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Prevalence and antibiogram of *Streptococcus mutans* in dental plaque and caries samples

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Abstract: *Streptococcus mutans* is a Gram-positive facultative anaerobic bacterium that belongs to the mutans streptococci group and is considered to be an important pathogen associated with human dental caries. In this study, 106 *S. mutans* isolates were isolated from 140 clinical samples. The 106 isolates comprised 56 isolates from soft caries lesion, 50 isolates from dental plaque samples, the latter included 30 isolates from plaque of caries active and 20 isolates from plaque of caries-free cases. The isolates showed sticky and rough colonies on TYCSB media. Isolates produced alpha hemolysis on blood agar. Isolates were catalase-negative and able to ferment mannitol, sorbitol, inulin and sucrose. The identification of *S. mutans* was confirmed by the detection of the *S. mutans*-specific glucosyltransferase gene (*gtfB*) using PCR specific primers for *S. mutans gtfB* gene. Isolates were tested for their sensitivity to diverse antimicrobial agents. It was found that 99% of the isolates were sensitive to ofloxacin and levofloxacin and 88.5% of isolates was sensitive to chloramphenicol, however, 80.2%, 75.5% and 65% of the isolates were resistant to ceftriaxone, cefotaxime and erythromycin, respectively. It was recorded that 90.5% and 85.8% of the isolates were sensitive to linezolid and tetracycline, respectively, and were highly resistant to clindamycin with a frequency of 95.3%. Based on the statistical analysis using the *chi-square* test, there was a significant difference in antimicrobial susceptibility profiles among the three groups with higher frequencies among soft caries lesion isolates ($P < 0.0001$). Therefore, the extensive use of antimicrobials in treatment and prophylaxis in dental caries must be controlled.

Keywords: *S. mutans*; caries; plaque; *gtfB*; antimicrobial susceptibility.

1. INTRODUCTION

The mouth consists of mucosal surfaces (lips, cheek, palate and tongue) and teeth which because of their different biological and physical properties allow the growth of various microbes[1]. The normal temperature of the oral cavity is 37°C which represents a good environment for bacteria to survive. Besides, saliva has a pH of 6.5–7 which is the most favorable pH for most bacterial species, saliva also keeps the bacteria hydrated and transports nutrients to different microorganisms[2]. Oral microbiota contains the microorganisms that are present in the human oral cavity which is considered the second-largest microbial community in the human body after the gut[3,4]. The genera *Streptococcus* and *Actinomyces* which are facultative anaerobes are acquired at birth and the hours after[5]. Species of *Streptococci* are present in the human oral cavity as their natural habitat; they belong to the viridans group streptococci (VGS). The viridans group streptococci are facultative anaerobic Gram-positive spherical or ovoid-shaped, non-spore-forming, non-motile and ferment carbohydrates with the production of acid but not gas. They are the major contributor to the formation of plaque, which is a highly complex ecosystem with many different types of bacteria[6]. The group of

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mutans streptococci (*Streptococcus mutans* and *S. sobrinus*) are considered to be the main causative agents of dental caries. These bacteria are the most common pathogens isolated from human dental diseases and their occurrence has been reported in epidemiological studies[7]. *Streptococcus mutans* is a facultatively anaerobic, Gram-positive cocci bacterium frequently found in the human oral cavity and is an important cause of tooth decay[8]. It is a mesophilic organism that can grow at temperatures between 18-40°C. *S. mutans* bacteria colonize the mouth; adhere to the teeth and form plaque, the soft, sticky film formed on teeth from food degradation. The biofilm is the proliferation and teeming of bacteria. *S. mutans* bacteria break down sugars and produce lactic acids which cause tooth decay; a process of demineralization or loss of calcium phosphate from the tooth structure then the tooth "softens" and eventually collapses forming a cavity. The teeth have a natural pit and fissure morphology that facilitates bacterial adherence and colonization. The proximal contacts of teeth also facilitate adherence of plaque and bacteria[9]. This study aimed to isolate, examine and compare the antimicrobial susceptibility profiles of *S. mutans* isolates from both dental plaque (plaque of caries active samples and the plaque of caries-free samples) and dental caries samples.

2. METHODS

2.1. Collection and processing of clinical samples

A total of 140 clinical samples were included in the present study. The samples were collected by the dedicated members in the dental clinics at Faculty of Dentistry, October University for Modern Sciences and Arts. Samples were collected from patients upon the approval of the review board of dental clinics and the ethics committee of Faculty of Dentistry, October University for Modern Sciences and Arts (MSA) (Ethics approval number: ETH38). The 140 samples comprised 70 samples from soft caries lesion and 70 samples from dental plaque, the latter included 35 samples from the plaque of caries active and 35 samples from the plaque of caries-free cases. Plaque samples were collected by swabbing the surface of the intact enamel and cervical margin of the teeth from caries-free and caries active patients. Soft caries samples were obtained from the outermost layer of carious dentin and removed with a sharp sterile excavator[10-13]. Swabs and soft caries samples were placed into an Eppendorf tube containing 1 ml brain heart infusion broth (LAB M, England) in the dental clinics then transferred to the microbiology laboratory within 10 min. In the laboratory, they were put in test tubes containing 10 ml brain heart infusion broth and incubated at 37°C for 24 hr.

