DOI: 10.36108/ujees/5202.70.0113
UNIOSUN Journal of Engineering and Environmental Sciences. Vol. 7 No. 1. March. 2025

Antibiotic Resistance Profile of Biofilm Forming *Streptococcus mutans* Associated with Dental Caries in Osogbo, Osun State, Nigeria

Efunwole, O. O., Olaitan, J. O., Wahab, A. A., Oluwajide, O. O., Oyedara, O. O.

Abstract *Streptococcus mutans* (*S. mutans*) has been implicated as the primary microbial pathogen associated with dental caries. The financial and economic burden, together with loss of productivity experienced due to dental caries, are of significant concern. This study aims to isolate *S. mutans* from decayed teeth and dental plaque samples obtained from government hospitals and their surrounding areas. *S. mutans* was isolated and presumptively identified with morphological, biochemical, and polymerase chain reaction (PCR) amplification of species-specific primers for *S. mutans*. The isolates were tested for their ability to produce biofilm and the presence of biofilm-forming genes (glucosyltransferases (*gtfB*) and (*gtfC*)). The susceptibility of the isolates to antibiotics of choice was carried out with Kirby Bauher disc diffusion methods. A total of ninety-six (96) *S. mutans* isolates were isolated and presumptively identified with morphology and biochemical tests. Sixty-three of the isolates (66%) were identified as *S. mutans* with specie-specific gene with a band at 1272 bp. Biofilm assays indicated that 53 of the isolates (84%) were biofilm formers, while 43 (68%) were strong biofilm formers. The biofilm genes 47 (75%) had the *gtfB* gene and43 (68%) possessed the *gtfC* gene were detected in the isolates. All the isolates were resistant to penicillin G and clindamycin and some were susceptible to piperacillin, imipenem, and ciprofloxacin. In addition, some of the isolates were resistant to Ampicillin while some were susceptible to Ampicillin. These findings suggest that imipenem, piperacillin, and ciprofloxacin could serve as best control methods for dental caries infection.

Keywords: *Streptococcus mutans*, biofilm, antibiotic sensitivity test, dental caries, PCR

I. Introduction

Enamel is the prominent and the most mineralized part of the tooth and is composed of 96% mineral and 4% of water and protein [1, 2]. The enamel is composed of calcium phosphate minerals, and the structural parts include the pulp, dentine, enamel and cementum. Early oral inhabitants of the mouth include *Streptococcuss obrinus, S. gordonii, S. mitis, S. oralis* and *S. mutans*. They attach to the tooth enamel and become opportunistic pathogens when the environment favours them [3]. Frequent consumption of fermentable carbohydrates and unhygienic oral conditions favour these microorganisms and create a protective biofilm that is shielded from saliva buffering [4]. The oral cavity is dental caries-free when the sugar intake is low or not regular. Also, the host saliva stabilizes the ecosystem by buffering the oral environment, supplying nutrition to the environment and producing antagonistic antimicrobial agents like mutation. *S. mutans* is considered the main etiology agent of dental caries due to its acidogenic and aciduric properties. The acidic bacterial products dissolve

Efunwole, O. O., Olaitan, J. O., Wahab, A. A.,
*(Department of Microbiology, Osun State University, Osogbo, **230284** Nigeria)*
Oluwajide, O. O.
(African Center of Excellence for Microbial Secondary Metabolites, Osun State University, Osogbo, 230284 Nigeria)
Oyedara, O. O.
*(Department of Biotechnology, Osun State University, Osogbo, **230284** Nigeria)*
Corresponding Author: abideen.wahab@uniosun.edu.ng
Phone: +2348034588086

the calcium phosphate mineral to demineralize the tooth and destroy the enamel, causing tooth cavities, fuzzy feeling on the teeth, chronic bad breath (halitosis) and red, swollen tender gums that bleed after brushing [5]. The combination of bacteria, acids and carbohydrates mix into a sticky, colourless film called biofilm.

[6], described dental caries as a "localized dissolution of tooth enamel, caused acidic fermented carbohydrates by-products caused by commensal bacteria present in the biofilm which covers the tooth surface". At a non-cariogenic condition, re-mineralization occurs when the pH is close to neutrality, oral commensals, which are peroxigenic, produce hydrogen peroxide (H_2O_2) and other antimicrobial products to prevent the overgrowth of *S. mutans* on dental biofilms. Bacteria adhere to tooth surfaces in order to colonize and accumulate with the assistance of glucosyltransferase (Gtf) and glucan binding protein (Gbp) [7]. The Gtf enzyme catalyzes the synthesis of water-soluble glucan (glucan of (1-6) linked glucose groups) and water-insoluble glucan (glucan of (1-3) bonded glucose groups) from sucrose breakdown, and Gbp is a glucan-binding receptor protein (Joel & Ramteke, 2022). GtfB and GtfC are highly homologous, sharing ~75% of amino acid sequences, and GtfD possesses 50% sequence identity. The enzymes gtfB (4.4 kb) and gtfC (4.3 kb)are in an operon arrangement separated by 198 bp [8]. In the absence of surface protein antigen, the least harm is caused to the enamel.

