

Isolation, Identification and Antimicrobial Susceptibility of some Bacterial Species Isolated from Dental Plaque

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Abstract

Dental plaque is a sticky bacterial film that develops on teeth producing plaque as a result. If it is not removed with regular dental cleanings and daily brushing, it can initiate oral health issues such as gum diseases and dental caries. This study aimed to determine the prevailing bacterial species found in some patients' dental plaque cases and dental caries and to analyze the antimicrobial susceptibility of the obtained bacteria. A total of 60 samples of dental plaque were taken from patients who attended the health centres in Baghdad with different ages using cotton swabs. All gained swabs were transported to the microbiology laboratory and cultured on culture media. According to standard laboratory diagnosis protocols, primary diagnosis was performed. VITE 2 Compact Automated- System (GP (and (GN (diagnosis cards used according to manufacturer company constructions (BioMérieux) to confirm the diagnosis. Antibiotic susceptibility test was done by using antibiotic disks according to Kirby- Bauer method. Out of 60 samples, forty-two (70%) samples showed growth on culture media, while 18 (30%) showed no growth, most of the isolates, 29 (69.0%) were Gram-positive bacteria represented by *Streptococcus mutans*, *Staphylococcus aureus*, and only 13 (30.9%) of isolates were Gram-negative includes *Klebsiella pneumonia* and *Escherichia coli*. According to the antibiotic susceptibility test results, Gram-positive bacteria showed high resistance against amoxicillin, Cefotaxim and erythromycin with percentages of 86.2% and 89.6% respectively. Gram-negative bacteria showed high resistance against amoxicillin and erythromycin with percentages of 76.9% and 69.2% against Cefotaxim.

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1. Introduction

Generally, the human body is colonized by a huge number of different microorganisms, and the human oral cavity is the main sources of these microorganisms. It was found that nearly seven hundred to one thousand microbial species colonize the mouth, incidence of oral or mouth diseases like periodontal diseases and dental caries stay thoroughly associated with oral microbes [1-4]: these microorganisms can enter the circulatory system of the human body via injured oral mucous membranes and as a result cause an increase in the levels of antibodies and then lead to raise the

cardiovascular illnesses risk [2]. Dental caries is a widespread human oral disease and a primary public health problem along with weighty financial loads [5]. The imbalance in microorganisms of dental plaque biofilms could lead to a rise in acid-tolerant and acid-making bacterial species such as streptococci. This can decrease the pH of plaque and, as a result, cause tooth demineralization, resulting in caries [6]. The human oral cavity is usually germ-free at birth: the bacteria are picked up from the mother during breastfeeding and the environment particularly [7]. Dental caries affects

about (60 – 90%) of schoolchildren and most adults [8]. Caries are identified as local damage to dental tissues by the actions of different bacterial species [9]. Bacterial plaque is the main causative factor of dental caries, which forms on tooth surfaces [10]. It forms on human tooth surfaces when different bacteria are found in the plaque, it becomes unprotected from fermentable carbohydrates because they produce acid, and this acid then lowers mouth pH, which causes demineralization in the enamel on the teeth. Remineralization actually will occur; but, if demineralization outpaces the remineralization process, hollows or cavities may develop in the human teeth [11]. The clinical etiology of tooth caries includes; the presence of plaque that contains bacteria that produce acid and a vast number of bacteria together, daily consumption of efficiently fermentable carbohydrates such as sugar, the absence of saliva production, or a reduction in the ability of saliva to work as a buffer and a genetic predisposition to caries [12]. The most notable bacteria that cause dental caries are streptococci, especially *Streptococcus mutans*, lactobacilli, and many other bacterial types [9]. Many opportunistic bacteria such as *Staphylococcus* species and other bacteria may be regarded as probable pathogens, particularly in persons with diabetes mellitus, neutropenia and AIDS [13]. *Staphylococci* are considered an impermanent resident in the oral cavity, in addition; they are supposed as the reason for different oral disorders like dental caries [14]. Many bacterial species including *Klebsiella pneumoniae* and *Escherichia coli* were found in dental plaque when they colonized the plaque dentures of dependent elderly [15]. The incidence of caries generally increases among persons who do not brush their teeth frequently [16]. This study was conducted to isolate, identify and study the antibiotic susceptibility of aerobic bacterial species that causes dental plaque and carries among Iraqi patients. Dental diseases with diabetes and cardiovascular are three diseases that receive the highest efforts of inhibition and cure worldwide [17]. The World Health Organization (WHO) stated that about (60–90%) of children in schools and approximately (100%) of human adults are suffering from cavities worldwide [18]: - for that reason, the prevention of caries plays a crucial role in public health control.

