, blue In color with a domed center. {6} Bacteria belonging to the genus Streptococcus are the first inhabitants of the oral cavity whichh can be acquired right after Oral streptococci produce an arsenal of adhesive birth, molecules that allow them to efficiently colonize different tissues mouth. Also, they have a remarkable ability to metabolize in thecarbohydrates via fermentation thereby generating acids as , byproducts

Streptococci are found In almost every location In the human body and are the dominant species In the human oral cavity and upper respiratory tract, Warm, moist, and rich In nutrients, the oral cavity provides an ideal environment for colonization by a community of bacteria, often In the form of a complex structure called biofilm, or plaque Streptococci are well known for their ability to assimilate a large array of carbohydrates by glycolysis, and for their enhanced tolerance to acidic pH. Employing the Embden-Meyerhof-Parnas (EMP) pathway, streptococci convert one

molecule of glucose or other equivalent carbohydrates (e.g. fructose) Into two molecules of pyruvate, with the concomitant generation of two molecules of ATP and two molecules of NADH. Under the conditions of excess carbohydrates and oxygen limitation, streptococci tend to perform homolactic fermentation, reducing pyruvate Into lactic acid and regenerating NAD from NADH. The production of lactic acid results In rapid acidification of the environment, and allows streptococcal species to outcompete acid-sensitive microorganisms. When faced with carbohydrate limitation or Increased oxygen tension, streptococci produce alternative fermentation products such as formate, acetate and ethanol. In fact, streptococci lack the capacity for oxidative phosphorylation and electron chain transport systems and depend exclusively on substrate-level phosphorylation for energy production. Therefore, most streptococci are considered facultative anaerobes and susceptible to growth Inhibition by oxygen{7}. Streptococcus mitis Is part of the normal flora of the oropharyngeal, nasal, gastrointestinal and genitourinary tracts. Classically, it shows low pathogenicity and virulence, can occur as a pathogenic microorganism in immunocompromised patients, mitis/oralis can cause a variety of infections that include blood stream infections, infective endocarditis, meningitis, and septicemia. Rare cases of urinary infection and VGS toxic shock,

Dental caries should be treated becausee most cases of streptococcal endocarditis are caused by poor oral {8}. Streptococcus mitis may cause life-threatening infections, particularly endocarditis. Meningitis with S. mitis is rare, caused severe clinical conditions Including sepsis and septic shock especially in neutropenic patients. {9}. Traditionally viridans streptococci have been considered to be susceptible to beta-lactam antimicrobial agents. S. mitis has been implicated as the etiologic agent in urinary infections. {10}. Streptococcus mitis biovar 1 is a typical representative of the commensal microbiota of the respiratory tract. It colonizes several surfaces in the oral cavity and pharynx after birth and is believed to remain a numerically important member of those ecosystems throughout life. Like a few other Streptococcus species. {11}. Streptococcus mitis and S. oralis are human oral colonizers, opportunistic pathogens, and species of the viridans group streptococci (VGS). {12}. Protein M Is considered as the main virulence factor, limiting phagocytosis, disturbing the function of complement, and being responsible for adhesion {13}. Streptococcus mitis is highly susceptible to therapy with penicillin G potassium. {14} But in some conditions show resistance to penicillin Not everyone needs antibiotics becausee Streptococcus mitis normal flora. This studies was Aim to detection and isolation of bacteria that are the main cause of tooth caries, also aimsto know if bacteria in oral cavity Streptococcus mutans or Streptococcus mitis.

Methods

Study Design

This study deals with Isolation of bacteria causing dental caries from the Forty samples (male and female) were collected from patients attending the clinics of the Department of Dentistry at Bilad Al-Rafidayn University College for the period 7/11/2022 to 4/5/2023.