Conventional studies of *S. mutans* are based on cultural methods to identify and characterize *S. mutans* in the oral cavity, but the challenges of cultural methods are inconsistent morphology based on the common culture medium used, the cost of the selective medium combined with high labour-intensive. A deep insight into the characteristics of *S. mutans* and the relevant antibiotic is pertinent to identifying the pathogen accurately [9]. A primer for identifying and targeting the specific genes of *S. mutans* and the biofilm-forming genes such as glucosyltransferases and glucan-binding protein can be employed for accuracy [8].

Therefore, the objectives of this study are to identify *S. mutans* with morphological, biochemical, and PCR amplification of species-specific genes and determine the susceptibility of the isolates to antibiotics.

II. Materials and Methods

A. Study Location and Collection of Samples

Samples were collected from Uniosun Teaching Hospital and its environs with protocol number (UTH/REC/2023/04/756). The total number of samples collected using size expression for prevalence study n= z2pq/d2, where q =1-p was 254 with an attrition figure of 10% following the work of [10]. One hundred and nineteen (119) samples of decayed teeth and one hundred and thirty-five (135) plaque samples were collected by the Doctors inside sterile bottles containing about 3mLs of sterile prepared normal saline. Decayed teeth samples from Inukan Specialist Hospital were labelled as IT (1-17), plaque samples labelled IP (1-24), decayed teeth from Uniosun Teaching Hospital were labelled UT (18-64) and plaque samples labelled (UP 25-71). Decayed teeth samples from Emmanuel Medical Center labelled ET (65-87) and plaque samples labelled EP (72-92). Osun State Hospital (OT 88-119), OP (93-135), respectively. Samples were transported to the laboratory immediately.

B. Isolation of *Streptococcus mutans*

The samples were homogenized and streaked on the surface of Mitis Salivarius Agar (MSA) plates

(Hi media) supplemented with 1% Potassium tellurite and modified with 0.5 IU/mL bacitracin (Sigma, USA), using a sterile inoculating loop and incubated for 48 hours at 37°C [11].

C. Morphological Identification of the Isolates

The plates showing distinct characteristics of *S. mutans* were subcultured, Gram-stained and observed under the microscope. Sucrose fermentation, catalase, oxidase and coagulase tests were also carried out on the isolates to determine their biochemical characteristics and later stored on Nutrient agar slants at 4°C inside the refrigerator [12].

D. Molecular Identification of Isolates

i. Genomic DNA extraction

From 24hr old culture, an inoculum was picked and suspended inside sterile plain bottles containing sterile brain hearth infusion broth and incubated for 48 hours. Two millilitres of each sample were transferred to 2mL Eppendorf tubes and labelled. Zymo research bacteria DNA extraction kit was used to extract the bacteria DNA following the manufacturer's instructions.

ii. PCR amplification of species-specific gene

Specially designed primers for *S. mutans* strain (forward primer 5'-TAT GCT ATT GGA GGT TC-3' and reverse primer 5'-AAG GTT GAG CAATTG AAT CG-3' were used for the identification. Genes amplification was done in a PCR condition of the first cycle of denaturation at 95 °C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, then annealing at 50 °C for 30 seconds, followed by extension at 72 °C for 40 seconds, and finally a terminal extension for 10 min. The amplicon generated from the PCR reaction was run in 1.5% agarose gel containing ethidium bromide and checked for the appropriate bands (1272 bp) under a UV transilluminator and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 1kb plus DNA ladder bp ladder [13].

E. Detection of Biofilm-producing Genes

The genomic DNA of each isolate was extracted with Zymo research bacteria DNA extraction kit. Biofilm production genes of *S. mutans* (Glucosyltransferases (*gtfS*) enzyme and glucan binding protein (GBP) were amplified with PCR amplification primer set of forward primer 5'AGCAATGCAGCCAATCTACAAAT3' and reverse primer 3'ACGAACTTTGCCGTTATTGTCA5' for *gtfB* and forward primer 5'ATGGCGGTTATGGACACGTT3' and reverse primer 3'TTTGGCCACCTTGAACACCT5' for *gtfC* in the PCR condition previously described**.** The amplicons were run in 1.5% agarose gel containing ethidium bromide and checked for the appropriate bands (1272 bp) under a UV transilluminator and photographed [14].

