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In-Vitro Investigation of the Chemical and Virulence Properties of Streptococcus Mutans Isolated from Individuals with Dental Implant Prostheses

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Abstract: ***Background*:** Iraqi society suffers from a number of health issues, including dental caries, necrosis, and gingivitis. The treatment of bacteria has become difficult because of their resistance to many antibiotics. One of the most prevalent species in the mouth that causes dental caries is Streptococcus mutans. ***Objectives*:** The aim of this research was to isolate and diagnose Streptococcus mutans and study other Virulence factors such as catalase and blood hemolysis. ***Materials and Methods*:** Using biochemical tests and phenotypic diagnostics. Using biochemical tests and phenotypic diagnostics. The study included the collection oral swabs from (54) patients infected with teeth dental caries (A group) and dental prosthesis wearing patients (B group) at specialized dental hospital and dental clinics at was it province and both sexes. ***Results*:** The number of isolates was 14(25.92%) belonging to Streptococcus mutans, were identified by cultural methods, biochemical tests, Api 20 Strep. system and Vitek 2 compact system. The findings indicated that the all of the isolates had a not capacity for catalase production.

Keywords: Streptococcus mutans, Api 20 Strep, Vitek 2 compact system, catalase test and blood hemolysis.

Research Paper

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1. INTRODUCTION

The success of dental restorations and implants is influenced by numerous factors, with surface characteristics and bacterial adhesion particularly important (Oztel *et al*., 2017). Biofilms on restoration surfaces contribute significantly to periodontal disease and peri-implantitis, which are leading causes of failure in restorative and implant treatments (Viitaniemi *et al*., 2017). Streptococcus mutans (S.mutans)contribute to dental caries and oral diseases by producing a polymeric matrix that adheres to hard surfaces, playing a key role in microbial adhesion to restoration or implant surfaces (Yu *et al*., 2016). Bacterial adhesion and plaque formation are influenced by surface material, microbial electrical charge, surface roughness, surface hydrophilicity or hydrophobicity, and bacterial membrane proteins. Increased surface roughness of restorations enhances bacterial adhesion. Dentists often need to reduce surface roughness to minimize undesirable effects when adjusting ceramic restorations for proper contour and occlusion. Glazing and polishing are effective methods for creating a smooth, glass-like surface, thereby reducing bacterial adhesion on zirconia restorations (Hannig. 1997; Al-Wahadni & Muir Martin. 1998; Al-Marzok & Al-Azzawi. 2015).

Periodontal disease is one of the most prevalent diseases in the world such as Dental caries and gingivitis. the acids present in the oral cavity disintegrate the enamel layer or damage the surface of the tooth root, the concentration of these acids is related to the human diet. as well as these acids cause damage to the supporting tissues of the teeth (Garcke & Nürnberg .2021; Elgamily *et al*., 2019; Poza-Pascual *et al*., 2021). Streptococcus mutans is one of the most important causes of dental caries. It is a Gram-positive, facultative anaerobe, as well as have ability to form biofilms, acid production and salt tolerance (Lemos *et al*., 2019). tooth caries is a complex, dynamic illness that causes the demineralization and remineralization of tooth hard tissues. It has long been linked to poor oral hygiene and sugar consumption. Caries can destroy the tooth crown and eventually expose the root surfaces of permanent teeth, and it can happen at any point of a tooth's existence. The start and course of dental caries are influenced by the relative abundance of protective and pathogenic substances (Munteanu *et al*., 2022). Acidogenic byproducts of bacterial metabolism

boost the carious potential of the biofilm by causing enamel demineralization and triggering defensive responses in the pulp and dentine, such as increased intratubular dentine and early inflammation. As enamel demineralizes more, bacteria will eventually infiltrate dentine, resulting in further demineralization and ultimately cavitation (Tedesco *et al*., 2020).