2. Study Materials and Methods

2.1. Samples Collection and Patients Data

In the current study, dental samples were collected from patients attending some health centres in Baghdad City. Sixty (60) dental plaque specimens were collected from several patients with varying dental health and suffering from dental caries from October 2023 to January 2024. Medicinal history was obtained from all study participants after obtaining their oral agreement using a questionnaire form including whether the patient smoked or not, whether the patient had used antibiotics for the last two weeks or not, and whether the patient used toothpaste and dental floss or not.

2.2. Laboratory Culturing and Identification of Obtained Isolates.

All dental plaque swabs were cultured to detect aerobic bacterial growth by inoculating in culture media including MacConkey agar culture media, Blood agar culture media, Nutrient agar media and Mannitol salt agar (Oxoid/England) and incubating at 37 °C for 24-48 hours aerobically. The plates were examined for growth after 24 hours of incubation [19]. The obtained bacterial colonies were diagnosed according to standard microbiology laboratory identification protocols depending on morphological characteristics and biochemical tests [20]. Vitek 2 System and Gram-positive -GP and Gram-negative-GN colourimetric cards (BioMérieux / France) were used to confirm the diagnosis of bacterial isolates.

2.3. Antibiotic Susceptibility

Antibiotics sensitivity test was done according to the Kirby- Bauer method designated in 1984 by Claus and Berkeley. Brain heart infusion broth media of five ml was poured into plane tubes and inoculated with a full loop of bacterial isolate; the inoculated tubes were incubated at (37°C) for 24 hours. After this, the inoculated broth was compared with the turbidity standard, and the density of the test suspension was adjusted to be equal to (0.5) tube of Maccfarland. Then, growing bacteria was transferred using a sterile cotton swab streaked on the Mueller-Hinton agar plate surface (Difco-USA). The inoculated plates were left to dry for nearly a minute at room temperature, and; selected antibiotic disks including (Amoxicillin/Clavulanic, Cefotaxim, Tigecycline, Erythromycin and cefepime) were placed on the plates containing bacteria with

sterile forceps and incubated at (37°C) for 24 hours in a reversed position. After overnight incubation of inoculated plates, the inhibition zone diameters were measured with a ruler in (mm). The results of the inhibition zone were determined depending on the Clinical and Laboratory Consensus Standards Institute (CLSI).

3. Results and Discussion

3.1. Samples and Patients Data

A total of 60 swabs were obtained from dental plaque during the current study; - only 42 samples showed bacterial growth while 18 samples showed no growth. As shown in Figure (1), most of the patients 43.5% were smokers, 77.5%, and 95% did not use toothpaste and dental floss respectively. Current study results found that 77.5% of study participants don't use toothpaste and don't brush their teeth daily; - in contrast, in a study conducted in Ethiopia, most patients (83.2%) brushed their

teeth every day also use toothpaste and charcoal [21]. Another study found that 64.1% of patients brush their teeth and only 5.5% were smoking cigarettes [22], These results disagree with the current study results, which show that 43.5% of patients were smokers. Environmental, socioeconomic and geographical variables affect the rates of dental caries worldwide; - despite developments, most kids stay to hold dental caries. Dental caries is a widespread dental condition that is a risk factor for tooth loss in children and adults [23]. It is one of the very common diseases in humans that depends on the presence of different microbes. The standard etiology of caries is based on four influential factors including; host, oral environment, time and oral microorganisms. Too much consumption of carbohydrates causes an increase of acid-resistant, and acid-producing mouth microbes [24].