F. Quantification of Biofilm in Isolates

Each isolate was inoculated in about 3mL of Brain heart infusion broth supplemented with 2% sucrose and incubated at 37°C for 48hrs, then adjusted to 0.5mcfarland using sterile normal saline. Then 2µL was picked from the dilution and again diluted in another 2µL of BHI broth containing 2% sucrose to make 100 fold dilution. The diluted culture of each isolate was mixed and distributed into wells of 96 microtiter plates at 200µL per well in triplicates and incubated at 37oC for 48 hrs. The culture was thereafter discarded by inverting the plate, and each well was washed 3 times with sterile saline

(0.9%). 200µL of methanol was added to each well and left for 20 minutes to fix the biofilm and then discarded, and the plate was left to dry. 200 µL of 0.1% crystal violet was added to each well and left for 15 minutes. After staining, the wells were washed three times with distilled water and left to dry. 200 µL of 33% glacial acetic acid was added later to each well to resolubilize or dissolve the stained biofilm, making it measurable. The optical density (OD) at 600 nm was measured using a microplate reader. The average OD of each test isolate was calculated, and the mean OD of the control wells (no bacteria) was subtracted to get the final biofilm measurement [15].

G. Antibiotic Susceptibility of Biofilm-producing *S. mutans*

This test was done according to the methods of [16]. McFarland standard of 24 hrs old culture of *S. mutans* was prepared and was swabbed on Mueller Hilton agar plates. Twelve different routinely used antibiotic discs, for the treatment of dental caries were placed on the surface of the Mueller Hilton agar medium using sterile forceps. The antibiotics include ciprofloxacin (5µg), Amoxicillin (20µg) clavulanic acid (10µg), Cefalexin (30µg), Piperacillin (30µg), Imipenem (10µg), Chloramphenicol (30µg), Penicillin G (1unit), Cefotaxime (30µg), Ampicillin (10µg) and Clindamycin (2µg), Erythromycin (15µg) and Kanamycin (30µg) (MASTDISCS AST product). The plates were incubated for 24 hours at 37°C for 24 hours, a vernier caliper was used to measure the diameter of the zone of inhibition for the antibiotic. Each isolate was interpreted as susceptible, intermediate and resistant to the antibiotic following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2023) and Clinical and Laboratory Standards Institute (CLSI) recommendations of 2024.

III. Results and Discussion

A. Results

i. Isolation and identification of *S. mutans*

A total of 96 *S. mutans* were isolated from the samples. From the teeth samples, 78 were isolated, while 22 were from plaque samples, with the highest occurrence from Uniosun Teaching Hospital Figure 1.

ii. Morphological and biochemical identification of isolates

The isolates showed the presence of hard-raised, convex, opaque, rough pale blue colonies and a glossy appearance on the culture medium. No observable growths in some plates. The isolates fermented sucrose by a colour change from red to yellow, catalase and coagulase-negative. Microscopically, they are Gram-positive cocci in chains Figure 2.

iii. Molecular identification of isolates

The results of the PCR amplification of the species-specific genes showed that Sixty-three (63) isolates were positive with a band at 1272 bp, and 43 isolates were negative with no amplification of the gene Figure 3.

iv. Detection of biofilm genes

The PCR amplification showed a total of 47 *S. mutans* were positive for the biofilm-producing genes glucosyltransferases (*gtfB*) at a base pair of 517 bp and 43 were positive for *gtfC* genes at a 578 bp, respectively Figure 4.

v. Quantification of biofilm in *Streptococcus mutans*

When the biofilm produced in 96-well microtiter plates was quantified with a microplate reader, a

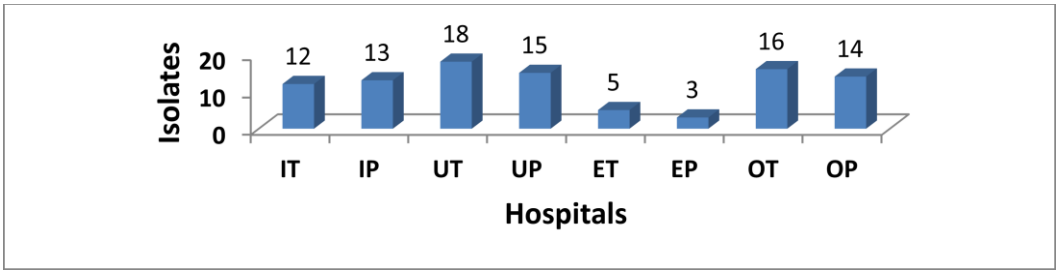

Figure 1: Frequency of Occurrence of *S. mutans* in the Different Hospitals Sampled
Key: IT: Inukan decayed teeth sample, IP: Inukan plaque sample, Uniosun Teaching Hospital decayed teeth sample, UP: Uniosun plaque sample, ET: Emmanuel decayed teeth sample, EP: Emmanuel plaque sample, Osun State Hospital