Laboratory prepared Culture media

Mitis salivarius agar prepared to render to the manufacturing company directions; the ingredients were dissolved in distilled water, pH was adjusted to 7.2 ± 0.2, then heated in the water bath to dissolve all ingredients entirely. The media was sterilized by autoclaving at 121°C for 15 min at 15 pounds/inch2, subsequently dispersed into sterile Petri dishes; otherwise, the media were incubated at 37 °C for 24 hours to confirm sterility

Blood agar base

Prepared according to the manufacturer's instructions (3.9 mg of blood agar base was added to 100ml of distilled water) and covered by cotton, then sterilized by autoclave at 121°C, 15 lbs. pressure, for 15 minutes, then transferred into a water bath at 50 °C. When agar was cooled to 50 °c, sterile blood was added aseptically and mixed well gently, and warmed to room temperature. 15ml was dispensed to sterile petri dishes aseptically, then stored at 2-8 °C in sealed plastic bags

to prevent moisture loss.It is an enriched bacterial medium that used to detect to ability of Streptococci mitis hemolysis.

Muller-Hinton aga

used to detect Antibiotic sensitivity to isolates Prepared according to sigma Aldrich com. Suspend 19g Mueller Hinton agar powder in 500ml of distilled water. Mix and dissolve them completely.Sterilize by autoclaving at 121°C for 15 minutes. Cool to 45- 50 c.Pour the liquid into the petri dish and wait for the medium to solidify.

Collection of specimens

Sample saliva and tooth extraction

Saliva and tooth extraction samples were collected at the clinics of the Bilad AL-Rafidain University Collage and diagnostic and allergy tests were performed on the bacteria taken from patients suffering from caries as shown in the figure 2 , the samples were taken before and after cleaning and transported to lab by Transport media, the samples of the tooth extraction were transported by a sterile Violet

Sample collection procedures include :

1- hands were washed and gloves were worn before collection

2-transport media was used and the area containing caries was scanned, the swab was taken from the patient before cleaning his teeth from Calcifications and after, as for the extraction tooth samples were transferred by a sterile Violetas the tooth was placed directly into violet to avoid contamination

3-The samples were taken to the laboratory as quickly as possible to prevent further growth of microorganisms .

4-samples were cultured immediately after collection (after 30 minutes of collection). If this is not possible, the specimen must be refrigerated at about 4" immediately until delivery to the laboratory and processed no longer than 18 hour after collection.

Identification of strptocoucs mitis

Gram s stain Examination

Accordindly to (58) all the bacterial isolates were examined by Gram's stain, shape, and color of the cells were observed via a light microscope by taking one bacterial colony and transported to the microscopic slide, fixed then stained by using Gram's stain. Cell arrangement and shape were observed with oil immersion.

Morphological Examination

Morphological examination based on morphological characteristics including colony color, shape, edges, and texture (as primary diagnostic tests) of isolates growth on Mitis salivarius agar base was incubated 37°C overnight .

Blood agar test:

its performed to detect bacteria capability to blood hemolysis, 10 dishes were taken and the test was done on them, and the results showed that the streptococcus mitis is not hemolytic, that maen it gama hemolysis

Muller-hinton agar:

used to detect antibiotics sensitivity to isolates, streptococci mitis was resistance and show Partyshall zone

Result and Disscusion

Characterization of patients with caries

Characterization of the stady group on dental caries, including, the patient's gender, age, job, housing, and time if before or after brushing his teeth, with the mention of observations, forty samples of patients were collected from each patient taken from him before and after the dental cleaning work The number of total total Isoletes before cleaning, streptococcus mitis 42.5% and

