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Isolation and diagnosis of the bacteria causing gingivitis in humans and testing for their drug sensitivity

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Abstract: The study aimed to isolate and identify the bacteria causing gingivitis. 100 samples were collected from patients with gingivitis during the period from September 2024 to January 2025. Samples were taken from the gingival area using sterile cotton swabs, The samples were diagnosed phenotypically and microscopically, as well as biochemical testing.To confirm the diagnostic results,use the Vitek-2 Compact System The antibiotic susceptibility testing for bacterial isolates was conducted using disk diffusion method, According to CLSI M100 35rd2 The results showed that the highest isolation rate for bacteria causing gingivitis was for for *S. aureus* (28%), followed by *S. epidermidis* (19%), *S. haemolyticus* (14%), and *St. sanguinis* (10%). The lowest isolation rate was for *St. thoraltensis* (5%), All bacterial isolates showed high sensitivity to the antibiotic ofloxacin. Most isolates showed resistance to the antibiotics azithromycin, clarithromycin, and erythromycin.

Keywords: Gingivitis, bacteria, Periodontal diseases, Antibiotics

I. Introduction

Periodontal diseases are one of the most common oral diseases in the world, as they affect the tissues surrounding and supporting the teeth The most common form of periodontal disease is gingivitis[1]. The most common cause of oral diseases is the accumulation of dental biofilms, which colonize the gingival and subgingival regions of the mouth[2]. Gingivitis is an inflammation confined to the gum tissue only, affecting up to 90% of the world's population[3]. Gingivitis can be plaque-related or non-plaque-related, and the main indicators of gingivitis include redness, sensitivity, and bleeding after brushing[4]. While periodontitis affects the supporting ligaments of the teeth and the alveolar bone, periodontitis may develop over time with the accumulation of dental plaque, bacterial imbalance, formation of periodontal pockets, which may eventually lead to tooth loss[5] According to the World Health Organization, it is estimated that periodontitis affects about 19% of the adult population worldwide. [6], and the main bacteria associated with gingivitis and periodontitis are Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythus[7]. The severity of gum disease in individuals depends on several factors, including genetic factors, smoking and poor oral hygiene[8].

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II. Materals and Methods

2.1 Sample Collection

One hundred Samples were collected from patients with gingivitis who visited the Al-Suwailih Health Center and outpatient clinics in Basra Governorate during the period from September 2024 to January 2025 using sterile transport medium swab.

2.2 Sample isolation and identification

The samples were spread on blood agar medium ,Maconkey agar and Manitol salt agar (MSA), and incubate at 37°for 24 hours. After that, the isolates were then diagnosed microscopically by Gram staining to observe the color and shape of the colonies as well as by biochemical tests including Catalase, Coagulase, Urease[9]. The diagnosis was confirmed by the Vitek-2 compact system[10].

2.3 Antibiotic sensitivity testing

The susceptibility of bacterial isolates to eight antibiotics was evaluated using the disc diffusion method. According to CLSI M100 35rd2,0.1 ml of bacterial suspension was diffused onto Mueller-Hinton agar (MHA) using a disposable cotton swab. Antibiotic disks were then distributed onto the surface of the cultured MHA using sterile forceps, and incubate at 37°C for 24 hours. After incubation, the inhibition diameters were measured using a ruler and compared with the CLSI 2025 standard Inhibition zone diameters were interpreted in alignment with Clinical and Laboratory Standards Institute (CLSI).

2.4 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 26 (IBM SPSS Statistics 26), where all experimental data were analyzed through descriptive analysis which included the mean and standard deviation at a probability level of 0.01.

III. Results

The results showed that out of 100 samples, 76 (76%) showed positive culture growth on the culture media, while 24 (24%) show negative culture growth. The highest isolation rate was for S. aureus (28%), followed by S. epidermidis (19%), S. haemolyticus (14%), and St. sanguinis (10%). The lowest isolation rate was for St. thoraltensis (5%) as shown in table (1).

Table 1: Shows the numbers and percentages of bacterial isolates

Bacterial isolates	Number of isolates	Percentage
Staphylococcus aureas	28	28%
Staphylococcus epidermidis	19	19%
Staphylococcus haemolyticus	14	14%
Streptococcus sanguinis	10	10%
Streptococcus thoraltensis	5	5%

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Figure 1: Growth of a: S.haemolyticus, b:S.aureus, c:S.epidermidis on mannitol salt agar

Figure 2: shows growth of *St. thoraltensis* and *St. sanguinis* on Mitis salivarius Agar

The biochemical tests showed that all Staphylococcus isolates were positive for the Catalase test and negative for the Coagulase test, except for *S. aureas* isolates, which were positive for the plasma coagulation test. All isolates were positive for the Urease test, except for *S. haemolyticus*, which was negative for the Urease test. *St. thoraltensis* was negative for the Catalase, Urease, and Coagulase tests, while *St. sanguinis* was positive for catalase test and negative for urease and Coagulase tests, as shown in Table (2) and Figure.