2.2. Isolation of *S. mutans* bacteria

The growth from the brain heart infusion broth was inoculated onto the surface of Tryptone-yeast-cysteine-sucrose-bacitracin (TYCSB) agar by streaking [tryptone 15 gm/L (Techno Pharmchem, India), yeast extract (Pronadisa, Spain) 5 gm/L, Disodium hydrogenphosphate.7 H_2O (Sigma, Germany) 1 gm/L, Sodium bicarbonate (Oxford, India) 2 gm/L, Sodium acetate trihydrate (Oxford, India) 20 gm/L, Sucrose 200 gm/L, L-cysteine (Sigma, Germany) 0.2 gm/L, Sodium sulfite (Oxford, India) 0.1 gm/L, Sodium chloride (Adwic, Egypt) 0.1 gm/L, Agar (Oxoid, England) 15 gm/L, Bacitracin (Sigma, Germany) 0.002 gm/L, Distilled water 1000 ml)], which is the most sensitive and selective media for the culture of *S. mutans*[14].

2.3. Identification of *S. mutans* isolates

All isolated bacteria were identified to the genus and the species levels. Bacterial isolates were identified microscopically (for microorganism characters like Gram reactions, shape and arrangement) and by biochemical reactions-based methods.

2.3.1. Microscopical examination and cultures characteristics of S. mutans

Fresh pure colonies of the tested isolates were used to prepare heat-fixed smears, which were subsequently stained by the Gram-stain technique. The stain reaction and microscopical characters of tested isolates were then observed.

2.3.2. Identification of S. mutans using biochemical tests

Isolates were identified to the genus level depending on the catalase test, the presence of this enzyme in a bacterial isolate can be evidenced when a small inoculum is introduced into hydrogen peroxide (El Nasr, Egypt) causing rapid production of water and oxygen which causes the appearance of froth[15]. Sugar fermentation test was performed on all isolates to test their ability to ferment four sugars: mannitol, sorbitol, sucrose and inulin (Loba Chemie, India). Brain heart infusion broth supplemented with the phenol red indicator (Loba Chemie, India) and with 10% of each of the four sugars was inoculated with the tested isolates and incubated anaerobically at 37°C for 72 hours. The color of media would change from red to yellow as compared with the controls indicating the ability of these isolates to ferment these sugars. Brain heart infusion broth medium was used as negative control and media inoculated with standard *S. mutans* strain ATCC 25175 was used as a positive control[16]. Blood hemolysis was tested using blood agar which differentiates organisms to alpha (greenish

discoloration around colony), beta (i.e., the clear zone around colony) or gamma (i.e., no hemolysis around colony) hemolytic microorganisms. The isolates were streaked on blood agar obtained from Kasr Al-Ainiy, School of Medicine, and plates were incubated for 24 hours at 37°C, then the type of hemolysis was observed[17].

2.3.3. PCR-based molecular identification of S. mutans isolates

The identification of *S. mutans* isolates was confirmed by PCR-based identification using pairs of primers targeting the *S. mutans gtfB* gene.

2.3.3.1. DNA extraction and preparation of PCR primers

Genomic DNA was extracted from examined isolates using the i-genomic BYF DNA Extraction Kit (Intron, KOREA) according to the manufacturer's directions. The PCR primer lyophilized powder, synthesized by Invitrogen (UK), was reconstituted using nuclease-free water (Thermo Scientific, USA) to achieve a concentration of 100 pmol/µl and then was adjusted to the working solution of 10 pmol/µl. The sequences of the primers are 5'- TTCGGGTGGCTTGGTTAAAGC -3' (forward primer) and 5'- TGCTTAGATGTCACTTCGGTTGT -3' (reverse primer) designed in this study using the primer designing tool available at NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The expected PCR amplicon size is 123 bp.

2.3.3.2. PCR reactions

The PCR reactions were prepared in total volumes of 20 µl, containing 1 µl of template DNA, 1 µl of F primer and 1 µl of R primer, 10 µl of Dream Taq Green PCR Master Mix (2×) (Thermo Scientific, USA) containing Dream Taq DNA polymerase, and the volume was completed to 20 µl by adding nuclease-free water. The PCR amplifications were carried out in a Veriti 96 well thermocycler (Applied Biosystems, USA) programmed for an initial denaturation at 94°C for 5 minutes and 40 cycles of denaturation at 94°C for 30 seconds, primer annealing at 53°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes.