rod20% as in Figure 3, the number of total total Isoletes after cleaning Streptococcus mitis 30% and Gram-negative rod 7.5% as in Figure 4, the number of total extraction total Isoletes in which Streptococcus mitis appeared 20% Staphylococcus 2.5% and Gram-negative rod 2.5% as in Figure 5, then the total total Isoletes were divided into male and female, the number of total total Isoletes male as 28 samples, the number of male total Isoletes before cleaning was 23 samples including streptococcus 63.5% and gram-negative rod 0.14% as in Figure 6, the number of male total Isoletes after cleaning was 16.4% including, Streptococcus mitis 28.5% and Gram-negative rod 10.7% 0 as shown in Figure 7, the number of male total Isoletes of thrush is 17.8% and all of them include Streptococcus mitis as shown in Figure 8, the number of total female samples was classified before cleaning for streptococcus bacteria 33.3% and Gram-negative rod 16.6% as in Figure 9 and the number of total Isoletes after cleaning for Streptococcus mitis bacteria 33.3% and gram-negative rod 8.3% as shown in figure10, the number of extraction total Isoletes for total females is 50% and the number of extraction total Isoletes for streptococcus bacteria includes 33.3% of the total percentage of females, Staphylococcus 8.3% and Gram-negative rod bacteria 8.3 as in Figure 11, This is Aggrement with the study of the source number 1,2,3,4 and 5.