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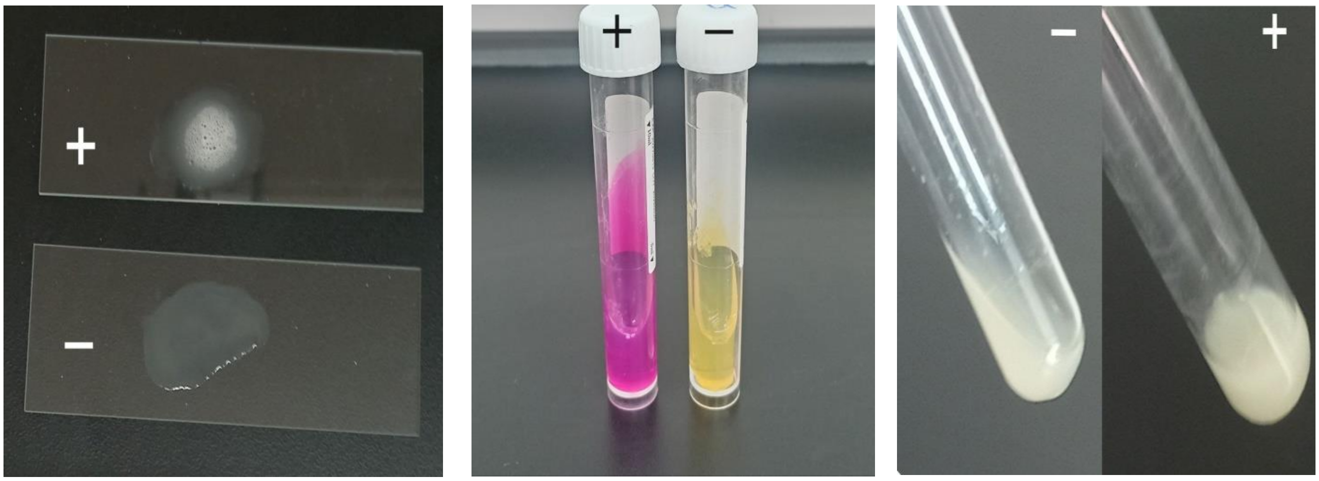

Figure 3. Biochemical tests of bacterial isolates

Table2: shows the Biochemical tests of the bacterial isolates

Bacterial isolates	Biochemical tests			
	Catalase	Urease	Coagulase	Hemolysis
Staphylococcus aureas	+	+	+	+
Staphylococcus haemolyticus	+	+	-	-
Streptococcus thoraltensis	-	-	-	+
Staphylococcus epidermidis	+	+	-	-
Streptococcus sanguinis	+	-	-	+

The results showed Gentamicin, Ofloxacin, and Chloramphenicol show the highest mean inhibitory effects (≥21 mm) with low variability, indicating broad and consistent activity. Erythromycin and Azithromycin show poor and inconsistent efficacy, suggesting possible resistance among isolates. Gentamicin, Ofloxacin, and Chloramphenicol differ significantly from Erythromycin and Azithromycin. no significant difference among Gentamicin, Ofloxacin, and Chloramphenicol.The results indicated that all bacterial isolates showed increased sensitivity to the antibiotic ofloxacin (OFX), *S. aureus* showed sensitivity to tetracycline, vancomycin, chloramphenicol, gentamicin, and ofloxacin, but resistance to erythromycin, azithromycin, and clarithromycin. *S. haemolyticus* showed sensitivity to tetracycline, vancomycin, chloramphenicol, gentamicin, ofloxacin, and erythromycin, but resistance to clarithromycin and azithromycin. *S. epidermidis* showed sensitivity to vancomycin, tetracycline, chloramphenicol,

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gentamicin, clarithromycin, azithromycin, and ofloxacin, while *St. thoraltensis* showed resistance to azithromycin, erythromycin, chloramphenicol, and tetracycline. *St. sanguinis* showed sensitivity to the antibiotics ofloxacin, vancomycin, tetracycline , and chloramphenicol, and exhibited total resistance to erythromycin, clarithromycin, and azithromycin as shown in Figure (4) and Table(3).

