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Isolation and diagnosis of Streptococcus mutans and Enterococcus faecalis from dental caries patients in Thi- Qar Governorate

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ABSTRACT

This investigation was conducted to isolate Streptococcus mutans and Enterococcus faecalis from dental caries patients in Thi-Qar governorate. A total of (100) samples were gathered from people aged 4 to 65 with the help of a dentist, samples were taken with aseptic techniques. The samples were transferred by specially sterilized containers. Traditional and molecular method were used for isolation and diagnosis bacterial isolates. Total of Forty-five (45%) samples were identified as Streptococcus mutans followed by 25 (25) isolates identified as Enterococcus faecalis.

Keywords: *Dental caries, Streptococcus mutans, Enterococcus faecalis*

INTRODUCTION

The oral microbiome plays a critical role in maintaining oral health Frequent dietary carbohydrate intake can lead to dysbiosis of the microbial community from over production of acid with selection for increases in acidogenic, acid-tolerant bacteria (Tanner et al.,2018). Tooth decay, commonly known as dental caries, is a microbiologically contagious disease of the teeth that causes localized disintegration and damage to the calcified structure, multiple variables contribute to the development of this disease including interactions within the plaque community, host physiology, food, fluoride, pH, and the structure of the tooth enamel (Yadav and Prakash, 2017).This illness is caused by biofilms and is characterized by acid damage to the enamel, which leads to localized demineralization, cavitation, and tooth disintegration (Bowen et al.,2018).

The ability of Streptococcus mutans to acid generation and tolerance ,synthesis of extracellular poly saccharide make it the most common causes of dental caries (Li et al.,2020).Towering levels of Streptococcus salivarius, Streptococcus sobrinus, and Streptococcus parasanguinis, alternative pathogens, were also linked to caries, notably in persons with no or depressed levels of S.mutans (Gross et al.,2012). Enterococcus faecalis has been detected in both root canal systems and it is one of the most frequently found in teeth with pulp necrosis periodical lesions. (Pinheiro et al ., 2003; . Rodríguez et al 2015). Our current study is a focus on isolating and diagnosing of Streptococcus mutans, Enterococcus faecalis from caries lesions and the surrounding dental plaque in relation to caries in patients of Thi-Qar Governorate.

MATERIAL AND METHODS

Samples collection

The study was conducted at the Shamiya Branch of the Specialized Dental Center in Thi- Qar Governorate, where a hundred patients were selected between July 2021 and December 2022. All patients were given a standardized questionnaire that focused on factors such as gender, age, geographic location, sugar consumption, and tooth brushing habits. Under strict aseptic conditions and the supervision of dental professionals, samples were collected from the patients. Samples were collected from various types of caries lesions, including pit and fissure cavity, smooth surface cavity, and root cavity. In addition, dental plaque that was in contact with the border of the caries lesion area was also collected using the following strategy:-

1- The patient's mouth was washed with distilled water more than once

2- Saliva is removed from the decay area with short blows of air

3- Dental caries and dental plaque was removed from the tooth that has been chosen. Each patient's sub-gingival and supra-gingival plaques were scraped from the gingival sulcus with a sterile gracey curette.

4- Root canal caries samples were collected from the patient in the Endodontic department , where contact with any gingivitis or abscess, if present, was avoided

5- Using the transport swab media to take the sample from the gracey curette

Isolation of microorganisms causes dental caries

All samples were cultured aerobic and anaerobic in brain heart infusion broth also in sabouraud dextrose broth for purpose of isolating yeast mitis salivarius agar (M.S. agar) was also used to isolate Streptococcus mutans related with dental caries as follows :

Culture media	The components	Purpose	References
M.S.B	90 g of the MS agar , 1 L of purified water , 1 mL of potassium Tellurite Solution 1% , 150g sucrose , 200 unit / L Bacitracin	For isolating S.mutans	(Al-Mizraqchi, 1998).
Modified media (Modified- 1 M.S.- S.O.B. agar)	MS 90 g, aztreonam, 20 mg, bacitracin 20 units and .(NaCl) 20 g dissolved in 1000 ml D.W.	For isolating E.faecalis	Modified method of (Hirasawa and Takada, 2002)

Also used selective Mutans-Sanguis agar for diagnosis S.mutans. All antibiotics were added after the MS containing NaCl was sterilized and cooled to 50°C. Rapid STR System was used for diagnosis of Streptococcus mutans ,while VITEK 2 system for diagnosis E. faecalis.

