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Genotyping and Prevalence Rate of Dental Caries Infection Isolated from Patients of Dental Hospital

Muslim Abbas Allu[1], Hassan Mohsen Hassan[2]

[1]Nursing Department, Technical Institute of Zakho/ Duhok, University of Duhok polytechnic muslim.allu@dpu.edu.krd
[2]General and laparoscopic surgeon, FIBMS, College of medicine, University of Dohuk, E-mail: hassan.hassan@uod.ac
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ABSTRACT

This Study was done from the period July 2018 Until June 2019 In the Dental hospital in Duhok, to isolate and diagnoses Streptococcus mutans bacteria form dental caries infections, the sensitivity of isolates to antibiotics,193 samples taken from dental caries infections of were obtained. the results showed that 29(15%) from Isolates belonged to S. mutans bacteria. the isolates varied in their sensitivity to antibiotic, Where the sensitivity was higher for Tetracycline, Erythromycin, Streptomycin, Ciprofloxacin, Chloramphenicol, Amikacin, Gentamycin, Cephalothin, whereas resistance to antibiotics Amoxicillin, Ampicillin, effect than antibiotics against S. mutans Bacteria. This refers to the possibility of benefiting from those to minimizing the risks of resistance of bacterial, Genotypes c, e, f and k were detected isolates corresponded to female and male with dental 29)%15(by PCR. Of the 193 samples, caries, and 17 (4.95%) were positive for S. mutans genotype c, 4 (3.35%)genotype f, genotype k, and genotype c and k, respectively. The aim of the present study was to determine the prevalence of Streptococcus mutans and its genotypes in female and male aged 1-50years and more than50 years without dental caries

Keywords: Streptococcus mutans, Prevalence rate, Dental caries, Antibiotic. Genotyping.

INTRODUCTION

The most common pathogens in the oral cavity in healthy people include Streptococcus spp., Granulicatella spp., and Veillonella spp. (Aas et al., 2005; Dewhirst et al., 2010), which can also cause dental and oral-maxillofacial infections under certain conditions, such as caries, periodontitis, the mouth cavity is sterile at birth but quickly acquires bacteria from the environment, notably from the mother during the first feeding [Daniyan and Abalaka, 2011]. Dental caries affects 60 to 90% of schoolchildren and the great majority of adults, making it one of the most prevalent diseases in the world [Schwendicke et al., 2016]. It is also a very common oral illness in several Asian and Latin American countries, although it seems to be less prevalent and less severe in the majority of African countries [Oluremi et al., 2010]. S. mutans are a major cause of tooth decay, positive gram anaerobic bacteria, the higher pathogenicity is due to the ability of bacteria to biofilms formation, acid production, and tolerance of acids and saltsTaketo. (K.; Naoki. N.; Saori. Y.; Yoshiaki. T.; Jun. I.; Yasutaka. H.; Hidenobu. S. (2016). S. mutans is one of the most common types associated with dental caries, survival in the natural environment, Streptococcus mutans (SM) is considered one of the main organisms in plaque that contributes to the initiation of caries. Despite being ubiquitous in the oral cavity, SM prevalence often indicates caries susceptibility and poor oral hygiene. [Nyvad, B.1993].

Caries is defined as the localized destruction of dental tissues by bacterial activity [Ozdemir, 2014]. The primary causative factor in dental caries is bacterial plaque, which forms on tooth surfaces from naturally occurring oral flora [Maripandi et al., 2011]. Dental plaque forms continuously on tooth surfaces, and when bacteria in the plaque are exposed to fermentable carbohydrates, they produce acid. Acid lowers the pH in the mouth, which causes the enamel on the teeth to undergo a process called demineralization. Remineralization truly occurs over time; however, if demineralization outpaces remineralization, cavities develop in the teeth [Chambers, 2012]. Numerous studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. [C. Shukla, R. K. Maurya, V. Singh, and M. Tijare,2016, M. R. Costa, V. C. Silva, M. N. Miqui, T. Sakima, D. M. Spolidorio, and J. A. Cirelli, 2007] Little is known about the effect of removable orthodontic appliances on oral colonisation by mutans streptococci. [Giovanna B., Manuela P., A Giannotti, Fusao O., Maria R. 2002]