Table3: Inhibition diameters of antibiotics against bacterial isolates

Bacterial isolates	Inhibition diameters(mm)							
	Tetracycline30 mg	Erythromycin15 mg	Chloramphenicol30 mg	Ofloxacin 5mg	Vancomycin30 mg	Gentamicin 10mg	Clarithromycin15 mg	Azithromycin 15mg
S. aureas	24	9	25	21	19	21	11	9
S. haemolyticus	25	25	22	25	19	23	10	0
S. epidermidis	25	8	22	20	17	24	29	21
S. thoraltensis	0	13	10	27	20	-	18	8
S. sanguinis	26	0	26	21	18	-	0	0
Mean	20.0	11.0	21.0	22.8	18.6	23.2	13.6	7.6
Sd	10.45	9.87	6.16	2.86	1.34	1.30	10.59	8.85

(-) sign indicates the antibiotic has not been tested on bacteria, Sd=Standard deviation

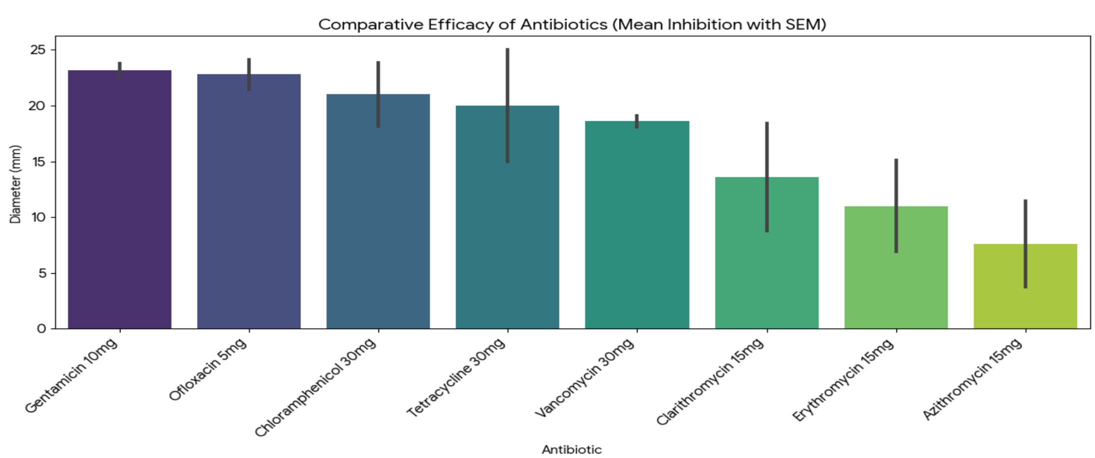

Figure 5. Shows Comparative efficacy of antibiotics

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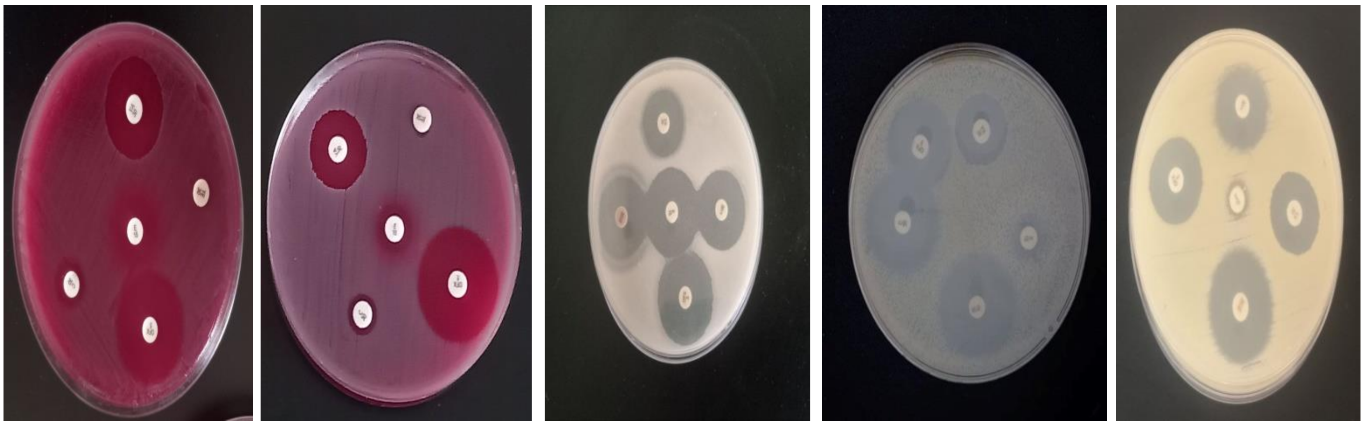