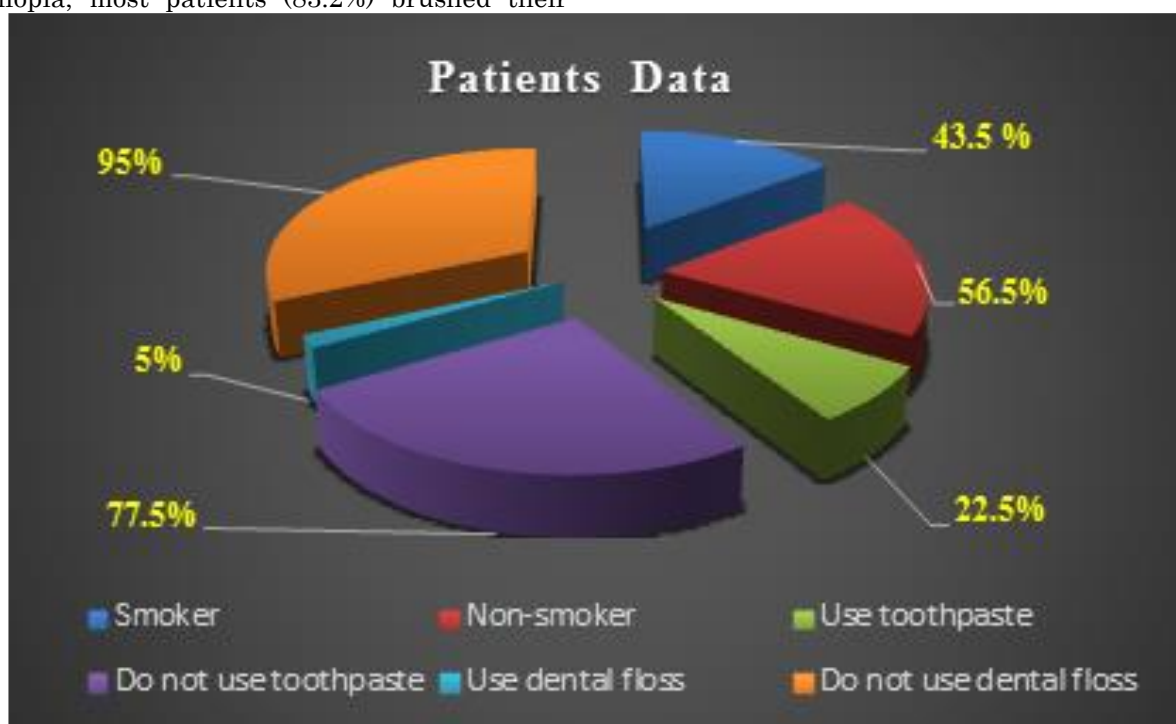


Figure 1. Patients Data Analysis

3.2. Identification of Bacterial Isolates.

Out of 60 samples obtained, forty-two showed growths on culture media. Most of obtained bacterial isolates 29 (69.0%) were Gram-positive represented by *Streptococcus mutans* 11 isolates (26.1%) and *Staphylococcus aureus* 18 isolates (42.8%), and only 13 (30.9%) of obtained isolates

were Gram-negative represented by *Klebsiella pneumoniae* 9 isolates (21.3%) and *E. coli* 4 isolates (9.5%), as shown in Figure (2). Figure (3) shows the shape, and color of some bacterial colonies that were cultured on different culture media. According to a local study conducted by

Mohammed et al. (2022), out of 112 obtained samples, 63.4% of samples showed growth on the culture media plates, while another 41 36.6% of samples did not show any growth [25]; this result was in agree with current study results when 70% of obtained samples were showed growth on culture media and only 30% of samples showed no growth. Current study results agree with the study conducted by Jubair. (2015) showed that the most frequent isolates that cause dental plaque was *S. mutans* 71.4% [26]. Another study conducted reported that Streptococci represented the majority of bacterial types isolated from study patients, which were isolated at 53.5%, followed by *Staphylococcus. aureus* at 33.8% [25]. The VITEK 2 compact is an automated, biochemical identification system that allows the accurate rapid identification of medically important bacteria and yeast through fluorescence technology [27]. Another study found that most bacterial species isolated from dental carriers were Streptococci species in percentage 68.1% [28]. In a recent study, *S. mutans* and *S.*