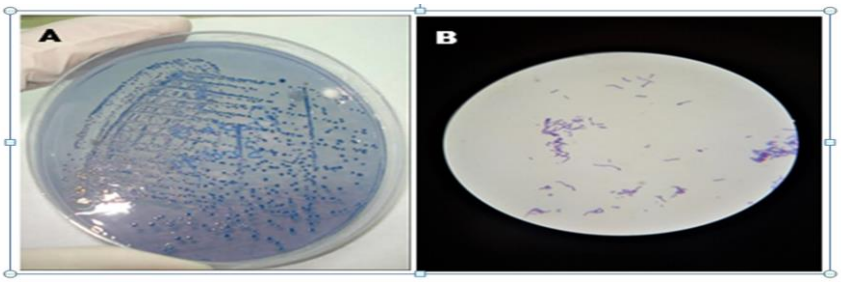

Figure 2. (A) Colony appearance and (B) microscopic view of *S. mutans*

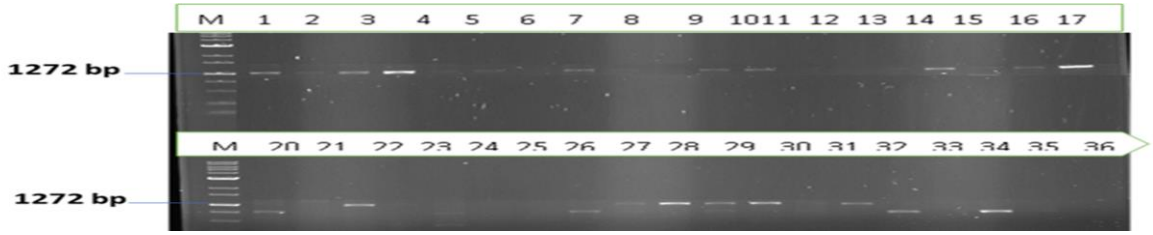

Figure 3: Gel electrophoresis image of PCR product of species-specific gene for *S. mutans*
Legend: Lane M: Ladder; Lane 1-8: Decayed teeth from inukan Specialist Hospital, Lane9-36: Decayed teeth from Uniosun Teaching Hospital, Lane37-49: Decayed teeth from Emmanuel Medical center, Lane 50-: 74Decayed teeth from Osun State Hospital, Lane 75-96: plaque samples

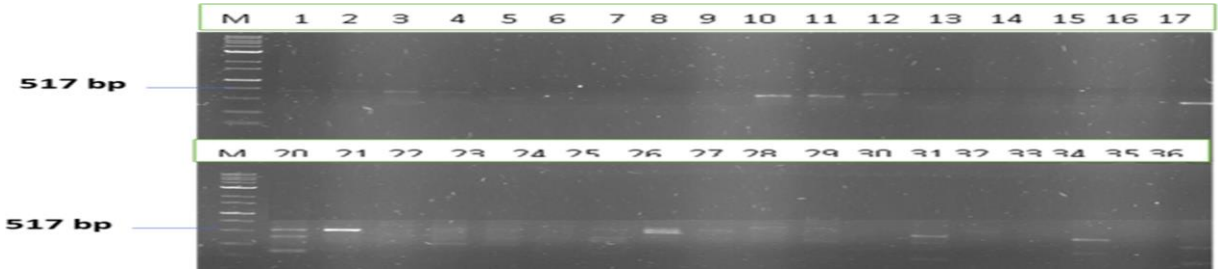

Figure 4: Gel electrophoresis image PCR product from *S. mutans* with *gtfB* gene
Legend: Lane M: molecular weight marker; Lane 1-8: Decayed teeth from inukan Specialist Hospital, Lane 9-36: Decayed teeth from Uniosun Teaching Hospital, Lane 37-49: Decayed teeth from Emmanuel Medical center, Lane 50- : 74Decayed teeth from Osun State Hospital, Lane 75-96: plaque samples

total of 43 (81.5%) isolates showed to be a strong biofilm former Table 1.