Figure 5. Antibiotic inhibition diameters of bacterial isolates

IV. Discussion

The results showed that S. aureas growing on blood agar formed yellow spherical colonies and had the ability to completely lyse the blood around the colonies, which were of the β-hemolytic type when grown on MSA medium, it exhibited large colonies with a golden yellow color due to its ability to ferment mannitol sugar, causing the medium to change color from pink to yellow due to the presence of red phenol. All S. aureas isolates were positive for the catalase test, which distinguishes them from the Streptococcus genus, due to their ability to convert hydrogen peroxide (H_2O_2) into oxygen and water via the catalase enzyme. All S. aureas isolates also showed positive coagulase test results for their ability to convert fibrinogen to fibrin via the coagulase enzyme[11]. S. epidermidis showed small to medium-sized white colonies that are non-hemolytic when cultured on blood agar, while on MSA, it appeared as pink colonies due to its inability to ferment mannitol. All isolates of S. epidermidis showed positive results in the catalase test and the urease test, and negative results in the coagulase test due to the absence of the coagulase enzyme, which is consistent with[12]. St. thoraltensis appeared as small, gray to white colonies that were partially hemolytic when grown on blood agar while showing small to medium-sized light blue colonies on Mitis salivarius agar medium. The catalase test was negative, consistent with[13]. St. sanguinis showed medium-sized circular colonies in black color on Mitis Salivarius Agar. The catalase test results were positive for the presence of the catalase enzyme, while the urease and coagulase tests were negative, which is consistent with a study by [14]

In the present study, the susceptibility of bacterial isolates to eight antibiotics was evaluated using the disc diffusion method. Statistical analysis demonstrated a significant effect of antibiotic type on inhibition zone diameter ($p = 0.016$), whereas differences among bacterial isolates were not statistically significant ($p = 0.600$). The results showed that Staphylococcus was the most frequently isolated bacterial species in cases of gingivitis, contradicting the findings[15], who concluded that Streptococcus was the most frequently isolated bacterial species causing gingivitis. Streptococcus plays a significant role in some oral infections and dental caries. This finding aligns with a

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study by[16], Those who observed the dominance of S.aureus due to its ability to form strong biofilms and produce β-lactamase. These virulence factors provide a competitive advantage within the oral environment, facilitating infectio, followed by S. epidermidis bacteria, which was isolated at a rate of (19%), contradicting a study conducted by [17] which found that the isolation rate of S. epidermidis bacteria was (9.09%). The current study contradicted the findings of [18], which indicated that the isolation rate of S. haemolyticus bacteria from patients with gingivitis was (8%).Our study results do not agree with the results of [19], where it was reported that the isolation rate of the bacteria st.sanguinis reached (44%). The results of [20] showed that st.sanguinis is strongly associated with gingivitis, and the results of their study showed that St.thoraltensis is one of the most common oral pathogens in patients with gingivitis, as it was isolated at a rate of (1.1%).

The results showed that the antibiotic ofloxacin, which belongs to the fluoroquinolones class, exhibited high inhibitory activity against all bacterial isolates. This is because fluoroquinolones inhibit the DNA gyrase enzyme, thus preventing DNA replication[21]. *S. aureus* showed high sensitivity to the antibiotic Chloramphenicol, followed Tetracycline, and resistance to the antibiotics Erythromycin and Azithromycin. This is because the bacteria are able to resist both antibiotics through two mechanisms. The first is through the secretion of the enzyme Esterase, which works to break down the lactone ring or works to change the structure of the antibiotic by transferring the active group of the antibiotic, such as ribosyl, acyl, and phosphoryl groups. The second mechanism depends on modifying the target of the antibiotic through the process of methylation on the 23S rRNA[22]. This contradicts the findings of[23], whose study showed that *S. aureus* isolated from gingivitis exhibited high sensitivity to erythromycin and resistance to tetracycline. It is consistent with the findings of[24], whose study showed that *S. aureus* isolated from patients with gingivitis showed sensitivity to tetracycline, chloramphenicol, and vancomycin. Chloramphenicol demonstrated inhibitory activity against Staphylococcus ssp by preventing the formation of new peptide bonds during protein synthesis through binding to the 50S ribosomal subunit, thereby inhibiting translation in the bacterial cell[25]. *S. haemolyticus* showed sensitivity to tetracycline, vancomycin, chloramphenicol, gentamicin, ofloxacin, and erythromycin, and resistance to clarithromycin and azithromycin. *S. haemolyticus* also showed sensitivity to vancomycin, a glycopeptide that inhibits bacterial cell wall synthesis by binding to the D-Ala-D-Ala end of the peptide chain[26]. *S. epidermidis* exhibited resistance to erythromycin, consistent with the findings of a previous study[27] *S. epidermidis* isolated from patients with gingivitis showed resistance to the antibiotic erythromycin.

Conclusion

S. aureus is the most isolated bacteria from patients with gingivitis. Gentamicin and ofloxacin exhibited the highest and most consistent activity, while macrolides showed widespread resistance.

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