aureus bacteria were isolated from 46.4% and 35.2% of dental caries samples respectively [21]. According to Vitek 2 confidence rates results, the current study GP and GN identification cards showed different confidence values: 25 isolates (59.5%) showed a confidence rate of 99-96% (excellent identification), twelve isolates (28.5%) showed a confidence rate 93-95% (very good identification), and only 5 isolates (11.9%) showed confidence value 89-92% (good identification). Gram-negative isolates include 2 genera and 2 species, and Gram-positive isolates include 2 genera and 2 species. Depending on other epidemiological studies data in Iraq, *Streptococcus. mutans* and *Streptococcus mitis* were distinguished with percentages of (40%) and (10.7%), respectively collected from one hundred patients with dental caries and identified by using the Vitek 2 system [29]. In a recent study, the most predominant bacteria isolated from dental caries was *S. mutans* in 37.4% of obtained isolates, while *S. aureus*, *E. coli* and *k. pneumonia* was isolated with percentages of 3%, 3.5% and 4.5% respectively [22].

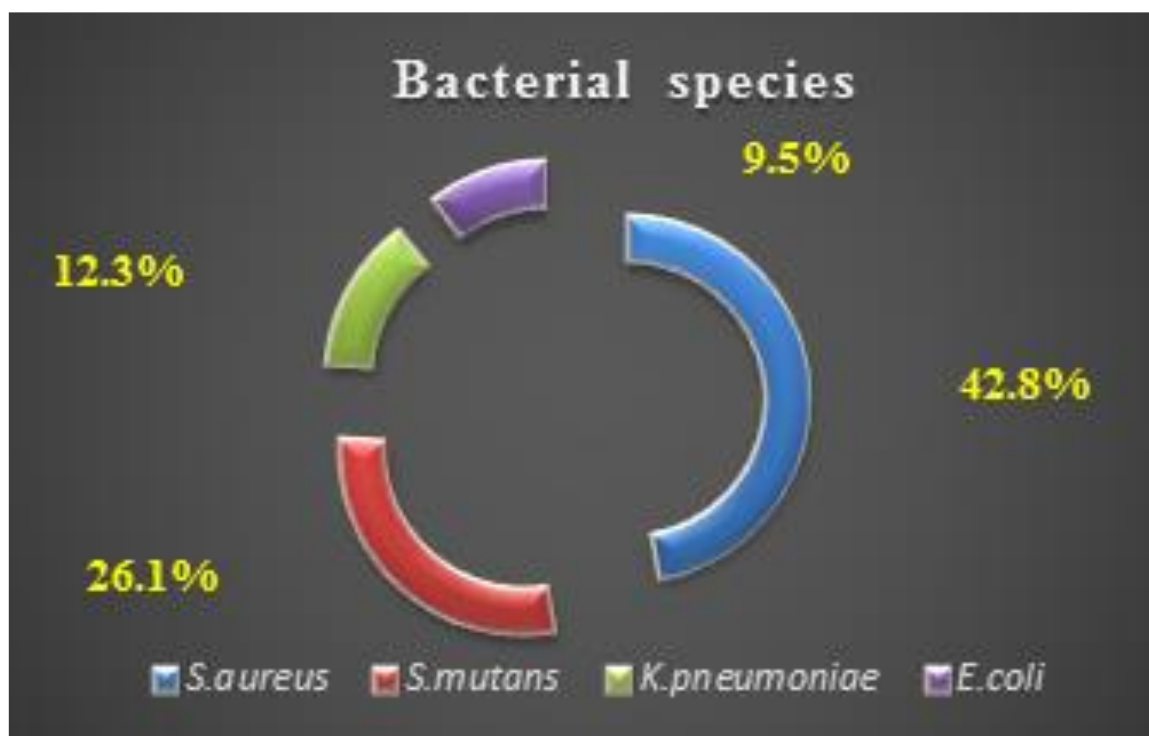


Figure 2. Percentages of Isolated Bacterial Species



Figure 3. Different bacterial types on different culture media. A and B: *E. coli* on MacConkey agar. C: *S. aureus* on Mannitol salt agar. D: *S. mutans* on Blood agar. E and F: *K. pneumoniae* on MacConkey agar.