Table 1: Quantification of Biofilm Produced by *S. mutans*

S/N	Isolate No	A	B	C	MEAN	OD = X-ODc
1	PC	1.371	1.366	1.375	1.3707±0.0045	1.369
2	NC	0.112	0.115	0.11	0.1123±0.0025	0.1106
3	12	0.463	0.457	0.436	0.452±0.0142	0.4503
4	2	0.477	0.465	0.443	0.4617±0.0172	0.46
5	4	0.479	0.375	0.451	0.435±0.0538	0.4333
6	11	0.574	0.479	0.563	0.5387±0.052	0.537
7	1	0.445	0.454	0.432	0.4437±0.0111	0.442
8	30	0.3	0.323	0.309	0.3107±0.0116	0.309
9	48	1.365	1.327	1.371	1.3543±0.0239	1.3526
10	38	0.325	0.379	0.351	0.3517±0.027	0.35
11	24	0.237	0.211	0.217	0.2217±0.0136	0.22
12	25	0.492	0.487	0.498	0.4923±0.0055	0.4906
13	35	0.505	0.492	0.521	0.506±0.0145	0.5043
14	29	0.623	0.579	0.548	0.5833±0.0377	0.5816
15	13	0.335	0.316	0.416	0.3557±0.0531	0.354
16	34	0.148	0.141	0.199	0.1627±0.0317	0.161
17	26	0.193	0.186	0.174	0.1843±0.0096	0.1826
18	37	0.095	0.241	0.217	0.1843±0.0783	0.1826
19	39	0.116	0.15	0.118	0.128±0.0191	0.1263
20	40	0.068	0.16	0.199	0.1423±0.0673	0.1406
21	44	0.225	0.277	0.213	0.2383±0.034	0.2366
22	47	0.086	0.095	0.078	0.0863±0.0085	0.0846
23	22	0.188	0.186	0.154	0.176±0.0191	0.1743
24	50	0.228	0.218	0.186	0.2107±0.0219	0.209
25	55	0.25	0.242	0.231	0.241±0.0095	0.2393
26	60	0.073	0.098	0.098	0.0897±0.0144	0.088
27	59	0.115	0.139	0.105	0.1197±0.0175	0.118
28	73	0.907	0.123	0.131	0.387±0.4504	0.3853
29	59	0.207	0.252	0.291	0.25±0.042	0.2483
30	92	0.133	0.14	0.146	0.1397±0.0065	0.138
31	71	0.322	0.311	0.318	0.317±0.0056	0.3153
32	62	0.505	0.478	0.426	0.4697±0.0402	0.468
33	85	0.06	0.088	0.102	0.0833±0.0214	0.0816
34	66	0.232	0.232	0.246	0.2367±0.0081	0.235
35	61	0.41	0.492	0.361	0.421±0.0662	0.4193
36	72	0.232	0.279	0.23	0.247±0.0277	0.2453
37	56	0.291	0.311	0.331	0.311±0.02	0.3093
38	75	0.257	0.309	0.313	0.293±0.0312	0.2913
39	91	0.129	0.15	0.171	0.15±0.021	0.1483
40	80	0.287	0.274	0.313	0.2913±0.0199	0.2896
41	65	0.195	0.18	0.203	0.1927±0.0117	0.191
42	89	0.155	0.154	0.191	0.1667±0.0211	0.165
43	90	0.289	0.318	0.297	0.3013±0.015	0.2996
44	95	0.311	0.429	0.333	0.3577±0.0627	0.356

vi. Antibiotic sensitivity patterns of isolated *Streptococcus mutans*

All the isolates were resistant to penicillin G and clindamycin. The isolates were strongly susceptible to piperacillin, imipenem, and ciprofloxacin. In addition, some of the isolates were resistant to Ampicillin while some were susceptible to Ampicillin. The Multiple Antibiotic Resistance (MAR) index for all the isolates was more than 0.2. MAR is calculated as the ratio of the number of classes of antibiotics to which a particular bacterial strain is resistant divided by the total number of antibiotics used Table 2.

B. Discussion

Dental caries is primarily caused by *S. mutans,* a bacterium that thrives in biofilms and produces acids that demineralize tooth enamel. *S. mutans* outcompetes other bacteria due to its acidogenic and aciduric properties [17]. Confirmative screening using species-specific PCR demonstrated that only 66% of the initially selected colonies were confirmed as *S. mutans*. This finding is in line with the work of [18], who observed a consistent band at 1272 bp for *S. mutans*. Among the confirmed isolates, 53 (84%), produced different categories of biofilm, strong, weak and moderate, supporting the report that *S. gordonii,* a commensal organism, can reduce the virulence properties associated with biofilm formation in *S. mutans* and inhibit sucrose-dependent biofilm formation and may also compete with *S. mutans* for colonization sites on tooth and demonstrate biochemical antagonism, which could reduce *S. mutans* numbers, hence reduction in biofilm formation [19]. The detection of biofilm-related genes, specifically *gtfB* and *gtfC*, in 47 and 43 isolates, respectively, suggests diversity in *S. mutans* strains. This indicates that not all strains possess protein antigens capable of causing harm to the host [20]. Meanwhile, the role of *S. mutans* as an etiological agent of dental caries is well established; its cariogenic potential is influenced by complex interactions within dental plaque biofilms rather than solely by the virulence properties of a single organism [21]. Incidentally, 47 confirmed samples possess biofilm genes and exhibited resistance to penicillin G and Clindamycin. This finding contrasts with the work of [22], who recommended amoxicillin and penicillin G as the most effective antibacterial agents for treating dental caries. The observed resistance may be attributed to the long-term use of penicillin, which can lead to the emergence of resistant strains [23], particularly among hospital patients. This is further supported by the Multiple Antibiotic Resistance (MAR) index for each isolate, which was found to be greater than 0.2. This may suggest that the patients have been exposed to the usage of antibiotics, and also biofilm production makes microorganisms resilient and resistant to antibiotics. Though few isolates were susceptible to amoxicillin-clavulanic acid, chloramphenicol, cefotaxime, and Ampicillin, Imipenem, piperacillin, and ciprofloxacin were most effective with different zones of inhibition, which were interpreted to be susceptible as recorded by EUCAST 2023 and CLSI 2023.