2. MATERIALS AND METHODS

2.1. Traditional Methods for Identification of *S.mutans* .

Subculture on the surface of blood-agar plates for further purification and incubated anaerobically for two days at 37 C°. The following methods were used for initial characterization of the isolates:

- Morphology of colony on blood agar.
- Gram-staining and microscopic examination.
- Catalase test.
- Rapid API 20-Strep.
- Using Vitek-2 Compact System.

2.2. Samples collection:

From fifty-four dental patients suffering from dental caries at from different ages and both sexes in specialized dental hospital and dental clinics at wasit province during swab was collected from patients infected with teeth dental caries (A group), dental prosthesis wearing patients (B group). Collected samples were thoroughly washed using phosphate buffer saline and used for culture procedure. Colonies grown on blood agar plates were identified and characterized following the standard microbiological lab protocol. S.mutans was identified based on (according to the information in Bergeys Manual of Determinative Bacteriology 9th ed., 1994) (Bergey.1994). Colonial morphology on blood agar, Gram staining, and specific growth characteristics were determined to follow the method described by Fingold and Baron (1986) (Fingold & Baron.1986). Collected twonty nine sample from (A group), and twenty-five sample from (B group).and detected of the virulence factors, such as the ability to adhere to the oral epithelial cells strains were examined.

2.3. Blood Agar Medium

The blood ager medium was prepared according to the manufacturer's instructions. After sterilization in an autoclave, the medium was cooled to 40-45°C, and then 5% fresh human blood was added. This medium is used to determine the ability of isolated bacteria to decompose blood and to identify the type of hemolysis.

2.4. Identification of S.mutans by API 20 E system

The bacterial suspension was prepared from purified isolated colonies by utilizing API 20E suspension medium and the turbidity was adjusted to 0.5 McFarland tube (1-1.5x108 CFU. ml-1). By using a sterile Pasteur pipette the bacteria suspension was transferred to the twenty microtubes and inoculated according to the manufactures instruction and incubation for 24 hours at 37°C, the isolates were identified by utilizing the numerical coding of the API 20E system for confirmatory identification at specie levels.

2.5. Bacterial Identification Using Vitek-2 Compact System

The Vitek 2 system was used in this study to diagnose S. mutans isolates. The bacterial isolates were biochemically identified according to the Vitek 2 system's results report. Following the manufacturer's instructions (Company: France Biomeriux), the bacterial suspension was prepared by transferring a quantity of the bacterial colonies to be diagnosed from a pre-purified, 18-24 hour culture to 3 ml of sterile saline (0.45% sodium chloride solution). Turbidity was controlled using a Densi-Chek turbidity meter. Gram-positive bacteria were identified using the ID-GP (identifier positive Gram) cassette, a completely closed system requiring no additional reagents. The label was placed on the cassette designed for use with the Vitek 2 system, inserted into the device, and automatically filled in a vacuum-sealed chamber. The cassette was then incubated. At 35.5°C, the culture is automatically colorimetric (with a new reading) every 15 minutes for a maximum incubation period of 8 hours. The data were analyzed using the Vitek-2 database, which allows for active identification of the organism starting 180 minutes after the start of incubation (Karagöz *et al*., 2015).

3. RESULTS AND DISCUSSION

3.1. Isolation and Characterization of S.mutans.

Specimen were collects from patients in specialized dental hospital and dental clinics as shows in table (1). Fourteen sample local were characterized depending on cultural and microscopic characteristic. Genus and species were characterized by using biochemical tests, API 20 E and Vitek 2 System test confirmatory test. This results of this test have been showed that all S.mutans were gram positive and under microscopic avoid or spherical in their shape, arranged in short or medium length chain these results are consistent with previous local and international studies of Streptococcus mutans (Pisarska *et al*.,2022; Abranches *et al*.,2018).And results of biochemical tests showed that all isolates were negative for catalase test (Abranches *et al* .,2018 ;Hussein. 2020). The growth of isolates under anaerobic conditions (5%CO2) was much better then incubating under aerobic conditions. This result matches previous research findings of Streptococcus mutans. (Flayyih *et al*.,2016; Hassan *et al*., 2024). Hemolysin test was used to investigate the production of blood enzyme. The results of blood agar hemolysis showed some S. mutans has the ability to hemolysis the blood (alpha type), these results were agreed with what was stated in previous studies (Zayed *et al*., 2021). Ferraro and Vieira examining factors which contributes to caries and how the factor differs in men

and women, there results demonstrate that caries risk factors for women include a different salivary composition and flow rate, hormonal fluctuations, dietary habits, genetic variations, and particular social roles among their family (Ferraro and Vieira. 2010).