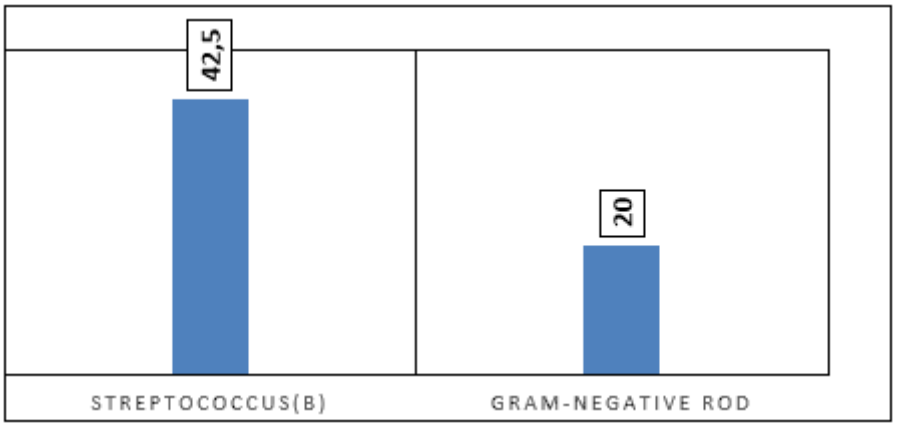


Figure 1. The number of isolates before cleaning

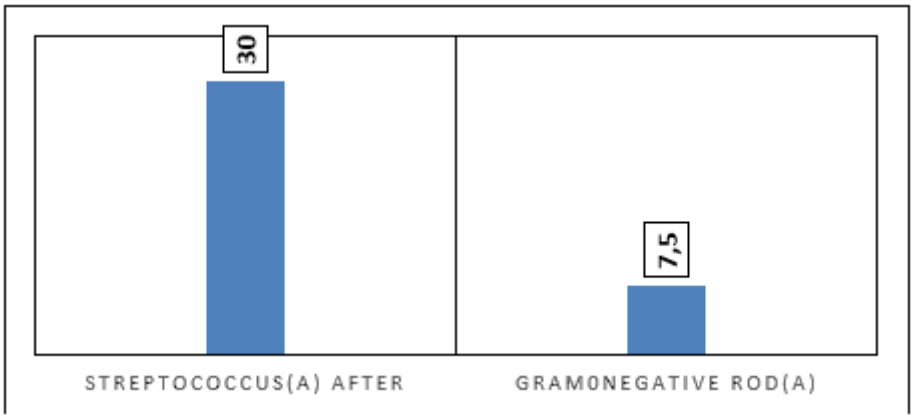


Figure 2. The number of isolates after cleaning

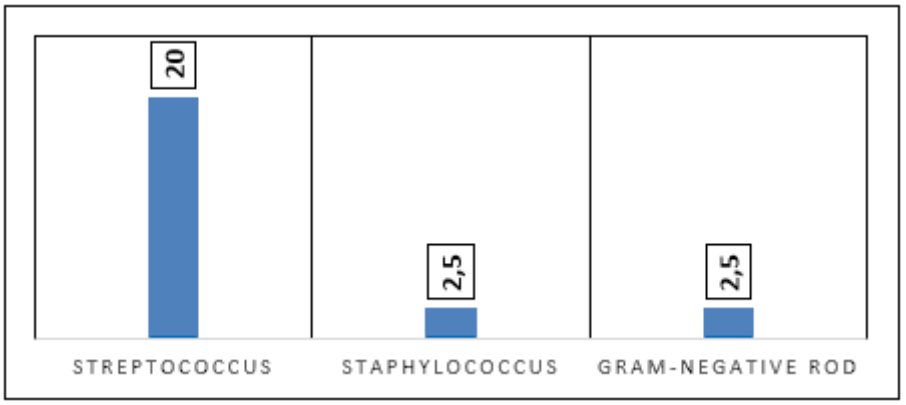


Figure 3: The number of isolates total Isoletes before cleaning

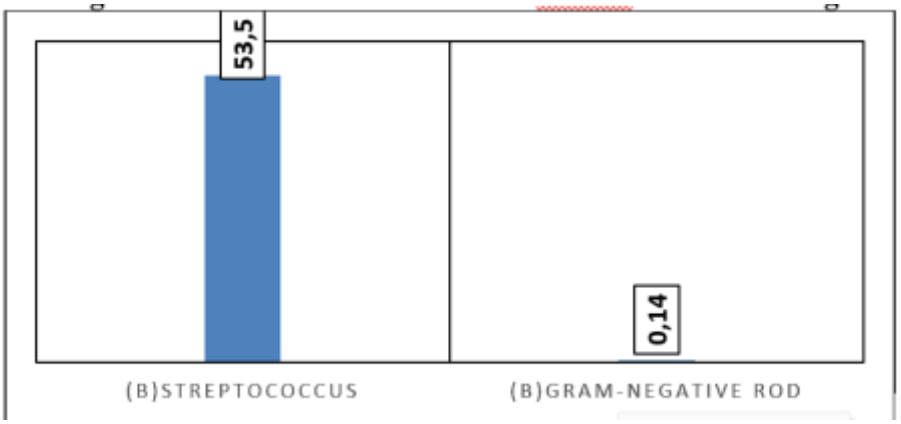


