Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 2927 – 2931
Received 15 April 2021; Accepted 05 May 2021.

Molecular Diagnosis of *S. Mutans* Isolated from Dental Caries in Thi-Qar Province

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ABSTRACT

The aim of this study was to determine the prevalence of *S. mutans* isolates that isolated from patient mouths of the dental caries (n=100) in Nasseriha city through the period February to June,2020 at Thi-Qar province. The isolates were identified as *S. mutans* according to the culture methods and biochemical tests, and to analyze by the polymerase chain reaction (PCR) technique to diagnosis those isolates molecularly by the 16s RNA gene and detected two serotype C and K genes. The results of current study showed the occurrence of *S. mutans* were 24% from all collected samples. The identification of *S. mutans* by 16s RNA gene recoded that all isolates (100%) of *S. mutans* harbored this gene. Whereas the results of serotype C and K genes showed 4.166% only had serotype C gene, while entirely isolates of *S. mutans* no contain serotype K gene.

Keyword: *S. mutans*, PCR, dental caries, 16s RNA, serotype C, serotype K

INTRODUCTION

Dental caries is an infectious pathological process, multifactorial, localized, post-eruptive, and transmissible, which finally destroys hard dental tissues (Selwitz *et al*., 2007), and it might lead to loss of tooth structure, thereby changes in functions of mouth (Schwendicke *et al*., 2015). Also this disease effected by a multifactorial aetiology, as environmental factors, food and hygiene habits and individual genetic susceptibility all play an important role in the development of this disease (van Houte, 1994). The initiation and development of this disorder are linked to the presence of abundant microorganisms which vary depending on the stage of the lesion. Increased caries prevalence is due to the acquisition of cariogenic bacteria *S. mutans* (Köhler *et al*., 1988). Several previous clinical studies have shown that *S. mutans* is considered as a primary etiological factor for dental caries (Marsh *et al*., 2015). *S. mutans* is strongly implicated in the onset of human dental caries, that resulted from the metabolic activation of the plague microorganisms, especially this bacteria was able to ferment the dietary carbohydrates(e.g. sucrose and glucose), which are remains between the teeth, for production the organic acids that lead to breakdown the hard tissue (enamel, dentin and cement) due to remove minerals of tooth surface demineralization process (Veale *et al*., 2016). The *S. mutans* was organized in biofilms existing in a dynamic balance with the organism immune defenses(Loyola-Rodriguez *et al*., 2008). Rogers, (1977) recorded that one of molecular microbiology had helped to identification of *S. mutans* and confirming that the acquisition of *S. mutans* is majorly from the mother transmitting to the child. It includes methods such as DNA hybridization, rDNA restriction analysis, autolysin gene, and 16S-23S rRNA inter spacer region (Chen *et al*., 2004) . The 16S rRNA gene analysis has been shown to differentiate between health and diseases by examining the composition of microbial communities (Clarridge, 2004) . Every bacterial 16S rRNA consists of 1500 nucleotides and several highly conserved regions within domain bacteria. Oral cavity of human is considered proper sterile at birth (Ion, 2013), but after 2-5 days a microbes begin to enter the mouth through feeding until it is reach during few months to more than a million bacterial cell (Metwalli, *et al*., 2013). The transmission of *S.mutans* form mother or people relevant to children will gave chance to these

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bacteria for cause early decay and clear necrosis in your teeth (Napimoga *et al.*, (2005), therefore these teeth are sensitive and weak at aging (Folayan *et al*., 2015). The aim of this study was identification of *S.mutans*, and detection of 16s RNA gene and two serotype C and K gene by PCR technique.

MATERIAL AND METHODS:

Collection the sample: Totally, 100 samples collected from people suffering from dental caries in the Al-Shameia dental center of Thi-Qar province during the period from February to June, 2020.

Laboratory methods

All isolates of *S. mutans* were identified depending on culture methods, microscopic characteristics and Biochemical tests (AL-Mudallal *et al*., 2008; Collee *et al*., 1996).

Extraction of bacterial DNA

Genomic DNA was extracted from all isolates by using Genomic DNA Extraction kit (Presto™ Mini gDNA Bacteria Kit /Korea).

PCR Protocol

Completely isolates of *S. mutans* (24/100) were used to amplification of 16sRNA, serotype C and serotype K genes. The specific primer pairs of three genes mention in table 1. The PCR program of 16sRNA gene as following: initial denaturation at 94°C for 3 min, followed by 32 cycles of denaturation at 94°C for 30sec, annealing at 56°C for 30sec, extension at 72°C for 1.5min and final extension for 10 min (Dunbar *et al*., 2001); for serotype C, as following: initial denaturation at 96°C for 2 min, followed by 25 cycles of denaturation at 96°C for 15sec, annealing at 61°C for 30sec, extension at 72°C for 1 min and final extension for 9 min (Shibata *et al*., 2003); while for serotype K as following: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min and final extension for 9 min(Nakano *et al*., 2004).

. Electrophoresis of PCR product was carried out in 1.4% agarose gel and the detection of a 1500bp, 727bp and 294 bp band indicate a positive result for 16sRNA, serotype C and serotype K genes, respectively.