2.3.3.3. Detection of the PCR amplified product by TAE (Tris-acetate-EDTA) agarose gel electrophoresis

PCRs products were resolved through TAE agarose gel (1.5 %) electrophoresis prepared using molecular biology grade agarose (GIBCO Bethesda Research Lab, U.S.A.) in 1× TAE buffer (Thermo Scientific, USA) stained with ethidium bromide (5 mg/ml) (Alliance Bio, USA). DNA fragments were electrophoresed by by horizontal agarose gel electrophoresis apparatus (Cleaver Scientific, UK) in 1× TAE buffer at 85 Volt for 45 minutes and visualized by placing on a Benchtop 2 UV trans-illumination (UVP, USA) and photographed directly. For the sizing of the separated DNA fragments, Gene Ruler 50 bp DNA (Thermo Scientific, USA) molecular weight markers were used.

2.4. Antimicrobial susceptibility testing

The determination of the sensitivity of collected isolates to antimicrobial agents representing different antimicrobial classes including chloramphenicol 30 µg, levofloxacin 5 µg and ofloxacin 5 µg (fluoroquinolones), erythromycin 15 µg (macrolides), cefotaxime 30 µg and ceftriaxone 30 µg (cephalosporin), clindamycin 2 µg (lincosamides), linezolid 30 µg (oxazolidinones) and tetracycline 30 µg (Bioanalyse, Turkey) was performed by the agar disc diffusion method[18]. The Kirby-Bauer method was used to determine antimicrobial susceptibility testing as follows[19].

2.4.1. Inoculum preparation

The inoculum suspension was prepared by taking colonies of isolates grown on brain heart agar media (Oxoid, England) for 48 hours in a candle jar at 37°C by a sterile plastic loop and transferred into a tube containing 5 ml of isotonic saline. Subsequently, the suspension was adjusted to match the 0.5 McFarland turbidity standard.

2.4.2. Inoculation of plates

A sterile swab was dipped into the inoculum tube. The swab was rotated against the side of the tube with pressure to remove excess fluid. The swab was used to spread the bacteria on the surface of the Muller Hinton agar (Oxoid, England) plate with 60 degrees rotation each time to ensure the even distribution of the inoculum. The antimicrobial discs were placed on the surface of the plate using an antibiotic disc dispenser (Oxoid, UK). The Petri dishes were enough to test about 4 discs. Each disc was pressed down to be in direct contact with the agar surface. The plates were incubated in a candle jar at 37°C for 24 hr. Then, the Petri dishes were held a few inches above a black background and the diameter of each inhibition zone was measured. The size of the inhibition zone was interpreted by referring to CLSI (2017) guidelines and the tested isolates were reported as sensitive (S) or resistant (R).

2.5. Statistical analysis

Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA). The frequencies were calculated to compare the prevalence and antimicrobial susceptibilities. Differences between the results of the isolates recovered from different groups of samples were determined using the *Chi-square* test. Differences were considered to be significant at a $P < 0.05$.

3. RESULTS

3.1. Identification and prevalence of *S. mutans* isolates

In the current study, 106 *S. mutans* isolates were isolated from a total of 140 clinical samples with a frequency of 75.7%. The 106 *S. mutans* isolates comprised 56 isolates from soft caries lesion samples (56/70, 80%), 50 isolates from dental plaque samples (50/70, 71.4%), the latter included 30 isolates from the plaque of caries active samples (30/35, 85.7%) and 20 isolates from the plaque of caries-free samples (20/35, 57.1%). After isolation of *S. mutans* isolates on TYCSB media, the isolates were identified as follows:

3.1.1. Macroscopic and microscopic examination of S. mutans isolates

The colonies of *S. mutans* isolates showed the characteristic sticky and rough appearance on TYCSB media. *S. mutans* isolates showed alpha hemolysis reaction on blood agar medium, which appeared as greenish discoloration. The bacterial cells on microscopic examination appeared as Gram-positive cocci with the characteristic chain arrangement.

3.1.2. Biochemical testing-based identification of S. mutans isolates

S. mutans isolates produced the characteristic biochemical behavior. In the catalase test, *S. mutans* isolates did not produce the characteristic froth upon the addition of H_2O_2, which indicated catalase-negative bacteria. The isolates were able to ferment the tested sugars mannitol, sorbitol, inulin and sucrose, and to change the color of phenol red indicator from red to yellow due to the production of acids from carbohydrate fermentation (Table 1).

Table 1. Biochemical tests results of *S. mutans* isolates**.**

Biochemical test	Catalase test	Mannitol fermentation	Sorbitol fermentation	Inulin fermentation	Sucrose fermentation
S. mutans isolates reaction	-ve	+ve	+ve	+ve	+ve

3.1.3. PCR-based molecular identification of S. mutans isolates

The identification of *S. mutans* isolates was confirmed by PCR-based identification using pairs of primers targeting the *S. mutans gtfB*. The target gene was amplified from all *S. mutans* isolates identified by phenotypic microbiological methods. Figure 1 shows representative agarose gel electrophoresis results of PCR amplicon size of 123 bp.