Therefore, proper identification of *S. mutans* is necessary for accurate control of dental caries infection.

Table 2: Susceptibility of isolates to antibiotics of choice according to EUCAST and CSLI breakpoint

Isolate code	IMI (10µg)		PEN G (1 unit)		CL (30µg)		CIP (5µg)		PRL (30µg)		CLI (2µg)		CEF (30µg)		AUG (30µg)		AMP (10µg)		CFX (30µg)		E (15µg)		K (30µg)		MARI
	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I	V	I	
12	19	S	0	R	11	R	28	S	29	S	32	S	25	S	33	S	23	S	19	S	32	S	10	R	0.25
2	6	R	0	R	0	R	23	S	30	S	11	R	16	S	10	R	6	R	6	R	11	R	24	S	0.66
4	11	R	0	R	0	R	31	S	15	S	21	S	32	S	37	S	26	S	11	R	21	S	13	R	0.33
11	15	R	21	S	21	S	34	S	22	S	20	R	19	S	31	S	21	S	15	S	20	R	6	R	0.33
1	6	R	6	R	6	R	29	S	20	S	20	R	6	R	10	R	6	R	6	R	20	R	6	R	0.83
30	6	R	0	R	0	R	24	S	18	S	6	R	6	R	20	S	6	R	26	S	6	R	18	S	0.58
48	10	R	0	R	0	R	23	S	21	S	6	R	6	R	11	R	20	S	21	S	6	R	11	R	0.67
38	6	R	0	R	0	R	24	S	29	S	6	R	6	R	6	R	6	R	6	R	6	R	8	R	0.83
24	15	R	0	R	0	R	26	S	31	S	15	R	23	S	13	R	22	S	6	R	15	R	10	R	0.83
25	6	R	0	R	11	R	33	S	29	S	17	R	15	S	30	S	6	R	13	S	17	R	16	S	0.5
36	6	R	0	R	0	R	33	S	24	S	6	R	6	R	20	S	22	S	18	S	32	S	11	R	0.5
25	10	R	0	S	0	R	29	S	29	S	6	R	6	R	11	R	22	S	6	R	30	S	30	S	0.5
35	6	R	0	R	0	R	27	S	6	R	6	R	6	R	6	R	6	R	12	R	6	R	14	R	0.92
29	15	R	0	S	12	S	28	S	13	R	15	R	23	S	13	R	6	R	16	S	20	R	6	R	0.5
13	6	R	0	R	0	S	26	S	6	R	17	R	15	S	30	S	32	S	18	S	16	R	6	R	0.5
34	24	S	0	R	0	R	20	S	26	S	6	R	6	R	20	S	6	R	11	R	20	R	6	R	0.67
26	30	S	0	R	0	R	24	S	29	S	6	R	6	R	11	R	19	S	18	S	29	S	18	S	0.42
27	29	S	0	R	0	R	31	S	27	S	6	R	6	R	6	R	20	S	16	R	26	S	11	R	0.58
38	31	S	0	R	12	S	26	S	29	S	15	R	23	S	13	R	6	R	18	S	6	R	6	R	0.5
40	30	S	0	R	0	S	23	S	30	S	17	R	15	S	30	S	6	R	17	S	6	R	6	R	0.42
44	35	S	0	R	0	R	29	S	20	S	6	R	6	R	20	S	22	S	19	S	6	R	6	R	0.5
47	30	S	0	R	0	R	29	S	27	S	6	R	6	R	11	R	22	S	6	R	20	S	20	S	0.5

