Genotypic Diversity Of *Streptococcus Mutans* in a Group of Caries-free and Caries active Normal and Mentally Retarded Egyptian children

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Abstract: ***Aim:*** To gain insight into diversity of *Streptococcus mutans* genotypes associated with different degree of caries among normal children and mentally handicapped children to help for planning a rational strategies for management and prevention of caries risk. ***Subjects and Methods:*** Genotyping for148 strains isolated from saliva of 58 Egyptian children caries free and caries active (30 Normal &28 Mentally Retarded child) aged 6-14 years using RAPD using three arbitrarily primers (P1,P2, P3). (RAPD) random amplified polymorphic DNA analysis was preferred as a valuable tool in *S.mutans* epidemiological studies, by virtue of its rapidity, efficiency and reproducibility in generating genetic fingerprints of *Streptococcus* isolates. ***Results:*** No correlation was observed between genotype numbers and *S.mutans* salivary levels.All children with caries active & free had colonized the same genotype (RAPD with P2).The other two genotypes RAPD patterns encoded P1 & P3 were separately carried by 68.9% & 86.2% respectively.17.2% from all subjects colonized with both genotypes RAPD patterns using P2&P3 together ,13.8% (8/58) carried the genotype related to P2 only & 68.9% (40/58) carried all genotypes together. Result highlighted that bacterial interactions might promote variants growth. Presence of the *S.mutans* genotype encoding P2 might initiate genotype encoded to P3 growth & both genotypes encoded to P2&P3 were relevant to genotype encoded P1 growth. The UPGMA method was used to group the units & instruct the Dendrogram. ***Conclusion:*** Genotypic finger printing of *Streptococcus mutans* can be used as a tool for assessment of caries risk for children from in order to targeted our effort to those for preventive approaches. In addition, Using RAPD contributed to precise picture of some virulent traits of *S. mutans* genotypes in the oral cavity . Bacterial interactions can play an important role in their virulence.

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Key words: *Streptococcus mutans*, PCR (arbitrarily primed PCR), RAPD, Dental Caries.

1.Introduction

Dental caries is a transmissible infectious disease in which *Streptococci mutans* (MS) play the major role . As in many infectious diseases, colonization by pathogens is required before the disease can occur. MS are generally considered to be the principle etiological agent of dental caries, Loeshe,(1986).As well as, it may colonize the host and induce dental caries more than other strains, Alaluusua, et al., (1996); Becer et al., (2002).

There is a range of virulence factors important for the establishment of MS in the complex microbial community of dental bio-film .Studies of virulence factors of *S. Mutans* and their correlation with species diversity are fundamental to understanding the role played by colonization by different genotypes in the same individual , and the expression of characteristics that may or may not influence virulence capacity and survival ability under different environmental conditions ,(Klien et al.,(2004)

It had been observed that children harbor one to five distinct genotypes of *Streptococcus mutans* at different ages; Emanuelsson, et al., 1998;Kozal, et al., (1999);Mattos-Graner, et al., (2001).

Information on the stability of colonization with this microorganism in children could help elucidate the natural history of the development of caries, Klien, et al., (2004).

Streptococcus mutans exhibits extensive genotypic diversity, few data are available about the stability of the genotypes detected at the time of initial acquisition. Previous studies suggested that early colonization of MS strains might be stable in the mouth for many years, although some genotypes

detected in childhood and could not be recovered in later years,(Emanuelsson,et al.,(2000); Lembo, et al., (2007).

Klien et al., (2004) identified a total of 52 distinct genotypes for normal children, but mentally retarded may have a less number of them. However, a tendency toward effective stability of genotypes may differ from child to another.

PCR based techniques are widely used effectively in differentiating *S. Mutans* colonies and distinguish a specific strain from others in the oral cavity, Paster, et al.,(2001). These methods typically use broad – range or universal primers to amplify DNA that has been extracted directly, Lesser, et al, (2002).A genetic finger-printing method for characterizing bacterial isolates.Several investigators suggested using random amplified polymorphic DNA (RAPD) analysis, for characterization of human isolates of *Streptococci mutans* bacteria as an effective method in differentiating between *Streptococcus mutans* colonies. Also it is reproducible , rapid, and considered to be a valuable tool in *Streptococcus mutans* epidemiology and transmission studies, Troung (2000); Emanuelsson,(2003).