Figure 4: The number of male isolates before cleaning

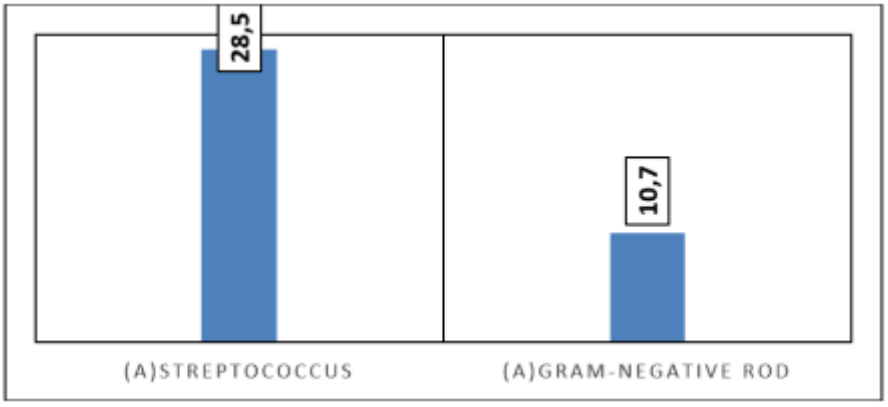


Figure 5:The number of male isolates after cleaning

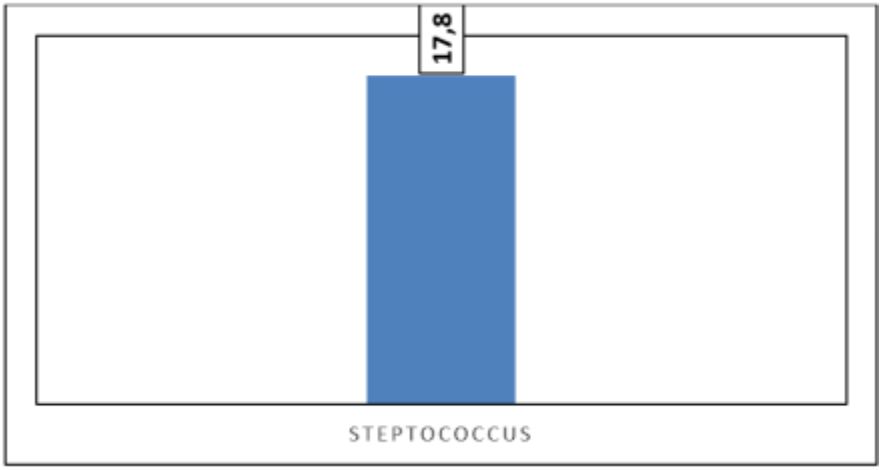


Figure 6: The number of male isolates for tooth extraction

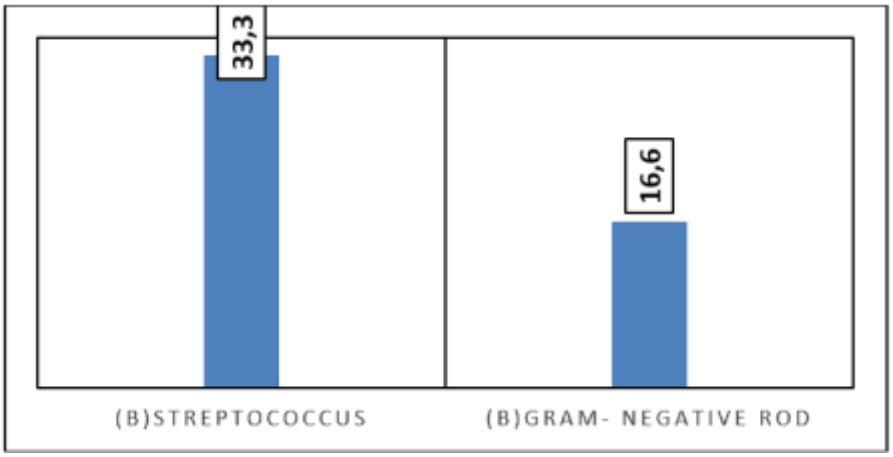
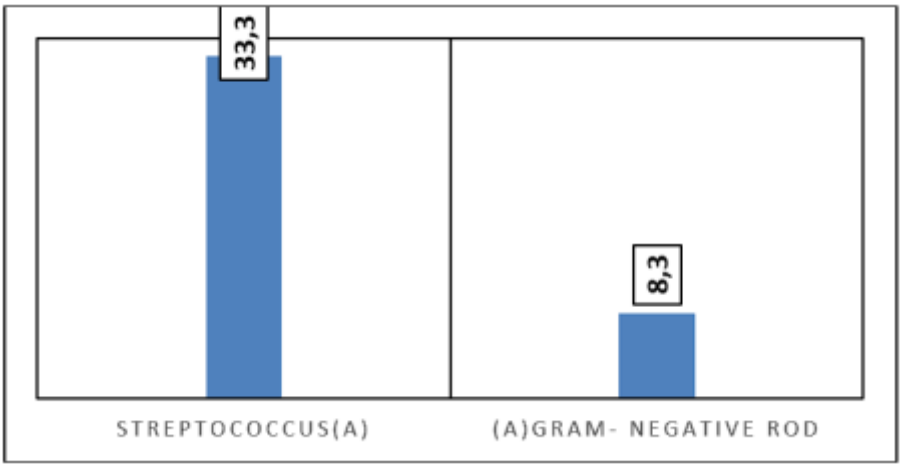