22	33	S	0	R	60	R	60	S	26	S	6	R	6	R	6	R	6	R	6	R	6	R	6	R	0.75
50	35	S	0	R	12	S	29	S	30	S	15	R	23	S	13	R	6	R	8	R	22	S	22	S	0.42
55	29	S	0	R	8	S	20	S	27	S	17	R	15	S	30	S	32	S	6	R	6	R	6	R	0.42
60	20	S	0	R	9	R	19	R	26	S	6	R	6	R	20	S	6	R	6	R	6	R	25	S	0.67
59	31	S	0	R	6	R	33	R	29	S	6	R	6	R	11	R	20	S	6	R	7	R	16	S	0.67
73	33	S	0	R	6	R	18	R	23	S	6	R	6	R	6	R	6	R	6	R	6	R	32	S	0.75
92	33	S	0	R	0	R	19	R	24	S	15	R	23	S	13	R	22	S	6	R	23	S	19	S	0.5
15	30	S	0	R	0	R	18	R	27	S	17	R	15	S	30	S	6	R	6	R	15	S	6	R	0.58
71	33	S	0	R	0	R	33	S	22	S	6	R	6	R	20	S	6	R	6	R	6	R	6	R	0.66
62	27	S	0	R	0	R	31	S	27	S	6	R	6	R	11	R	10	R	16	S	7	R	6	R	0.58
85	37	S	0	R	0	R	29	S	24	S	6	R	6	R	6	R	6	R	6	R	6	R	6	R	0.75
66	31	S	0	R	0	R	26	S	26	S	15	R	23	S	13	R	15	R	17	S	8	R	23	S	0.5
61	25	S	0	R	0	R	29	S	27	S	17	R	15	S	30	S	6	R	18	S	6	R	15	S	0.42
72	20	S	0	R	0	R	21	S	26	S	6	R	6	R	20	S	22	S	18	S	22	S	20	S	0.33
56	33	S	0	R	0	R	22	S	27	S	6	R	6	R	11	R	6	R	6	R	6	R	24	S	0.66
75	29	S	0	R	0	R	29	S	23	S	6	R	6	R	6	R	6	R	6	R	6	R	6	R	0.66
91	23	S	0	R	14	S	31	S	26	S	15	R	23	S	13	R	20	S	6	R	20	S	6	R	0.42
80	30	S	0	R	0	R	25	S	27	S	17	R	15	S	30	S	6	R	6	R	6	R	20	S	0.5
65	33	S	0	R	0	R	34	S	26	S	6	R	6	R	20	S	6	R	6	R	23	S	6	R	0.58
89	30	S	0	R	0	R	31	S	25	S	6	R	6	R	11	R	22	S	6	R	27	S	18	S	0.5
90	37	S	0	R	0	R	25	S	25	S	6	R	6	R	6	R	20	S	6	R	6	R	11	R	0.66
95	31	S	0	R	0	R	11	R	13	S	15	R	23	S	13	R	6	R	6	R	25	S	6	R	0.66

Key: **CIP: Ciprofloxacin(5µg), AUG: Amoxicillin (20 µg clavulanic acid (10 µg), CEF: Cefalexin (30µg), PRL: Piperacillin (30µg), IMI: Imipenem (10µg), Chloramphenicol (30µg), PEN G: Penicillin G(1unit), Cefotaxime (30µg), Ampicillin(10µg) and CLI Clindamycin (2µg), E: Erythromycin (15µg) and K: Kanamycin (30µg).**
V= Value; I= Interpretation; R= Resistance; S= Susceptibility. The diameter measured in millimeters (mm)

IV. Conclusion

In conclusion, the results of this study revealed the characteristics of *S. mutans* that cause dental caries. The conventional procedures for the detection of cariogenic species usually take a long time and are cumbersome. Phenotypic, biofilm-forming ability and modern molecular characterization are reliable and help in determining the bacteria diversity and uniqueness, among others. With the aforementioned techniques, other tests like antibiotic susceptibility can be reliably determined and directed to the right therapy. This technique is a very important research tool in this modern technology.

References

[1] Gil-Bona, A., & Bidlack, F. B. "Tooth enamel and its dynamic protein matrix". *International Journal of Molecular Sciences*, Volume 21, Number 12, 2020, p. 4458.

[2] Liu, H., Li, B., Gao, M., Li, Y., Yang, X., Li, F., Cai, Z., Liu, Y., Liu, S., Zhao, H & Guo, L., "Restoration of human tooth enamel". *BMEMat*, 2025, p.e12139.

[3] Bertolini, M., Costa, R. C., Barão, V. A. R., Villar, C. C., Retamal-Valdes, B., Feres, M., & Silva Souza, J. G. "Oral microorganisms and biofilms: new insights to defeat the main etiologic factor of oral diseases". *Microorganisms*, Volume 10, Number 12, 2022, p. 2413.

[4] Karasu, G. K., & Huntington, P. "*Feeding Horses: An Evidence-based Approach to Equine Nutrition*". CRC Press, 2025.

[5] Rosengard, H. C. "Oral Manifestations of Systemic Diseases, 2022.