Mental disability is an essential factor for harboring dental plaque initialing dental caries especially when there is bad oral hygiene and improper diet counseling. In addition, the degree of mental retardation IQ may be one of the factors influencing dental health of those children with special needs ,Gabre(2000). Nevertheless, not all the mentally handicapped had a high caries index for example those having Down syndrome might show less caries index, Abbas & Rashed (2006). Recently a great effort will be targeted to those children with special needs because not all of them have had the chance for dental advice. So they are in need for well organized preventive programs targeted to those at high risk to dental caries.

It is evident that incomplete knowledge of the oral microbial population will contribute to extension of the caries lesion enforces an empirical approach to therapy rather than specific antimicrobial therapy that might allow more conservative treatment options. Because of all controversies found in etiological factors of dental caries, where not all children having proper oral hygiene and proper diet counseling might show low caries index but may be because of the genotypic diversity of *Streptococcus mutans* present in their oral cavities, Chhour, et al., (2005).

Therefore, this study was carried out to gain insight into the diversity & abundance *of S. mutans* genotypes associated with caries-free and caries-active normal and mentally retarded children in order to help for planning a rational strategies for management and prevention of caries risk. Hope to be implemented from their primary primary stage of life.

2. Materials and Methods

2.1. Materials:

2.1.1 Subjects:

Subjects studied were 58 Egyptian children, with age range 6-14 years. Thirty normal child selected from the out patients clinics of Pediatric Dentistry and Dental Public Health Department, Faculty of Oral & and Dental Medicine, Cairo University. Twenty eight mentally retarded (MR) child with mild retardation regarding to their IQ Intelligent Quotient, data were collected from their file, they were selected from the outpatient clinic of the Human Genetics Department, National Research Centre .All children were subjected to dental examination. The study was approved by Ethical Committee and informed consents were obtained from all children's parents whom children were participating in the study.

2.1.2. Samples:

Stimulated saliva was collected in sterile tube containing sterile 0.9% Na Cl solution.

2.1.3.Microbiological Media:

a-Mitis-Salivarius agar supplemented with 20% sucrose and 0.2 U bacitracin (Sigma Chemical Co.)
b-Nutrient broth (Difco)

2.1.4. Primers:

10-mer primers (Kit A, Roth, Germany) were screened for the amplification of template DNA using Random Amplified Polymorphic DNA (RAPD) Reactions. Table (1) described code and nucleotide sequence of primers used and G+C content, in isolates studied *"S.mutans"*

Table 1. Code and nucleotide sequence of primers used and G+C content in isolates studied *"S.mutans"*

Primer	5'-sequence-3'	G+C(%)
P1	CAGGCCCTTTC	70
P2	TGCCGAGCTG	70
P3	CCCGTCAGCA	70

2.2. Methods:

2.2.1. Dental examination:

Dental caries examination for each child was performed by one of the authors (H.A) according to the criteria of (WHO,1997) ,they were divided into two groups :

a- Caries free group: Who had no caries i.e. their caries index=0.

b-Caries active group: who had more > 4 decayed or filled tooth ,&missed due to caries or indicated for

extraction due to caries using dmft for deciduous dentition & DMFT for the permanent teeth.

2.2.2. Microbiological Study:

All samples were transported refrigerated to the laboratory to be processed within 2 hours of collection. Samples were gently shaken and diluted in tenfold steps with repeated homogenization on vortex mixer for 10 seconds between successive dilution. Aliquots of 0.1 ml of the dilution were inoculated, in duplicate onto Mitis-Salivarius agar plates supplemented with 20% sucrose and 0.2 U bacitracin. Plates were incubated at 37° C for 48 hours in an atmosphere of 10% CO2. There-after, a stereoscopic microscope was used to verify *S.mutans* presence.
Each single –spore *S.mutans* isolate was grown for 6 days at 28°C in 5ml nutrient broth (Difco) in 15 ml falcon tubes. *S.mutans* were collected by filtration ,and ground with mortar and pestle in liquid nitrogen and stored quickly at - 20°C .