Figure 7: The number of female isolates before claning



Figer8 : The number of female isolates after cleaning

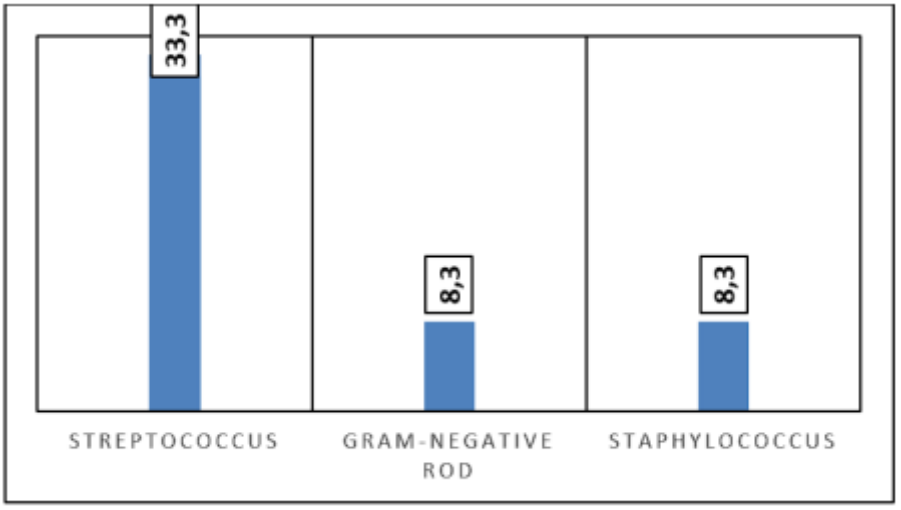


Figure 9 : The number of female isolates for tooth extraction

The following table shows the ratio of the number of tota isolates

	extraction	after	befor
streptococcus	20%	30%	42.5%
staphylococcus	2.5%	------	------
Gram-negative rod	2.5%	7.5%	20%

The following table shows the ratio of the number of total male isolates

	extraction	after	befor

streptococcus	17.8%	28.5%	53.3%
staphylococcus	-----	-------	----------
Gram-negative rod	-------	10.7%	0.14%

The following table shows the ratio of the number of total female isolates

	extraction	after	befor
streptococcus	33.3%	33.3%	33.3%
staphylococcus	8.3%	-----	------
Gram-negative rod	8.3%	8.3%	16.6%

Identification of mitis in the study groups

Microscopic Examination(gram stain)

Mitis isolates were examined by Gram stain. The results of the examination showed that isolates were gram- positive coccobacilli, occurring in singly, or in short chains and occasionally arranged in diplococcic Figure (10).

Figure 10: microscopic mitis under 100X

Microscopic Mitis in gram stain showing gram positive coci under 100x, which not have cytoplasmic membranous and not have cell wall interpreting the results of a gram stain firest step I crystal violet which stains cells purple or blue in the stepe to lodine is mordant makes dye less soluble so it adhrers to cell wall so that cells remain purple or blue step 3 Alcohol add to cell lead to decolorizer washes away stain from cell wall and cells are colorless In the step 4 Add Safranin is counterstain allows dye adherence to cells which appear pink or red

Identification the morphological on media growth

In the present study, colonize characterization after culturing on mitis salivarius agar base after incubation at 37C for 24H, streptococcus mitis show small, hard colonies and flat, blue In color with a domed center, it shows thikness and ragged(3), as shown in FIGURE13,This is Aggrement with the study of the source number 6.

Figure 11: Streptococcus mitis morphological on media growth

Sensitivity and resistance to antibiotics

Disk diffusion test was employed to determine antibiotic susceptibility of Streptococcaceae mitis, The results revealed that 10 isolates, that was Sensitive for penicillin and ancomyein, amoxiclin, trimethoprim This is Aggrement with the study of the source number 14

Conclusion

1. The main aim of cleaning teeth is to get rid of caries caused by bacteria that target the oral cavity and coexist in it, and therefore the process of cleaning teeth played a role in reducing the percentage of bacteria
2. Caries-causing bacteria are found in patients who neglect their daily oral and dental hygiene more often than healthy patients
3. streptococcus mitis, the most common bacteria in oral cavity
4. All the isolated bacteria were non - hemolytic, i.e., gama hemolysis

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