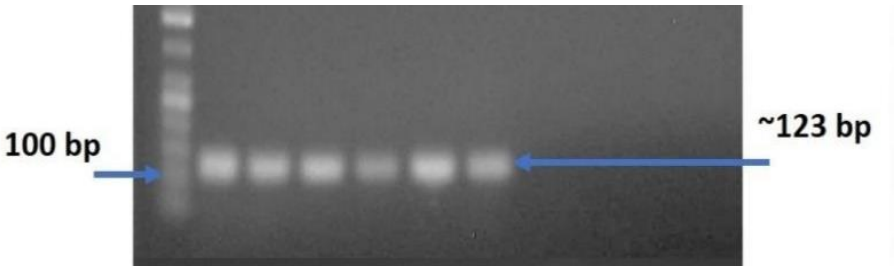

Figure 1. PCR-based identification of *S. mutans* isolates. *S. mutans* isolates produced a single 123-bp DNA fragment.

3.2. Antimicrobial susceptibility testing

All *S. mutans* isolates were tested for their sensitivity to diverse antimicrobial agents using the disc diffusion method (Table 2, Figure 2).

Table 2. Antimicrobial susceptibility testing of *S. mutans* isolates.

Antimicrobial agent	OFX (5 µg)	LEV (5 µg)	CRO (30 µg)	CTX (30 µg)	E (15 µg)	C (30 µg)	LNZ (30 µg)	DA (2 µg)	TE (30 µg)
Sensitive	105[1] (99.1%)[2]	105 (99.1%)	21 (19.8%)	26 (24.5%)	37 (35%)	94 (88.5%)	96 (90.5%s)	5 (4.7%)	91 (85.8%)
Resistant	1 (0.9%)	1 (0.9%)	85 (80.2%)	80 (75.5%)	69 (65%)	12 (11.5%)	10 (9.5%)	101 (95.3%)	15 (14.2%)

[1]Number of isolates

[2]Percentage correlated to the total number of isolates (n=106). OFX, ofloxacin; CRO, ceftriaxone; LEV, levofloxacin; CTX, cefotaxime; E, erythromycin; C, chloramphenicol; LNZ, linezolid; DA, clindamycin; TE, tetracycline.S, sensitive; R, resistant

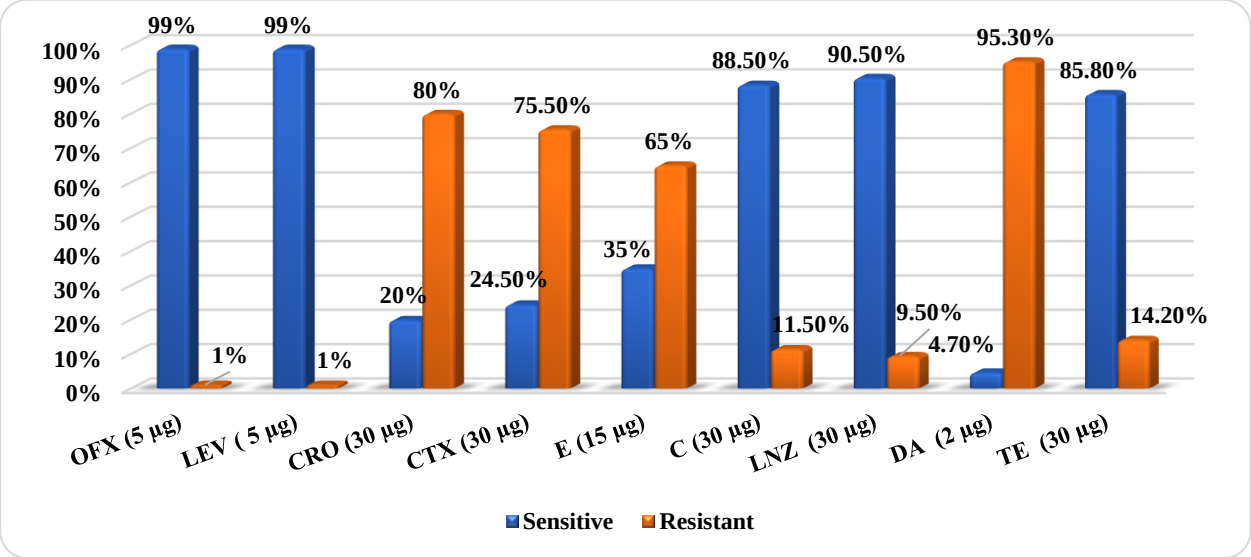

Figure 2. Antimicrobial susceptibility testing of all *S. mutans* isolates.

Antimicrobial susceptibilities of *S. mutans* isolates were grouped according to the source of samples from soft caries lesion, the plaque of caries active and plaque of caries-free cases (Table 3, Figures 3, 4 and 5).

Table 3. Antimicrobial susceptibilities of *S. mutans* isolates recovered from each sample type.