2.2.3. Genomic DNA extraction of *S.mutans*

DNA was extracted from each single –spore isolate by PCR with previously described primers *(Oho,et al., 2000).*

2.2.4 RAPD using arbitrary primers

DNA amplifications for screening of final typing were carried out in buffer (50 mM KCl, 1.5 mM MgCl2 , 10 mM Tris HCl, pH 8.8) containing 200 mM (each) dATP, dCTP, dGTP, and dTTP; 0.50 mM primer; 2.0 U of *Taq* DNA polymerase (Promega); and 50 ng of template DNA. The temperature cycling program used with a thermocycler (Gradient thermalcycler, Biometra, Germany) was as follows: 2 initial cycles consisting of 94°C for 4 min, 35°C for 2 min, and 72°C for 2 min, followed by 35 cycles consisting of 94°C for 30 s, 35°C for 1 min, and 72°C for 2 min and a final extension step consisting of 72°C for 5 min. A negative control (without DNA) was included in each AP-PCR run. Amplicons obtained were compared after electrophoresis on an agarose 1.5% gel, ethidium bromide was used in staining.. A 100 bp DNA ladder served as a molecular –size marker in each gel .Individual Ap-PCR amplicons were marked and individual bands were visually compared. In order to assess the colonal diversity of *S.mutans* isolates, a matrix of similarity using Dice's similarity coefficient was built based on data obtained with the three primers in RAPD reactions. A Dendrogram was constructed using UPGMA method with the aid of NTSYS [numerical and multivariate analysis system program (Exeter Software, Setauket ,Ny) *Williams, et al., (1990)*].

3. Results

The present molecular study was undertaken to define the profile of *S.mutans* associated with dental caries. Saliva of a total of 58 N and MR children (10 normal child, 8 mild grade mentally retarded child with caries free where their caries index was = 0) and (20 normal child, 20 MR child with high caries activity where caries index was > 4 respectively. 148 *S.mutans* isolates associated with their activity, their genotyping were screened
S. mutans polymorphisms were detected by comparing amplification pattern coded by primers P1.P2, and P3. The analysis of these profiles generated a Dendrogram that separated the isolates into groups of high genetic similarity based on reliable bootstrap values.

Our results indicated that it was a convenient and reliable way for using PCR methods with a different range of universal primers to amplify DNA as a good discriminative ability in differentiating between *S.mutans* genotypes.

Although high variations in the spectra of the clinical strains tested in the present study, statistical analysis revealed that there was no significance between spectrum of activity and caries experience of the host or the level of *S.mutans* in the oral cavity of normal children and Mentally retarded children as shown in (table 2 and 3) respectively

Table 2. Mean , SD and P value of *S.mutans* level for normal children with free and high caries scores

Normal children	Mean and SD of *S.mutans*	P
Caries free children	119158± 1731.995	
Caries active children	29425± 472.003	
		0.456

P>0.05

Table 3. Mean , SD and P value of *S.mutans* level for Mentally retarded children with free and high caries scores

Mentally retarded children	Mean and SD of *S.mutans*	P
Caries free children	2534± 4977.52	
Caries active children	1176 ± 161.03	
		0.601

P>0.05

On translating the Dendograms of the UMAGE codes of isolates which was represented for each group whether normal or mentally retarded, caries free or caries active codes were described as follows in table (4) , Fig.(1)