Table 1: Types of sample, number of sample, Number of isolate and Percentage of *S.mutans* isolated from human.

Type Sample	The number of sample	Number of isolate	Percentage
oral cavities of normal subjects	29	8	27.58%
dental prosthesis wearing patients	25	6	24 %
Total	**54**	**14**	**51.58 %**

3.2. A PI 20-Strep.System:

The result of Api-20 Strep. test has revealed that only 7 isolates from 18 samples were identified as *Streptococcus mutans* The present study deals with *S.mutans* isolation and identification, these were done according to cultural features, microscopic examination, biochemical tests and using the Api 20 Strep system which is specific foridentification of Streptococcaceae from other bacteria. **As in table 2**

Table 2: API-20E tests, used for identification of *S. mutans*

Active ingredients	Symbol	*S.mutans*
Ortho NitroPhenyl-Bd Galactopyranside	ONPG	+
L-arginine	ADH	-
L-Lysine	LDC	+
L-Ornithin	ODC	-
Trisodium citrate	CIT	-
Sodium thiosulfate	H2S	-
Urea	URE	-
L-tryptophane	TDA	-
L-tryptophane (indole production)	IND	+
Sodium pyruvate	VP	-
Gelatin (bovine origin)	GEL	-
D-Glucose	GLU	-
D-Mannitol	MAN	+
Inositol	INO	+
D-Sorbitol	SOR	+
L-Rhamnose	RHA	+
D-Saccharose (sucrose)	SAC	+
D-Melibiose	MEL	-
Amygdaline	AMY	-
L-Arabinose	ARA	-

3.3. Biochemical testing of bacterial isolates (Vitek 2 System test)

The bacterial isolates were biochemically identified according to the results report of the automated Vitek 2 system. As per the manufacturer's instructions (BioMerieux Company France) (BioMerieux.2010). The final identification of the isolated bacteria was performed using ID-GP and GN cards containing 64 specialized tests for bacterial identification. The diagnostic probability for the identified isolates was 93%. The Vitek 2 test was used in this study. To test for the presence of S. mutans bacteria in patients clinically diagnosed with dental caries, the VITEK2 Gram-Positive identification kits were routinely used in the clinical laboratory. Over 90% of isolates were correctly identified at the species level without any further testing. The VITEK2 Gram-Positive identification kits provide reliable results for the identification of Gram-positive cocci under routine laboratory conditions (Funke and Funke-Kissling, 2005). A study by (Ligozzi *et al*., 2002) demonstrated that the VITEK2 system is user-friendly, offering rapid (15-4 hours) and accurate means of determining as in table 3.

Table 3: VITEK 2 microbial identification system and GP card available was used for the automated identification of S.mutans