Antimicrobial agent	The sample source of *S. mutans* isolates (n =106)[1]					
	Soft caries lesion (n = 56)[2]		Plaque of caries active (n = 30)		Plaque of caries-free (n = 20)	
	S	R	S	R	S	R
OFX (5 µg)	56 (100%)[3]	0 (0%)	29 (97%)	1 (3%)	20 (100%)	0 (0%)
LEV (5 µg)	56 (100%)	0 (0%)	29 (97%)	1 (3%)	20 (100%)	0 (0%)
CRO (30 µg)	3 (5%)	53 (95%)	11 (37%)	19 (63%)	7 (35%)	13 (65%)
CTX (30 µg)	3 (5%)	53 (95%)	16 (53%)	14 (47%)	7 (35%)	13 (65%)
E (15 µg)	10 (18%)	46 (82%)	18 (60%)	12 (40%)	9 (45%)	11 (55%)
C (30 µg)	49 (87%)	7 (13%)	30 (100%)	0 (0%)	15 (75%)	5 (35%)
LNZ (30 µg)	47 (84%)	9 (16%)	30 (100%)	0 (0%)	20 (100%)	0 (0%)
DA (2 µg)	0 (0%)	56 (100%)	0 (0%)	30 (100%)	7 (35%)	13 (65%)
TE (30 µg)	47 (84%)	9 (16%)	30 (100%)	0 (0%)	13 (65%)	7 (35%)

[1]Total number of *S. mutans* isolates recovered in this study.

[2]Number of *S. mutans* isolates recovered from each sample type.

[3]Percentage correlated to the total number of isolates from each sample type.

OFX, ofloxacin; CRO, ceftriaxone; LEV, levofloxacin; CTX, cefotaxime; E, erythromycin; C, chloramphenicol; LNZ, linezolid; DA, clindamycin; TE, tetracycline; LNZ, linezolid; DA, clindamycin; TE, tetracycline.S, sensitive; R, resistant.

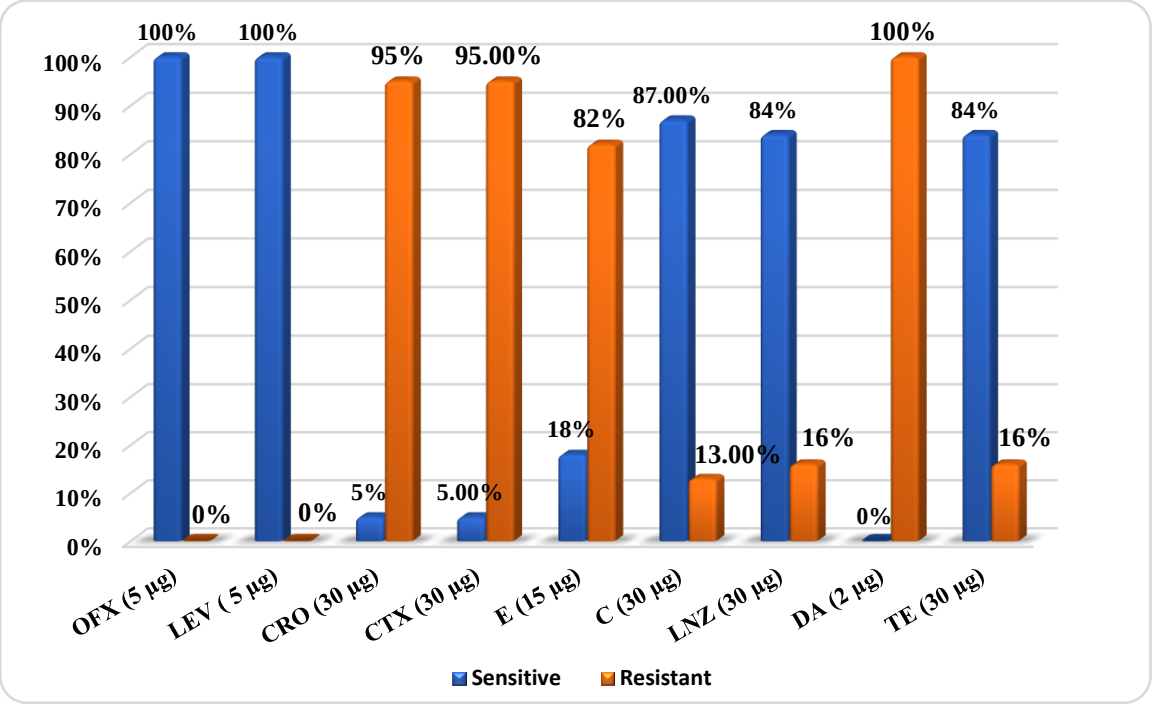

Figure 3. Antimicrobial susceptibility testing of *S. mutans* isolated from soft caries lesion samples.