Mutans streptococci are classified serologically on the basis of the chemical composition of its exopolysaccharides, also known as cell surface serotype-specific rhamnose–glucose polymers (RGPs). Six genetic groups and seven serotypes are recognized: S mutans serotypes c, e, f, k; S. sobrinus serotype g, S. downei serotype g, S. rattus serotype b, S cricetus serotype an and S. macacae serotype c (isolated from monkeys) (Sánchez L, Acosta E.2007and Nakano K, Nomura R, Nakagawa I, Hamada S, Ooshima T.2004). Serotype k has been found in patients with infective endocarditis, a complication that can lead to heart valves replacement (Martin M, Longman L, Forde M, Butterworth M.2007). Importantly, the frequency of serotype k was significantly higher (75%) in samples of dental plaque from patients with infectious endocarditis compared with oral samples from healthy patients (20%), suggesting a possible association with the development of cardiovascular disease (Nakano K, et, 2007, Nakano K, et2008 Nakano K, et, 2010, Nakano K, et,2007).

The present study was to determine the prevalence of Streptococcus mutans and its genotypes in female and male aged 1-50years and more than50 years without dental caries

MATERIAL AND METHODS

193 samples of dental caries infections were collected from patient in the Dental hospital in Duhok, During the period from July 2018 Until June 2019, For Isolation of Streptococci mutans Bacteria were spread the samples on the surface of Mitis Salivarius Bacitracin Agar plates using sterile swabs, inoculated plates were incubated an aerobically for 48 hours at 37 °C, identification of bacteria on the base of Morphology, Cultural characteristic (Tadesse, A. and Alem, M. 2006).

The sensitivity of S. mutans isolated were do against 10 Antibiotic according to Disc Diffusion Method (Morello, J.; Mizer, H. and Granato, P. 2006). And determination the minimum inhibitory concentration (MIC) of Tetracycline and Erythromycin for 29 S. mutans isolates, and compare the results with the (CLSI. 2014).

Genotypes c, e and f of isolates identified as S. mutans were detected using the multiplex PCR (Shibata Y, Ozaki K, Seki M, Kawato T, Tanaka H, Nakano Y, et al.2003). Primers used in the PCR assay were SC-F, SC-R, SE-F, SE-R, SF-F, SF-R. Each 50 µL reaction mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 2 mM de MgCl2, 0.2 mM of each dNTP, 0.5 µM of each oligonucleotide primer, 1U Taq DNA polymerase and 50-100ng DNA. Samples were amplified in a thermocycler iCycler (Bio-Rad). PCR conditions were described previously (30). S. mutans genotype k was identified by conventional PCR using a set of specific primers CEFK-F and KR based on the sequence of genes rgp F and rgpE and the PCR conditions previously described.

Statistical Analysis Statistical analysis was carried out using to analyze the data statistically and determine the statistical differences on the base SPSS program.

RESULTS

The study involved 193 samples of Dental caries for isolation and diagnosis of S. mutans for age groups, the age group was (1-5) years Most vulnerable group where the number was 55(28.50%), and the lowest age group more 50 years 11(5.69%). Statistical analysis showed a significant difference between age groups of tooth decay and no significant difference in relation to the number of isolates and age groups, the percentage and number of dental caries caused by were 98 (50.78%) female higher than those of males 95(49.22%) Table1.

Table 1: Number and Percentage of Dental caries & isolates of S. mutans bacteria by age and sex.

Age	Dental caries		S.mutans	
	Number	%	Number	%
1-5	55	28.50	9	31.04
6-15	44	22.79	7	24.14
16-25	36	18.66	6	20.69
26-40	32	16.58	4	13.79
41-50	15	7.78	2	6.89
More than 50	11	5.69	1	3.45
Total	193	100	29	100
Sex	Dental caries		S.mutans	
	Number	%	Number	%
Male	95	49.22	12	41.38
Female	98	50.78	17	58.62
Total	193	100	29	100

Check the sensitivity of 10 antibiotics of all bacterial isolates. All S. mutans isolates showed 100% complete resistance to Amoxicillin (86.20%), Ampicillin (82.75%), and. and the sensitivity of Gentamycin (86.20%), Tetracycline (86.20%), Erythromycin (89.66%), Streptomycin (68.96%), Ciprofloxacin (82.75%),

Cephalothin (75.86%) and Chloramphenicol (72.41%), and medium resistance for Amikacin (75.86%), Table2.

Table 2: Sensitivity test of S. mutans isolate to antibiotics.