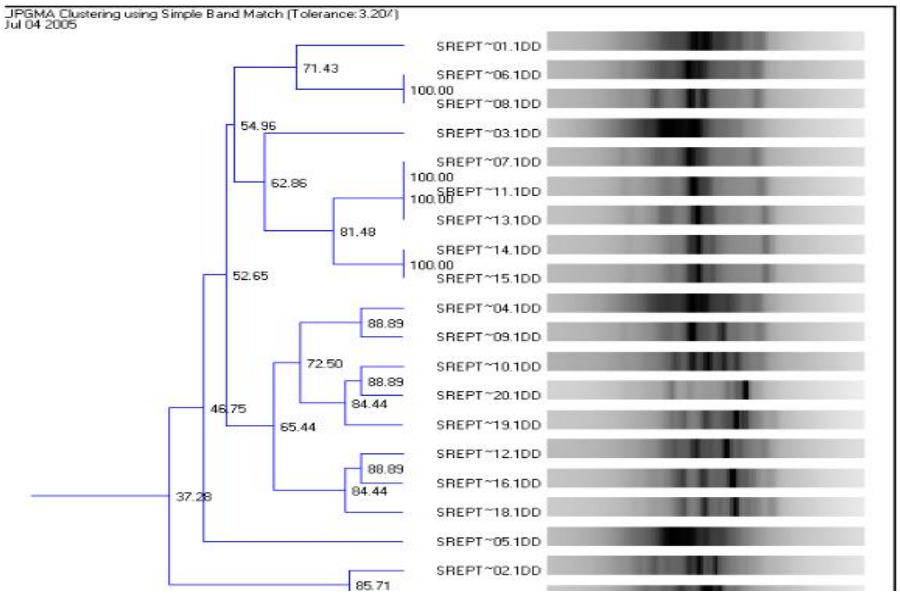

Fig. 1. Dendrogram using UPGMA cluster analysis using simple band match (Tolerance 3.20%) for *S. mutans* strains related to RAPD amplification pattern using primer P2 isolated from saliva of normal and mentally retarded subjects

With regard to the distribution of the different genotypes within different caries experience represented in table (5) showed that children were colonized with the same *S.mutans* genotype (RAPD pattern coding the primer P2.Whereas, those coding to primers P1 & P3were colonized in 68.9%(40/58) and 86.2% (50/58)respectively . On the other hand, A genotype of *S.mutans* (RAPD pattern encoded by primer P2 was detected in all individuals with caries free and caries active.

Amplification pattern encoded by primer P1 colonized oral cavity of caries active and caries free was respectively for the two group of children 70%(14/20), 62.5%(5/8) & 80%(16/20)& 50% (5/10).Moreover,, pattern encoded by primer P3 colonized them by 100% (20/20), 6/8 (75%)& 15/20 (75%),9/10(90%) respectively ,as presented in table (6)

Table 4. Code of isolate for each group

Normal caries active child	Normal caries free child	Mentally retarded caries active child	Mentally retarded caries free child
5,8,10, 16,18,	3,12,14, 15,19,20,	1,6,21	2,4,7,9, 11,13,17,

Table 5. The total Number of different genotypes found in all subjects

	RAPID amplification pattern using primers (p1,P2,P3		
	P1	P2	P3
No. of Individuals	68.9% (40/58)	100% (58/58)	86.2% (50/58)

Table 6. Distribution of the RAPD amplification pattern using primerP1&P2&P3 among normal and MR children with caries active and caries free

Distribution of the RAPD amplification pattern using primerP1&P2&P3	No. of genotypes isolated from the two groups examined			
	Isolated from Mentally retarded Children		Isolated from Normal Children	
	Caries active (N=20)	Caries free (N=8	Caries active (N=20)	Caries free (N=10)
P1	70% (14)	62.5% (5)	80% (16)	(50%) (5)
P2	100% (20)	100% (8)	100% (20)	(100%) (10)
P3	100% (20)	75% (6)	75% (15)	(90%) (9)
Total	36.5% (54/148)	12.8% (19/148)	34.5% (51/148)	(16.2%) (24/148)
	148			

Results denoted that, presence of the *S.mutans* genotype encoding P2 might promote genotype growth of other encoded to P3. As well as, both genotypes encoded to P2 & P3 were relevant to genotype coded P1growth. This notice was suggested due to notice in table (7) were 17.2% of all subjects colonized with both genotypes RAPD pattern using primers P2&P3 together, 13.8% (8/58) carried the genotype related to P2 only & 68.9% (40/58) colonized with all genotypes together(fig 2,3,4).