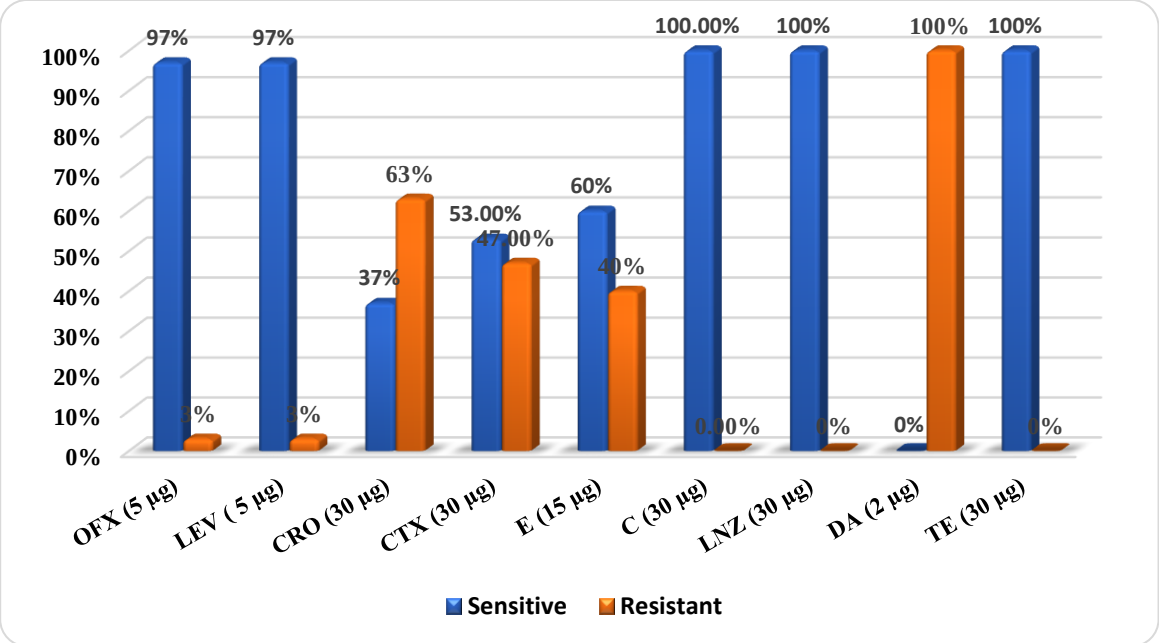

Figure 4. Antimicrobial susceptibility testing of *S. mutans* isolated from the plaque of caries active patients.

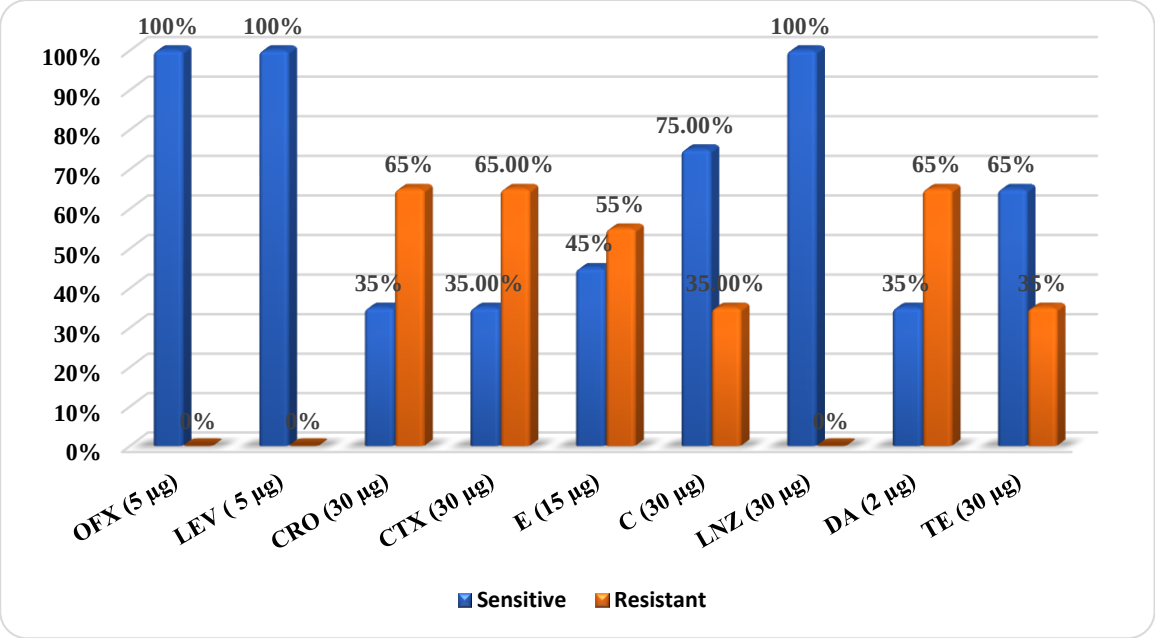

Figure 5. Antimicrobial susceptibility testing of *S. mutans* isolated from the plaque of caries-free samples.