Diagnosis by molecular method

The following primers were used for molecular diagnostic purposes to confirm the identification of bacterial isolates. The nucleotide sequences were analyzed and processed using NCBI-Blast Alignment identification http://www.ncbi.nlm.nih.gov/BLAST /Blast.cgi).

TABLE 1: List of the Primers

Name of Primer	Primers sequences	Size
Streptococcus sp. Str1	F 5' GTACAGTTGCTTCAGGACGTATC'3 R 5' ACGTTCGATTTCATCACGTTG '3	185bp

Streptococcus mutans	F 5' AGCCATGCGCAATCAACAGGTT'3 R 5' CGCAACGCGAACATCTTGATCAG'3	403bp
16s RNA	F5'- AGAGTTTGATCCTGGCTCAG- 3' 5'- GGTTACCTTGTTACGACTT- 3’	1250 bp

RESULT

S.mutans was found to be the most common bacterial species causing dental caries (45 isolates) , as it was detected in 45% of the patients with caries.The second most frequent bacterial strain discovered in patients with dental caries was E. faecalis, accounting for 25% of the cases as shown in Table 2 .

TABLE 2: Number and percentage Bacterial species isolates

Bacterial isolates	No	Percentage of patients
St.mutans	45	45%
E.faecalis	25	25%

S. mutans colonies that were round or spherical in shape and about 2-3mm in size appeared pale-blue and were raised or convex and tightly adhered to the surface of the selective M.S.B. agar plates. Certain colonies exhibited an irregular shape and had a surface with a rough or frosted-glass-like appearance. When cultivated on mutans-sangius agar, S. mutans colonies exhibit an irregular, rough, and heaped appearance that resembles frosted glass Figure (1). The Modified-1 M.S.-S.O.B. agar displayed colonies of E.faecalis that had an irregular shape, were flattened, had a lobed appearance, and were firmly attached to the agar surface. The colonies were also observed to be light blue in color.as shown in Figure (2).

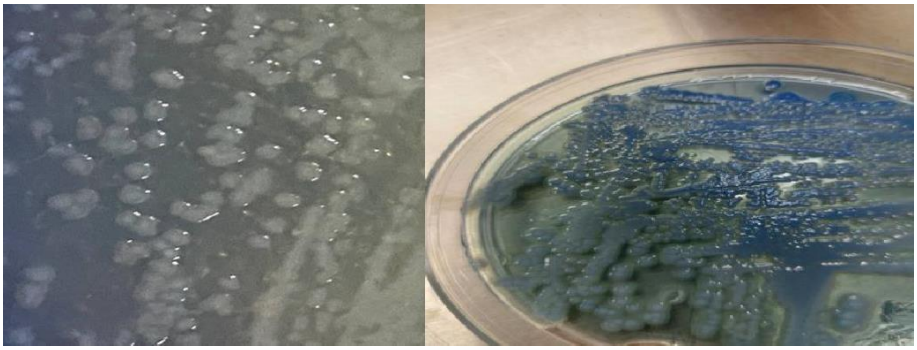

FIGURE 1: S.mutans on M.S.B. agar and mutans – sangius agar

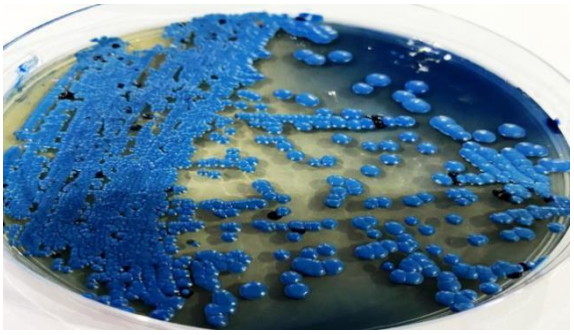

FIGURE 2: Enterococcus faecalis colonies on Modified- 1 M.S.- S.O.B. agar

S. mutans isolates were verified using the RapIDTM STR System, whereas the diagnosis of E. faecalis isolates was confirmed through the use of VITEK2. AS shown in Figure (4).

ARG	ESC	MNL	SBL	RAF	INU	GAL	GLU	NAG	PO4	TYR	HPR	LYS	PYR	HEM
-	+	+	+	+	+	+	+	-	-	-	-	+	-	-

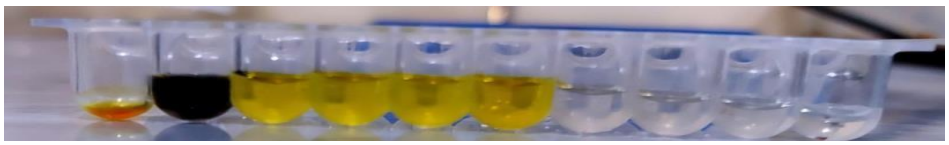

FIGURE 4: Result of identification by RapIDTM STR System

The DNA is extracted from sample isolates by using Genomic DNA extraction Kit .DNA appeared visually through horizontal gel electrophoresis using a 1% agarose gel before and after PCR product using 16SrRNA gene, Figure (5,6).