Table 7. Distribution of the RAPD amplification pattern using primer P1,P2,P3 among all subjects

Distribution of the RAPD amplification pattern using primers used	Number of individuals
All primers	**40(68.9%)**
P2	**8(13.8%)**
P2&P3	**10 (17.2%)**

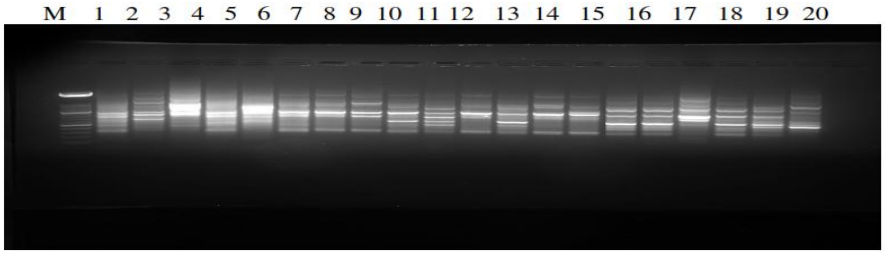

Fig 2. The Random Amplified Polymorphic DNA (RAPD) for *S.mutans* isolated using primer P1
M molecular weight marker (100 base pairs DNA Ladder .Roche)
Lanes 8,12,14,15,20 represent amplification pattern obtained from S.mutans isolated from Caries free normal children
Lanes 3,5,10,16,18 represent amplification pattern obtained from S.mutans isolated from Caries active normal children
Lanes 2,4,7,9,11,13 represent amplification pattern obtained from S.mutans isolated from Caries free mentally retarded children.
Lanes 1,6,17,19 represent amplification pattern obtained from S.mutans isolated from Caries active mentally retarded children

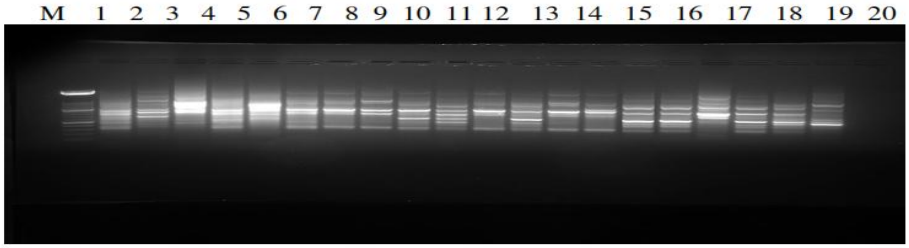

Fig 3. The Random Amplified Polymorphic DNA (RAPD) technique was performed to study DNA of the *S.mutans* isolated using primer P2
M molecular weight marker (100 base pairs DNA Ladder .Roche)
Lanes 8,12,14,15,20 represent amplification pattern obtained from S.mutans isolated from Caries free normal children
Lanes 3,5,10,16,18 represent amplification pattern obtained from S.mutans isolated from Caries active normal children
Lanes 2,4,7,9,11,13 represent amplification pattern obtained from S.mutans isolated from Caries free mentally retarded children.
Lanes 1,6,17,19 represent amplification pattern obtained from S.mutans isolated from Caries active mentally retarded children.

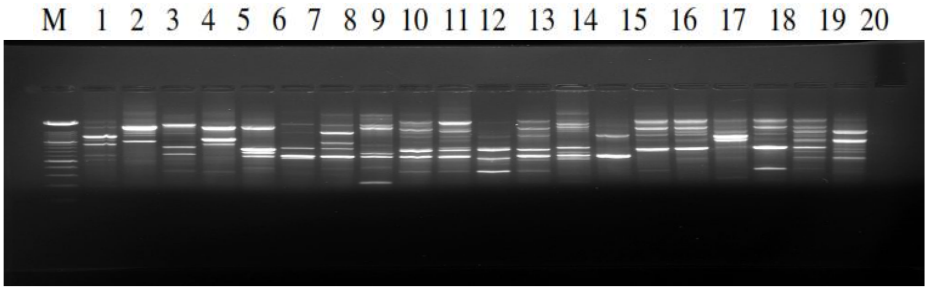

Fig 4. The Random Amplified Polymorphic DNA (RAPD) technique was performed to study DNA of the *S.mutans* isolated using primer P3
M molecular weight marker (100 base pairs DNA Ladder .Roche)
Lanes 8,12,14,15,20 represent amplification pattern obtained from *S.mutans* isolated from Caries free normal children
Lanes 3,5,10,16,18 represent amplification pattern obtained from *S.mutans* isolated from Caries active normal children
Lanes 2,4,7,9,11,13 represent amplification pattern obtained from *S.mutans* isolated from Caries free mentally retarded children.
Lanes 1,6,17,19 represent amplification pattern obtained from *S.mutans* isolated from Caries active mentally retarded children.