Based on our statistical analysis using *chi-square* test, we found that there was a significant difference in antimicrobial susceptibility among the three groups ($P < 0.0001$). It was found that 99% of the isolates were sensitive to ofloxacin and levofloxacin and 88.5% of isolates was sensitive to chloramphenicol, however, 80.2%, 75.5% and 65% of the isolates was resistant to ceftriaxone, cefotaxime and erythromycin, respectively. It was recorded that 90.5% and 85.8% of the isolates were sensitive to linezolid and tetracycline, respectively, and the isolates were highly resistant to clindamycin with a frequency of 95.3%. The susceptibility rates to quinolones (ofloxacin and levofloxacin) of 97% - 100% were almost similar in the three groups. The resistance rates to each ceftriaxone, cefotaxime and erythromycin among soft caries lesion isolates were higher than the rates among the plaque of caries active or plaque of caries-free isolates. The sensitivity rates to both chloramphenicol and tetracycline were high among isolates from the plaque of caries active samples, followed by soft caries lesions, then the plaque of caries-free samples. The resistance rate to clindamycin was 100% among isolates from soft caries lesions and the plaque of caries active samples, while it was 65% among those isolates from the plaque of caries-free samples.

4. DISCUSSION

S. mutans, a member of the viridans streptococci, is considered to be the main cause of dental caries, which has the highest incidence among all oral diseases[20]. Dental caries is best described as a complex biofilm-mediated disease that can be mostly attributed to frequent ingestion of fermentable carbohydrates such as glucose, fructose, sucrose and maltose, along with bad oral hygiene and inadequate fluoride exposure[21]. The ability of *S. mutans* to form biofilm on the tooth surface, also called dental plaque, is considered one of its important virulence properties[22]. *S. mutans* can generate acidic end products which are not only the direct down causative factor for demineralization of tooth surfaces but also may influence the caries-related microbial flora during the cariogenic process. In addition, *S. mutans* has also developed a mechanism to combat the destructive nature of the acidic environment (aciduricity)[23]. The current study aimed to isolate and examine the antimicrobial susceptibilities of *S. mutans* isolates from dental plaque and dental caries samples. In this study, *S. mutans* isolates were identified based on microscopic examination showing Gram-positive cocci that are arranged in chains. In addition, the isolates were able to produce alpha hemolysis on blood agar media as previously confirmed for *S. mutans*[24]. Furthermore, the colonies showed the characteristic sticky and rough appearance on TYCSB media as showed in the previous study of Vildósola *et al.*[25]. Based on biochemical testing, *S. mutans* isolates were catalase-negative, able to ferment the sugars mannitol, sorbitol, inulin and sucrose and to change the color of phenol red indicator from red to yellow due to acid production from carbohydrate fermentation which agreed with the studies of Al-Jumaily *et al.* and Vos *et al.*[16,26]. To confirm identification, *S. mutans* isolates were examined by PCR using specific pairs of oligonucleotide primers targeting the *S. mutans gtfB* gene, which can be considered specific primers for identification of *S. mutans*[27,28]. The phenotypically identified *S. mutans*

isolates produced a detectable PCR amplicon of 123 bp.

Concerning the antimicrobial susceptibility profile, *S. mutans* isolates were tested for their sensitivity different antimicrobial classes including chloramphenicol, levofloxacin and ofloxacin (fluoroquinolones), erythromycin (macrolides), cefotaxime and ceftriaxone (cephalosporin), clindamycin (lincosamides), linezolid (oxazolidinones) and tetracycline. Comparing the antimicrobial susceptibilities of *S. mutans* isolates from different groups of samples, there was a significant difference between the three groups s($P <$ 0.0001). Regarding ofloxacin and levofloxacin, soft caries *S. mutans* isolates showed 100% sensitivity to ofloxacin which agreed with Patidar *et al*.[29], in addition to 100% sensitivity to levofloxacin. While the isolates from the plaque of caries active samples showed 97% sensitivity to ofloxacin which was consistent with Oyiborhoro *et al*.[30] and were also 97% sensitive to levofloxacin which agreed with De *et al*. and Dhotre *et al*. [31,32]. The isolates from the plaque of caries-free samples showed 100% sensitivity to each ofloxacin and levofloxacin, although these types of isolates were not examined before according to our knowledge. The high sensitivity to fluoroquinolones among isolates can be explained by fluoroquinolones are infrequently used by dentists in treating dental and/or oral diseases in Egypt. Regarding cephalosporin class, ceftriaxone and cefotaxime, soft caries *S. mutans* isolates showed a high resistance frequency of 95% which agreed with Karikalan *et al*.[33]. The *S. mutans* isolates from the plaque of caries active samples showed 53% sensitivity to cefotaxime which agreed with Salman *et al*.[34] and 63% resistance to ceftriaxone. In addition, isolates from the plaque of caries-free samples showed 65% resistance to each ceftriaxone and cefotaxime. The high resistance rate to cephalosporins is due to the overuse of these safe antimicrobials in developing countries like Egypt.