3.3. Antibiotic Susceptibility Profile

An antimicrobial susceptibility test was performed for all obtained bacterial isolates against five antibiotics to examine the most efficient treatment against this bacterial species as shown in Figure (4). According to Table (1), the results showed that most Gram-positive and Gram-negative species showed resistance against Amoxicillin / Clavulanic, Cefotaxim and Erythromycin while showing less resistance toward Tigecycline antibiotic. *S. mutans* isolates showed high resistance against amoxicillin and Cefotaxim with a percentage of 90.9% for both. Current study results agreed with the local study found that most *S. mutans* isolates were resistant to Amoxicillin and Erythromycin, with a percentage of 90% and 70% respectively. The most effective antibiotic against *S. mutans* was Cefotaxim with a sensitive percentage of 88% [26]. The current study reported that *S. mutans* isolates showed 72.7%

sensitivity toward Cefotaxim; these results also agree with a recent study conducted in Ethiopia found that 83.6 % of dental caries bacteria were sensitive to Cefotaxim antibiotic [21]. Most Gram-positive and Gram-negative bacteria that cause dental plaque were susceptible to ceftriaxone and ciprofloxacin, *Escherichia coli* and *Klebsiella pneumoniae* showed 100% sensitivity to ceftriaxone antibiotics [22]. Generally, the fluctuation in results may be referred to the effect of samples by various factors including patients age, sample size, samples collection period and how much these antibiotics were used in the population

Table 1. Numbers and Percentages of Bacterial Species Resistance Against Different Antibiotics.

Bacterial species	Antibiotics									
	Amoxicillin Clavulanic amox		Cefotaxim CTX		Tigecycline TGC		Erythromycin ERY		Cefepime FEP	
	R	S	R	S	R	S	R	S	R	S
<i>S. aureus</i>	15 83.3	3 16.6	16 88.8	2 11.1	7 38.8	11 61.1	17 94.4	1 5.5	8 44.4	10 55.5
<i>S. mutans</i>	10 90.9	1 9.09	10 90.9	1 9.09	5 45.4	6 54.5	9 81.8	2 18.1	3 27.2	8 72.7
<i>K. pneumoniae</i>	7 77.9	2 22.2	8 88.8	1 11.1	1 11.1	8 88.1	8 88.8	1 11.1	4 44.4	5 55.5
<i>E. coli</i>	3 75	1 25	1 25	3 75	0 0	4 100	2 50	2 50	1 25	3 75

*R= Resistance, *S. aureus*= *Staphylococcus aureus*, *S. mutans*= *Streptococcus mutans*.

*S= Sensitive, *K. pneumoniae*= *Klebsiella pneumoniae*, *E. coli*= *Escherichia coli*.



Figure 4. Results of antibiotic susceptibility test shows the inhibition zones of used antibiotics including: FEP, ERY, amox, CTX and TGC against some bacterial isolates under study.

4. Conclusions

Dental diseases are a public health problem and the most prevalent diseases worldwide. *Streptococcus* spp. and *Staphylococcus* spp. are the main bacterial types isolated from patients with tooth caries at different ages due to exposure to many agents such as improper teeth brushing, daily smoking and diabetes disease. The incidence of caries generally rises in those who do not brush their teeth daily and regularly with fluoride toothpaste. Tooth brushing, smoking, the material used for brushing, and oral cleanliness status were linked with the prevalence of dental caries. It is essential to support health education on improving dental or oral health. Also, education on correct dietary lifestyles, daily teeth brushing with fluoride toothpaste and continuous use of dental floss might

encourage the control and prevention of dental caries. Additional studies are required to understand other factors that play a role in the occurrence of dental caries among different ages.

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