4. Discussions

In this study we have applied RAPD analysis directly to cultures of *Streptococci mutans* for characterization of isolates .Our results showed that this is convenient and reliable way in identifying *Mutans Streptococcus* isolates directly from cultures, Saarela, et al.,(1996) ;Truong, et al. (2000); Gronroos et al.,(2000) suggested the same conclusion and added that the technique is suitable for epidemiological studies on *S.mutans*.

Although high variations in the spectra of the clinical strains tested in the present study, statistical analysis revealed that there was no significance between spectrum of activity and caries experience of the host or the level of *S.mutans* in the oral cavity of normal children and Mentally retarded children as shown in(table 3 and 4) respectively. This may be due to the increased numbers of isolates taken in this study compared with the previous studies, so when we analyzed a great number of isolates, increase in the possibility of detecting different genotypes reflecting no significance for a specific one. Many studies analyzing genotypic diversity within oral bacterial species have been showing similar results ; Li et al.,(1994); Gronroos et al.,(1998) ;Mattos et al.,(1998) ;Longo, et al.,(2003) showed that it is useful in identifying the colones persisted in the oral cavity.

Genetic differences may relate to differences in virulence between MS strains. One important characteristic of Mutans in promoting dental caries development is the ability to adhere firmly to the tooth in the presence of sucrose. This will be noticed with regard to the distribution of the different genotypes within different caries experience revealed from our results in table 4. Supported by ALaluusua et al., (1996) .They suggested that caries active children with high sucrose consumption carry greater ribotype diversity of *S. mutans* compared with caries free children..In this respect Lembo et al., (2007) also expressed the presence of *S. mutans* in subjects with low caries experience due to the adherence firmly of it to tooth surface in the presence of sucrose , which action mediated mainly by the enzymatic action of glucosyl-transferase enzymes and that adhesion was considered fundamental for the virulence of *S.mutans* in pathogenesis of dental caries. On the other hand, Kreulen,et al., (1997) showed no correlation between caries activity and genetic diversity. We believe that this study has clearly illustrated the complexity of *Mutans Streptococcus* colonization in the same cavity where P2was found mostly in all children's oral cavities. Other action might be the nonspecific factors

of salivary constituents is mucosal immune system ,Li and Caufield (1995). Although A genotype of *S.mutans* RAPD pattern encoded by primer P2 was detected in all individuals with caries free and caries active Amplification pattern encoded by primer P1 colonized oral cavity of caries active and caries free was respectively for the two group of children. Moreover, pattern encoded by primer P3 colonized also in the two groups. This finding was augmented by Emanuelsson, et al., (2003) as they reported the possibility of several different genotypes of *S.mutans* to colonize a tooth site, and also the same genotype colonizes several sites in the same oral cavity. Same result was found when P1 promote genotype growth of other encoded to P3. As well as, both genotypes encoded to P2 & P3 were relevant to genotype coded P1growth. This notice was suggested due to notice in table (7) were 17.2% of all subjects colonized with both genotypes RAPD pattern using primers P2&P3 together,13.8% (8/58) carried the genotype related to P2 only & 68.9% (40/58) colonized with all genotypes together.

Thus, when only one genotype is present in an individual, this meant that this type would be integrated with another increase its virulence that is way not all children eating similar cariogenic diet showed same caries experiences. Moreover, for those mentally retarded children with a more complex flora, it is possible to miss rare or transient genotypes in a single pooled sample. So further studies should be done to correlate the genotyping of *Streptococcus mutans* with the cariogenic diet eaten by those children, as well as, their oral hygiene measures and rate of plaque formation.