Table (1): Primers sequences used for genes amplification

Name	Primer sequence 5' - 3'	Size (bp)	reference
16sRNA	F: GGTTACCTTGTTACGACTT R: AGAGTTTGATCCTGGCTCAG	1500	(Dunbar *et al*., 2001)
serotype C	F:CGGAGTGCTTTTTACAAGTGCTGG R;AACCACGGCCAGCAAACCCTTTAT	727	(Shibata *et al*., 2003)
serotype K	F:ATTCCCGCCGTTGGACCATTCC R:CCAATGTGATTCATCCCATCAC	294	(Nakano *et al*., 2004)

RESULTS AND DISCUSSION:

The present study recorded that 24/100 (24%) of completely collected samples from patients suffering the dental caries identified as *S. mutans*. The dental caries disease was remained as predominant disease international, saddling the billions of persons, especially children with pain and then poorer quality of life and general health (Schwendicke *et al*., 2015), and due to different initial colonization and acidogenic properties, that important for detection and differentiation of this bacteria (Hirose *et al*., 1993). Also Abd Al-Zahra & Saleh, (2018) recorded that the *S.mutans* a main etiologic bacteria of human dental caries, and it was unusual

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pathogen of forming the biofilms on the rigid tissues of the human oral cavity. This results were corroborated with results of local study performed by Moza'al, (2019) showed that 25% of isolates identified as *S. mutans*.

The molecular results showed entirely isolates contain 16s RNA gene, as shown in Fig (1). The identification of *S.mutans* isolates by using molecular technique with 16S rRNA gene was further accurate than bacteriological and biochemical assays. Rampini *et al*., (2011) demonstrate that the PCR amplification of 16S rRNA gene was sensitivity, specific, and used for diagnosing the bacterial isolates given the negative results of on culture, also useful for identification of bacterial pathogens in patients pretreated with antibiotics. However, the conventional procedures for identification of bacteria were occasionally inaccurate, therefore this type of difficulties might be resolved by using molecular technique like PCR, that was simpler, more rapid, species-specific and accurate for identification of bacterial species. Thus, the PCR method would facilitate the process of identifying isolates from clinical samples and be more useful than the conventional methods. The results of study by Kothari *et al*., (2019) suggested that the PCR technique was appropriate for investigating the spreading of *S. mutans*; and the sequence of 16S rRNA gene was painstaking to be a useful aid in phylogenetic characteristics (Stackebrandt *et al*., 2002).

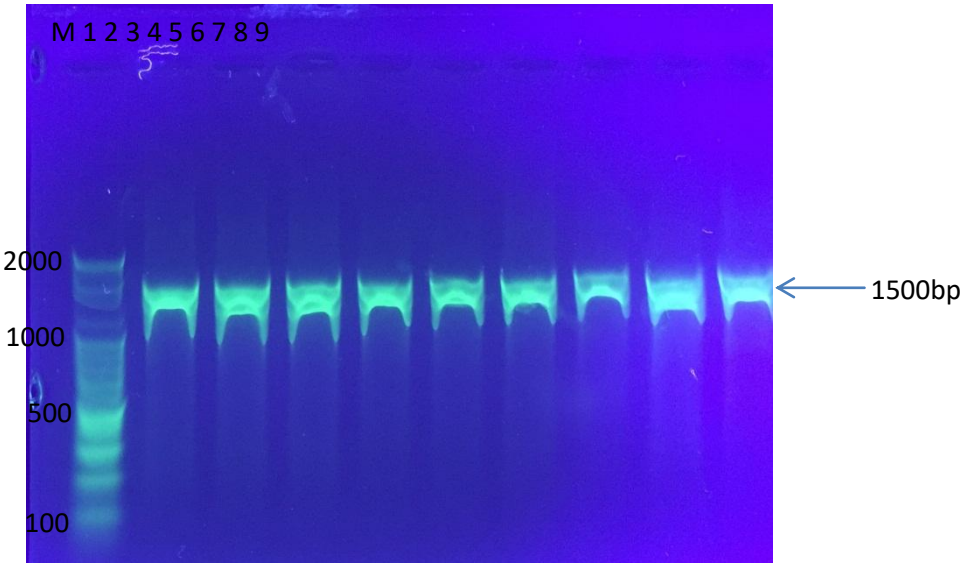

Fig.(1): Agarose gel electrophoresis of 16sRNA gene amplification, M: ladder, 1-0 : positive results.

The current study differenced from locally studies like: Abd Al-Zahra & Saleh, (2018) showed 28/33 (84.8) of *S.mutans* had this gene; while the study performed by Adhraa *et al*., (2016) mentioned that 22 isolates (100%) were identified as *S.mutans* by using 16S rRNA gene. The PCR results of serotype C and K genes showed only one isolates (1/24; 4.166%) of *S. mutans* had serotype C gene, while all isolates no had the serotype K gene. The serotype k of *S. mutans* isolates were revealed to be less vulnerable to phagocytosis process (Nakano *et al*., 2004). Also the identification of *S. mutans* by PCR technique, numerous methods had been proposed (Hoshino *et al*., 2004; Sato *et al*., 2003) and this technique for differentiation between serotypes c, e, and f had been developed (Shibata *et al*., 2003). The current results were differenced from the results of studies Nakano *et al*., (2004) and Arevalo-Ruano *et al*., (2014) showed that the frequency of serotype *k* was 5% and 4.2% respectively . Also the serotype K had been establish in saliva and blood (Nakano *et al*., 2010). The present results was agreed

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with results of study performed by Arevalo-Ruano *et al*., (2014) displayed the prevalence of *S. mutans* genotype C was (8.5%).

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