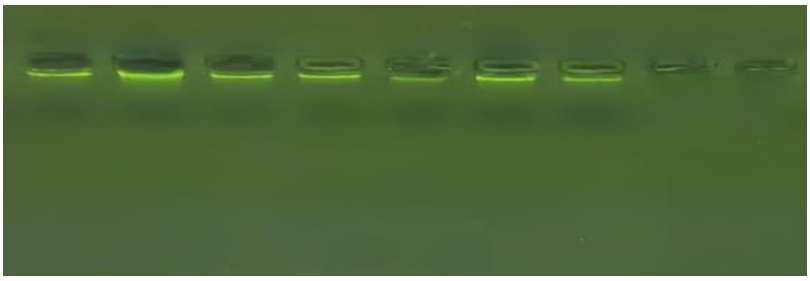

FIGURE 5: Gel electrophoresis of genomic DNA extraction

The results of the PCR to 16s gene 16 S.mutans isolates and 4 E.faecalis isolates was (99%) after homology search was conducted using (BLAST) program which is available (NCBI)online at (http://www.ncbi.nlm.nih.gov).

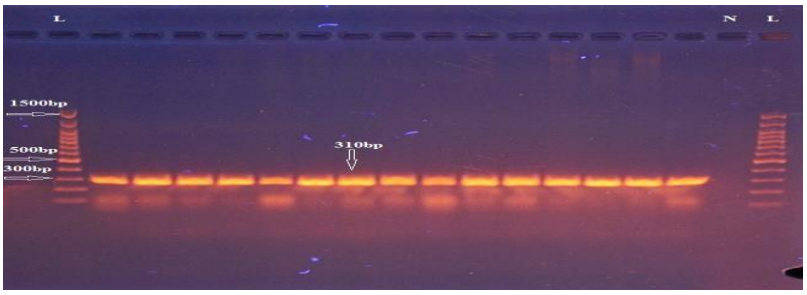

FIGURE 6: PCR product the band size

DISCUSSION

The occurrence of dental caries is a notable concern for public health and a commonly encountered non-communicable disease and most prevalent ailments observed in humans (Bassa et al.,2023). In an effort to prevent

contamination by microorganisms from various sources, more cautious measures were taken during sampling than those employed by Vishnu et al., (2022) and Turagam and Ealla, (2018). This was done to avoid the presence of oral microbiome in the caries area and adjacent dental plaque, as well as microorganisms from food residues that could interfere with the caries site. Furthermore, contact with the gums was avoided when collecting samples from decayed roots, as gums contain both normal flora and microorganisms that can cause inflammation. it was observed that Streptococcus mutans was the predominant species responsible for causing dental caries, accounting for 45 isolates or 45% of the affected patients. This finding is consistent with Abd Al-Zahra and Saleh's (2018) study in Thi-Qar Governorate, which also reported S. mutans as the cause of dental caries in 40.25% of patients. However, our current study used, even for the first time, a novel selective media, Mutans-sanguis agar, and this explain the higher percentage of S.mutans isolate (45%) from patients with dental caries compared to the percentage of isolates in previous study). S.mutans can synthesize significant amounts of extracellular glucan polymers from sucrose, which support its permanent colonization of hard surfaces and the development of the extracellular matrix(Oliveira et al., 2022).Total of 25 isolates belong to E.faecalis .The result current study corresponds with Mohammed et al., (2022) findings, where E. faecalis was also detected in dental caries patients from Baghdad and Diyala cities. Nevertheless, the isolation frequency of E. faecalis in Mohammed's study was lesser than the present research. The virulence of E. faecalis is attributed to their capacity to attach to surfaces of the host and create biofilms(Karayasheva and Radeva, 2017).In addition to their remarkable resistance to antibiotics. In addition to harboring multiple virulence and resistance genes, E. faecalis has a remarkable ability to transfer and transmit many of these genes via horizontal gene transfer mechanisms (Paganelli et al.,2012)

CONCLUSION

Streptococcus mutans was the main bacteria that isolated from patients with dental caries at age from 4-65 years followed by Enterococcus faecalis

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