Well No	Test	Symbol	Amount / well (mg)	Result of S. mutans
2	Ala-Pro-Arylamidase	APPA	0.036	-
3	Adonitol	ADO	0.1875	-
4	L-Pyrrolydonyl-Arylamidase	PyrA	0.018	-
5	L - Arabitol	lARL	0.3	-
7	D – Cellobiose	dCEL	0.3	+
9	Beta - Galactosidase	BGAL	0.0384	+
10	H_2S prodection	H_2S	0.036	-
11	Beta – N -Acety –Glucosamindase	BNAG	0.1875	
12	Glutamyl Arylamidase pNA	AGLTp	0.018	-
13	D - Glucose	dGLU	0.3	+
14	Gamma - Glutamyl – Tresferase	GGT	0.3	-
15	Fermentation /Glucose	OFF	0.0384	+
17	Bate – Glucosidase	BGLU	0.0024	+
18	D – Maltose	dMAL	0.0408	+
19	D – Mannitol	dMAN	0.0324	+
20	D – Mannose	dMNE	0.3	+
21	Beta – Xylosidase	BXYL	0.0228	-
22	Beta – Alanine Arylamidase pNA	BAlap	0.45	-
23	L– Prolin arylamidaes	ProA	0.036	-
26	Lipase	LIP	0.0024	+
27	Palatinose	PLE	0.0408	-
29	Tyrosine Arylamidase	TyrA	0.0324	-
31	Urease	URE	0.3	-
32	D –Sorbitol	dSOR	0.0228	+
33	Saccharose / Sucrose	SAC	0.45	+
34	D--Tagatose	dTAG	0.036	-
35	D – Trehalose	dTER	0.3	+
36	Citrate (sodium)	CIT	0.054	-
37	Malonate	MNT	0.15	-
39	5 – KETO –D -Gluconate	5KG	0.3	
40	L –Lactate alkalinisation	lLATK	0.15	+
41	Alpha - Glucosidase	AGLU	0.036	-
42	Succinate alkalinisation	SUCT	0.15	
43	Beta – N – Acetyl - Galactosaminidase	NAGA	0.0504	+
44	Alpha Galactosidase	AGAL	0.036	+
45	Phosphatase	PHOS	0.050	-
46	Glycin arylamidas	Gly A	0.012	-
47	Ornithine decarboxylase	ODC	0.3	-
48	Lysine decarboxylase	LDC	0.0306	-
53	L – Histidine assimilation	IHISa	0.186	+
56	Coumarate	CMT	0.126	-
57	Beta – Glucoronidase	BGUR	0.0378	-
58	O /129 resistance (comp.vibro)	O129 R	0.0105	+
59	Glu – Glu Arg Aryamidas	GGAA	0.0576	-
61	L – Malate assimilation	IMLTa	0.042	+
62	Ellman	ELLM	0.03	-
64	L – Lactate assimilation	ILATa	0.087	+

The results showed that patients who did not use toothpaste were more susceptible to tooth decay caused by S. mutans in both sexes, and the current findings are consistent with what was demonstrated previously (Peros *et al*., 2012). The high concentration of fluoride in toothpaste resulted in a reduction in the number of Streptococci mutans bacteria in saliva, and mutanous nodules were inhibited by changes in salivary pH and buffering capacity. Using toothpaste as an adjunct to brushing or using mouthwash may help improve the

effectiveness of oral hygiene practices. Approximately 30% of patients were found to have this effect. The difference in percentages between the current study and previous studies may be due to differences in sampling methods, sample types, and transport media.

S. mutans a primary etiologic agent of human dental caries, is especial pathogen at forming biofilms on the hard tissues of the human oral cavity. The ability of S.mutans to biofilm formation on teeth surface, dental caries to synthesize extracellular glucans is a critical virulence factor involved in the pathogenesis of dental caries in humans and animals. Dental caries remains the most prevalent disease worldwide, burdening billions of people, especially children, with pain and subsequently poorer quality of life and general health (Schwendicke *et al*., 2015). The results of the phenotypic characteristics of the S. mutans isolates in this study are consistent with those phenotypic characteristics of the S. mutans isolates described by (Flayyih *et al*., 2016). But they also differ from the studies conducted by regarding alpha-hemolysin on blood agar as a hemolysis test (EL-Sherbiny *et al*., 2014; Flayyih *et al*.,2014).

4. CONCLUSION

The results of the current study showed that the percentage of isolation of S. mutans isolated from the mouth 51.58 %. The dominance of S. mutans bacteria as a major cause of oral infections in humans, especially tooth decay, which plays a significant role in increasing the spread of bacteria in the mouth. Colony morphology, microscopy, and biochemistry found Streptococcus mutans. GP-ID cards and 64 biochemical assays indicated 26% of clinical sample isolates were St. mutans using automated Vitek 2 and Api20E system.

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