The present study showed that soft caries *S. mutans* isolates exhibited high sensitivity frequency of 87% to chloramphenicol which is consistent with Karikalan *et al*.[33]. In this study, the sensitivity rate of isolates from the plaque of caries active samples was 100% to chloramphenicol which disagreed with the results of Salman *et al*.[34] that recorded a sensitivity rate of 42.4%, and this difference may be because of the different outline of antibiotic usage across different countries as Salman *et al*. study was from India. The high sensitivity in this study is partly attributed to that chloramphenicol is not used nowadays excessively than in previous ages. Isolates from the plaque of caries-free samples showed 75% sensitivity to chloramphenicol. Regarding erythromycin, soft caries *S. mutans* isolates showed resistance frequency to erythromycin of 82% which agreed with Jubair (2015)[35] and this is inconsistent with the results of Patidar *et al*.[29] that recorded that 46% of *S. mutans* isolates were resistant to erythromycin. This variation in susceptibility rate could be attributed to the different patterns of antibiotic usage across different countries as that study was performed in India and maybe because that study was from 2013 and the excess use of erythromycin led to the emergence of more resistant strains. Notably, in Patidar *et al*. study, the authors considered the soft caries isolates and plaque isolates collectively as one group, whereas in the current study, they were considered as two separate groups. The isolates from the plaque of caries active samples showed 60% sensitivity to erythromycin which agreed with Oyiborhoro *et al.*, De *et al.*, Salman *et al.* and Al-Shami *et al*.[30,31,34,36]. Isolates from the plaque of caries-free isolates showed 55% resistance rate to erythromycin.

Soft caries *S. mutans* isolates showed a high sensitivity rate to tetracycline of 84% which agreed with Karikalan *et al*. and El Sherbiny *et al*.[33,37], but this finding disagreed with Patidar *et al*.[29] that recorded a low sensitivity rate to tetracycline. The isolates from the plaque of caries active samples showed 100% sensitivity to tetracycline. Isolates from the plaque of caries-free isolates showed 65% sensitivity to tetracycline. This difference maybe because of the less use of tetracycline in Egypt. In addition, soft caries *S. mutans* isolates showed high sensitivity to linezolid of 84%. The isolates from the plaque of caries active samples showed 100% sensitivity to linezolid which agreed with De *et al*.[31]. Isolates from the plaque of caries-free isolates showed 100% sensitivity to linezolid. Regarding clindamycin, soft caries *S. mutans* isolates showed high resistance to clindamycin rate of 100%, which may be explained by the excessive use of this drug in the treatment of dental diseases by dentists in Egypt. The isolates from the plaque of caries active samples in this study showed 100% resistance to clindamycin which disagreed with the results of Al-Shami *et al*.[36] and this difference may be due to the excessive use of clindamycin in the treatment of dental diseases and/or prophylaxis. The isolates from caries-free showed 65% resistance to clindamycin, the decrease in the resistance of the plaque of caries-free isolates to clindamycin compared to isolates from the plaque of caries active samples and those isolates from caries lesion samples maybe because of the less frequent use of clindamycin in individuals not having caries.

5. CONCLUSION

Collection, isolation and subsequent identification of *S. mutans* isolates were performed in the current study based on conventional microbiological methods. The identification was confirmed by molecular-based PCR

assay using specific pair of primers targeting the *S. mutans gtfB* gene, which can be used as a specific assay to identify *S. mutans* species. *S. mutans* isolates were tested for their antimicrobial susceptibility against diverse classes of antimicrobial agents following CLSI guidelines. It was found that 99% of the isolates were sensitive to ofloxacin and levofloxacin and 88.5% of isolates was sensitive to chloramphenicol, however, 80.2%, 75.5% and 65% of the isolates were resistant to ceftriaxone, cefotaxime and erythromycin, respectively. It was recorded that 90.5% and 85.8% of the isolates were sensitive to linezolid and tetracycline, respectively, and the isolates were highly resistant to clindamycin with a frequency of 95.3%. *S. mutans* isolates showed a significant variance in the antimicrobial susceptibility patterns of the isolates recovered from soft caries lesion, plaque of caries active patients and plaque of caries-free individuals with higher resistance rates among soft caries lesion isolates ($P < 0.0001$). Therefore, the extensive use of antimicrobials in dental caries conditions and/or as prophylaxis in dental diseases must be controlled. In addition, appropriate prescribing and rational use of antimicrobial agents is essential.

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Conflicts of Interest: The authors declare that they have no competing interests.

Ethical Statement: The samples were collected from patients by the dedicated members in the dental clinics at Faculty of Dentistry, October University for Modern Sciences and Arts, upon the approval of the review board in the dental clinics and the ethics committee of Faculty of Dentistry, October University for Modern Sciences and Arts (MSA) (Ethics approval number: ETH38).

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