5. Conclusions

1) The finding of the present study suggested that the primer used for genotyping of *Streptococcus mutans* were suitable for identification of genotypic diversity of those microorganisms in saliva of normal and mentally retarded children . Nevertheless, further studies should be done to evaluate large numbers of individuals with different population.
2) Presence of a certain *S.mutans* genotype contributed to a precise picture to some virulence traits of *Streptococcus mutans* variant in the oral cavity within a sample of different categories among Egyptian children .And highlighted to the bacterial interaction which might lead to initiation of growth of other variants.
3) There is no significance between the two groups either normal or mentally retarded both will have common isolates of *Streptococcus mutans* . So further studies is needed to correlate their salivary chemistry with the bacterial genotypes in their saliva.
4) Genotypic finger printing of *Streptococcus mutans c*an be used as a tool for assessment of caries risk for children from in order to targeted our effort to those for preventive approaches.
5) Most of dental practice is concerned with the treatment of the children once the clinical dental disease has emerged , much less opportunity or capacity exists to prevent disease development at its preclinical stage .An improved modern genetics intervention is needed to predict caries risk before its occurrence.

References

1. Loesche, WJ. (1986): "Role of *Streptoccocu*s in Human dental decay." *Microbial Rev*. 50, 353-380.
2. Alaluusua, S., Mättö, J. , Grönroos, L., Innitä, S., Torkko, H., Asikainen, S. , Jousimies- Somer, H. and Saarela.M. (1996): "Oral colonization by more than one clonal type of *mutans Streptococcus* in children with nursing-bottle dental caries." *Arch. Oral Biol.* (41):167-173
3. Alaluusua, S., Gro¨ nroos, L., Zhu, X., Saarela, M., Matto¨ , J., Asikainem,S. & Fukushima, K.: (1997). "Production of glucosyltransferases by clinical *mutans Streptococcal* isolates as determined by semi-quantitative cross-dot assay." *Arch Oral Biol* (42), 417–422.
4. Becker M.R., B.J. Leys, E.J. Moeschberger, M.L. Kenyon, S.G. Galvin, J.L. Boches, S.K. Dewhirst, F.E. and Griffen, A.L. (2002): "Molecular analysis of bacterial species associated with childhood caries." *J.Clin.Microbiol*. (40), 1001-9.
5. Klein MI, Florio FM Pereira AC, Hofling JF, Goncalves RB (2004): "Longitudinal study of Transmission diversity, and stability of Streptococcus mutans and Streptococcus sorbrinus genotypes in Brazilian nursery children." *J Clin Microbiol* 42, 4620-4626.
6. Kozal, K. Nakayama, R.,Tedjosasongko, U.,Kuwahara, S.,Suzuki, J.,Okada, M.,and Nagasaka, N. (1999): "Intra-familial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission." *Microbiol. Immunol*.43:99-106.
7. Mattos-Graner, R.O.,Li, Y.,Caufield, P.W.,Duncan, M.,Smith, D.J. (2001): "Genotypic diversity of *mutans Streptococci* in Brazilian nursery children suggests horizontal transmission." *J.Clin.Microbiol*. (39):2313-16.
8. Lembo, F.L., Longo, P.L., Ota-Tsuzuki, C., Rodrigues, C.R.M. , Mayer,M.P.A. (2007): Genotypic and phenotypic analysis of *Streptococcus mutans* from different oral cavity sites of caries-free and caries-active children. Oral Microbiol Immunol.;22 (5):313-17
9. Chhour, K.L.,Nadkarni, A.M.,Byun, R.,Martin, E.F.,Jacques, A.N.,and Hunter, N. (2005): Molecular