Antibiotics	Resistant		Sensitive	
	Number	%	Number	%
Amoxicillin	25	86.20	4	13.80
Ampicillin	24	82.75	5	17.25
Cephalothin	7	24.14	22	75. 86
Gentamycin	4	13.80	25	86.20
Streptomycin	9	31.04	20	68.96
Chloramphenicol	8	27.59	21	72.41
Tetracycline	4	13.80	25	86.20
Ciprofloxacin	5	17.25	24	82.75
Amikacin	7	24.14	22	75.86
Erythromycin	3	10.34	26	89.66

genotypes in female and male with and without caries, Overall, prevalence of S. mutans was 15 % (Table 3). In the group without dental caries S. mutans was not detected. Prevalence of S. mutans genotype c 17 (4.95%) genotype f and genotype k 4

(3.35%) The subjects harboring S. mutans genotype c, had an average DMF-T of 6.7

(SD ± 1.6) and the subjects with genotypes f, k and co-infection of c and k had a DMF-T of 6. Oral examination of one patient with genotype c and k showed the presence of dental caries in permanent teeth.

Table 3. Streptococcus mutans and its genotypes in Female and male with and without dental caries.

Groups	No.(%)
Female and male with dental caries	193)%100(
S. mutans detected	29)%15(
Genotype c	17 (4.95%)
Genotype f	4 (3.35%)
Genotype k	4 (3.35%)
Genotype c and k	4 (3.35%)

DISCUSSION

Number and Percentage of Dental caries & isolates of S. mutans bacteria aged 1-5 was higher comparison with other age groups and infected percentage in female was a higher comparison with male The reason for the high rate of dental caries in children is the weakness of the levels of prevention of teeth in addition to the availability of sugars in foods and liquids and even In medications they eat especially if they are prolonged and continuously, they also practice returning children as well as low rates of breastfeeding by mothers and the spread of artificial feeding, which has a significant impact on the appearance of caries in children at an early age ,These results are similar to those found by(AL-Sultani et. al. AL-Sultani. H.F.F; AL-Azawi. A.M; and ALShammari. H.A.2013). in Babel governorate when they found that the infection was higher in children with aged (5-7) years, the results were consistent with the findings of Khamise (Khamis, M.H. 2010).in Najaf governorate, which found that males (25.2%) in permanent teeth and higher in females (31.2%), the results did not agree with the findings of the researcher AL-Mosawi (AL-Mosawi. S. M. 2014). Most bacterial isolates showed high resistance to more than one antibiotic, due to the ability of bacteria to produce enzymesthe findings of the study ALmosawi (ALMosawi. S. M. 2014). The isolates showed sensitivity to Gentamycin, Tetracycline, Erythromycin. Table 5. The higher resistance of bacterial isolate may be due to the wide and random usage of several antibiotics by patients, and resistant factors with genetic and non-genetics origins that isolates possess them. The isolates showed higher resistance to Amoxicillin and Ampicillin.

prevalence of S. mutans genotype c 17 (4.95%) genotype f and genotype k 4 (3.35%) was lower, this study reports for the first time the presence of genotype k in Dohuk. Prevalence of genotype k was 8 (6.70%), similar to reports from Japan and Thailand (1-5%) (Nakano K, et.2004, Nakano K, et. 2007, Taku Y, Kaseko T., et.2011 Lapirattanakul J, Nakano K, et.2009), but lower than reported in México (16.9%) (Espinosa L, et.2012). Streptococcus mutans serotype k has been found in saliva and blood isolates (Nakano K, et.2004, Nakano K, et.2010). and its ability to survive for longer periods in blood, due to resistance to phagocytosis (Tsuda H, et.2000). Also, this new serotype has been related with bacteremia, systemic inflammation and was described as one of the risk factors of infective endocarditis and hemorrhagic stroke (Nakano K, et.al.2011). Thus, future studies in Colombia are required for understanding the role of S. mutans genotype k in the development of cardiovascular infective disease.

CONCLUSION

This study may be useful for the development of oral care products such as dental cleaning materials and can be used a mouthwash and treatment of tooth decay diseases and can be used as alternative treatment of antibiotics and prevent the side effects that may be result from antibiotics usage as a treatment. Thus, future studies are required for understanding the role of S. mutans genotype k in the development of cardiovascular infective disease.

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