[6] Soni, T., Sidana, R., & Sidana, A. "Recent Advancements in the Management of Dental Caries". *IOSR Journal of Dental and Medical Sciences*, Volume 22, Number 10, 2023, pp. 12–17.

[7] Rezaei, T., Mehramouz, B., Gholizadeh, P., Yousefi, L., Ganbarov, K., Ghotaslou, R., Taghizadeh, S. & Kafil, H.S., "Factors associated with *Streptococcus mutans* pathogenicity in the oral cavity". *Biointerface Res Appl Chem*, Volume 13 Number 4, 2023, p. 368.

[8] Zhang, Q., Ma, Q., Wang, Y., Wu, H., & Zou, J. "Molecular mechanisms of inhibiting glucosyltransferases for biofilm formation in *Streptococcus mutans*". *International Journal of Oral Science*, Volume 13, Number 1, 2021, p. 30.

[9] Zhou, X., Li., Y & Wang, R. "Advances in the study of *Streptococcus mutans*: Pathogenicity and Antibiotic Resistance". *Frontiers in Microbiology*, Volume 12, 2021, p. 1234.

[10] Zayed, S. M., Aboulwafa, M. M., Hashem, A. M., & Saleh, S. E. "Biofilm formation by *Streptococcus mutans* and its inhibition by green tea extracts". *Amb Express*, Volume 11 Number 1, 2021, p. 73.

[11] Alves L. A. & Ribeiro R. A. "Evaluation of Mitis Salivarius Agar for selective Isolation of *Streptococcus mutans* in clinical

samples". *Journal of Microbial methods*, Volume 185, 2021, pp. 105-112.

[12] Patel, R., & Shah, D. "Phenotypic and Biochemical Characterization of *Streptococcus mutans* in Dental Caries". *Journal of Oral Microbiology*, Volume 13, Number 1, 2021, pp. 45-52.

[13] Klein, D. "Advances in convectional PCR techniques for bacterial identification: A focus on *Streptococcus mutans*". *Journal of Molecular Diagnostics*, Volume 23, Number 4, 2021, pp. 567-578.

[14] Moshe, S., Avshalom T & Doron S. "Expression of biofilm-associated genes of *Streptococcus mutans* in response to glucose and glucose B*". Journal of Medical Microbiology*. Volume 56, Number 1, 2007, pp. 34-45

[15] O'Toole, G. A. "Microtitre Dish Biofilm Formation Assay". *Journal of Visualized Experiments* Volume 47, 2011, p. 2437.

[16] Bauer, A., Kirby, W. W., Sherris, J. C. & Turck, M. "Antibiotic susceptibility testing by a standardized single disc method". *American Journal of Clinical Pathology*.Volume 45, 1966, pp. 493-496.

[17] Bojanich M. A & Orlietti M. D. "Virulence Factors of *Streptoccusmutans* related to dental caries in *Staphylococcus* and *Streptooccus*". 2020, pp. 1-20

[18] Zainab H. A., Ali K. A., & Ali M. S. "Isolation and Identification of *Streptococcus mutans* from Dental Caries by Using Sm479 gene". *Indian Journal of Public Health Research and Development*, Volume 13, Number 1, 2018, pp. 447 – 454.

[19] Sangha, J. S. Barrett P., Curtis, T. P., Metris A., Jakubovics, N. S. & Ofiteru, I. D. "Effects of glucose and lactate on *Streptococcus mutans* abundance in a novel multispecies oral biofilm model". *Microbiology spectrum*, Volume 12, Number 4, 2025.

[20] Fang, Y., Chen, X., Chu, C. H., Yu, O.Y., He, J & Li., M " oles of *Streptococcus mutans* in human health: Beyond Dental caries". *Frontiers in Microbiology*, Volume 15, 2024.

[21] Martinez-Martinez, R. E., Fujiwara, T., Patiño-Marin, N., Hoshino, T., Wilson, M., & Loyola-Rodríguez, J. P. "Comparison of oral streptococci biofilm in caries-free and caries-affected preschool Mexican children". *Acta Odontológica Latinoamericana*, Volume 25, Number 1, 2012, pp. 27-32.

[22] Jain, P., & Pundir, R. K. "Antibiotic sensitivity pattern of *Streptococcus mutans* against commercially available drugs". *J Pharm Res*, Volume 2, Number 7, 2009, pp. 1250-1252.

[23] Høiby, N., Ciofu, O., Johansen, H. K., Song, Z.J., Moser, C., Jensen, P. Ø., Molin, S., Givskov, M., Tolker-Nielsen, T. & Bjarnsholt, T., "The clinical impact of bacterial biofilms". *International Journal of Oral Science*, Volume 3, Number 2, 2011, pp. 55-65.