analysis of microbial diversity in advanced caries J.Clin. Microbiol.
10. Gronroos L, Saarela M,Matto J,Tanner-Salo U ,Vuorela A, Alaluusuna S Mutacin,(1998): Production by Streptococcus mutans may promote transmission of bacteria from mother to child . Infect Immun. 66;2595-2600.
11. Gronroos L, Alaluusua S. (2000): Site-specific oral colonization of mutans streptococci detected by arbitrarily primed PCR fingerprinting .Caries Res. 34:474-480.
12. Emanuelsson, I.R.,Li Y.,and Brattall, D.(1998): Genotyping shows different strains of mutans streptococci between father and child within parental pairs in Swedish families. Oral Microbiol. Immunol.(13):271-77
13. Emanuelsson IR, Thornqvist E (2000): Genotypes of mutans streptococci tend to persist in their host for several years. Caries Res 34, 133-139.
14. Emanuelsson, R.M. , Carlsson, P. , Hamberg, K. , Bratthall, D. (2003): Tracing genotypes of mutans streptococci on tooth sites by random amplified polymorphic DNA (RAPD) analysis Oral Microbiol Immunol. Feb;18 (1):24-9.
15. Hala M.A.,Mervat A.R (2006): Assessment of Dental and Oral Health condition of Mentally Handicapped children in relation to their parent Knowledge, Attitude and Behavior toward dental health .presented in the first international conference of the Egyptian Society of Pediatric Dentistry &children with special Needs& accepted for publication in Egyptian Dental Journal March.
16. Li Y,Caufield PWm (1998): Arbitrarily primed polymerases chain reaction fingerprinting for the genotypic identification of mutans streptococci from humans. Oral Microbiol Immunol.13 :17-22.
17. Gabre, P.(2000): Studies on oral health in mentally retarded adults Swed Dent. J. Suppl. (142):1-48.
18. Kreulen,C.M.,deSoe,H.J.,.Hogeveen.R.,Veerkamp,JS. J.,(1997):*Streptococcus mutans* in children using nursing bottles. ASDC.J Dental Child.(64) :107-111.
19. Lesser,T.D.Amenuvor,J.Z.,Jensen,T.K.,Lindecrona,R. H.,Boye, M.,and Molter, K(2002): Culture-independent analysis of gut bacteria : the pig gastro-intestinal tract microbiota revisited. App.Environ.Microbiol.(68):673-90.
20. Longo,P.L.,MattosGraner,R.O,Mayer,M.P.A., (2003): Determination of mutacin activity and detection of mutA genes in Streptococcus mutans genotypes from caries –free and caries active children. Oral Microbiol,Immunol.(18); 144-49.
21. Li,Y.,CaufieldP.W.,(1995): The fidelity of initial acquisition of streptococcus mutans by infants from their mothers. J Dental Res.(74): 681-78.
22. Oho, T., Yamashita, Y., Shimazaki, Y., Kushiyama, M., and Koga, T. (2000): Simple and rapid detection of *Streptococcus mutans* and *Streptococcus sobrinus* in human saliva by polymerase chain reaction. Oral Microbiol. Immunol. (15): 258-62.
23. Paster ,B.J.,Boches, S.K.,Galvin, J.L.,Ericson, R.N.,Lau, C.N., Levanos, V.,Sahasrabudhe, A., and Dewhirst F.E.(2001): Bacterial diversity in human sub gingival plaque. J. Bacteriol. (183): 3770-83.
24. Saarela, M., Hannula, J., Mättö, J., Asikainen,S. and Alaluusua,S. (1996): Typing of mutans streptococci by arbitrarily primed polymerase chain reaction. Arch. Oral Biol. (41):821-26.
25. Smith,D.G.King,W.F.,Akita,H.,Taubman,M.A. (1998): Association of salivary immunoglobulin A antibody and initial *mutans streptococcal* infections. Dental Microbiol. Immunol. (13) 278-85
26. Truong, T.L.; Ménard, C.; Mouton, C.; Trahan, L. (2000): Identification of mutans and other oral streptococci by random amplified polymorphic DNA analysis. *J. Med. Microbiol.,* (49): 63-71.
27. World Health Organization, Oral Health Surveys. (1997): Basic methods, *Oral health survey: basic methods.* Geneva, WHO P 1-66.
28. Williams,J.G.K.,Kubelik,A.R.,Livak J.K.,Rafalski,J.A., and Tingey, S.Y.(1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. (18): 6531-35.