ISSN: 1812–1217

Prevalence of Anaerobic Bacteria in Periodontitis in Relation to Pocket Depth

Suhad Muwafaq Hamdoon
BDS, MSc (Assist Lec.)
Department of Dental basic Science
College of Dentistry, University of Mosul

Ghada Younis Abdul-Rahman
BSc,MSc,PhD (Assist Prof.)
Department of Dental basic Science
College of Dentistry, University of Mosul

الخلاصة

الأهداف : تهدف الدراسة الى تقييم انتشار البكتيريا اللاهوائية في اللويحةِ الجرثومية تحت اللثوية للمرضى المصابين بالتهابات ما حول السن وعلاقتها بعمقِ الجيب . **المواد وطرائق العمل** : انجزت هذه الدراسة على 97 عينة من اللويحة الجرثومية تحت اللثوية وتم قياس عمق الجيوب وعزل وتشخيص لبكتريا اللاهوائية ومقارنة تواجدها بالنسبة لعمق الجيوب **النتائج**:أظهرت الدراسة انتشار كبير للبكتريا اللاهوائية في المرضى المصابين بالتهابات اللثة مقارنة بغير المصابين، كما أظهرت الدراسةُ إن هناك انتِقال كبير منَ البكتيريا الاختيارية اللاهوائية الموجبة لصبغة كرامُ السائدة في العينات ذوات أعماق الجيوبِ السطحية إلى البكتيريا الإلزامية اللاهوائية السالبة لصبغة كرامُ السائدة في العيناتِ عميقة الجيوب. **الاستنتاجات**: ان استخدام الوسط الزرعي اللاهوائي يوفر معلومات جيدة حول قابلية الافراد للاصابة بالتهابات اللثة

ABSTRACT

Aims: **:** The study evaluated the prevalence of anaerobic bacteria in the subgingival plaque in periodontitis in relation to pocket depth. **Materials and Methods** :The study was performed on 97 sub-gingival plague sample , pockets depth were measured , anaerobic bacteria were isolated and identified in relation to pockets depth. **Results:** In this study high prevalence of anaerobic bacteria in the periodontal pockets of patients suffering from periodontitis compared with the gingival sulcus of healthy subjects with marked shifting from mainly Gram positive facultative anaerobic bacteria in shallow pockets to mainly Gram-negative strict anaerobic bacteria in the deep pockets.**Conclusion:**. Anaerobic culture used in this study provided information about the susceptibility of the individuals to develop periodontal diseases. on bone.

Key words: Pocket depth Anaerobic bacteria, Periodontitis**.**

HamdoonSM, Abdul-Rahman Gh Y. Prevalence of anaerobic bacteria in periodontitis in relation to pocket depth *Al–Rafidain Dent J*. 2014; 14(2):320- 328 .

Received:12/12/2013 ***Sent to Referees:*** 12/12/2013 ***Accepted for Publication:*** 1/6/2014

INTRODUCTION

Periodontal diseases can be defined as an apical extension of gingival Inflammation to involve the tissue supporting the tooth, including periodontal ligaments and bone.(1) They are poly microbial infections associated with local accumulation of dental plaque, a sub-gingival pathogenic periodontal flora, and calculus.(2) Periodontitis is an important global health problem which involves adult population over 35-40 years of age. The variance and severity of these diseases is influenced up to 90% by age and oral hygiene .(3) In studying gingivitis and periodontitis, researchers often employ measures of plaque accumulation, inflammation, probing depth, microbial assessment, or clinical attachment levels.(2) The bacterial etiology of periodontal disease is complex, with a variety of organisms responsible for initiation and progression of disease. The microorganisms of dental plaque are capable of initiating the mechanisms of destruction of periodontal tissues. Although over 400 different bacterial species have been detected in the oral cavity, only a limited number have been implicated as periodontal pathogens many of these organisms may also be present in periodontally healthy individuals and can exist in communal harmony with the host.(4) Anaerobic bacteria are believed to be the predominant causative factor in periodontitis .(5) In the initial stage of gingivitis, Gram-positive and facultative organisms predominate, including *streptococci*; *Actinomyces spp*. increase together with proportions of capnophilic species such as *Capnocytophaga spp*. In chronic periodon-

titis the sub-gingival plaque has two distinct zones: a zone of Gram-positive cocci and bacilli close to the tooth surface, and a zone of Gram-negative and anaerobic organisms next to the gingival crevice. In active pockets *Porphyromonas.gingivalis, Actinobacillus. actinomycetemcomitans, Prevotella.intermedia,* and *Fusobacterium .nucleatum* may also present.[6] Aim of the study: Isolation & identification of anaerobic bacteria in sub gingival plaque samples in patients with periodontitis Counting of anaerobic bacteria in sub gingival plaque sample in patients with periodontitis in relation to various periodontal pockets-depths compared with healthy individuals.

MATERIALS AND METHODS

The study was performed on 76 adult patients who diagnosed as periodontitis attended the Dental education hospital, department of periodontics , College of dentistry at Mosul University for diagnosis and treatment .Patients were selected for the presence of periodontal tissue alteration ; bleeding on probing ,redness, swelling, suppuration ,plaque and calculus accumulation and periodontal pocket formation .Excluded criteria include the presence of any systemic conditions that might affect the progression of periodontitis ,not receiving antibiotics during the last 3 months ,not submitted to any periodontal therapy at least 3 months ago and females not being pregnant. Twenty one control subjects were gathered from dental staff and students, they selected with absence of any signs and symptoms of periodontal diseases ,had good oral hygiene , no plaque ,no calculus ,they were also free from systemic diseases ,didn't have antibiotics & didn't submitted to periodontal treatment 3 months ago ,and female being not pregnant .Dental examination was performed on the dental chair at the department of periodntics under artificial light .Clinical variables were evaluated and collected by the same examiner under supervision of periodontist. After the completion of clinical examination and before taking the sub-gingival plaque sample supra gingival plaque was removed by cotton pledgets, and supra gingival calculus was removed by sickle scalar, teeth were isolated well to prevent salivary contamination ,pocket depth was measured by periodontal probe after teeth scaling , sub gingival plaque samples were obtained from the patients by inserting single sterile paper points size 50 (Dia dent Co.)for 30 seconds in a selected pocket or in the gingival sulcus of healthy control subjects . The paper points was inserted and removed by sterile twizzer and placed immediately in a sterilized screw-capped vials containing (4) ml thioglycolat broth as reducing transporting medium for anaerobic bacteria ,the samples transported directly to the laboratory,0.1 ml of each Sub-gingival plaque sample was taken by micropipette ,and cultured by spreading evenly on freshly prepared blood agar plate ,which incubated anaerobically using anaerobic jar+gas generating system 7 for 48-96 hours at 37C. [7]Simultaneously the same sample is cultured on Dentaid-1 ,which incubated in anaerobic candle jar for isolation of *Actinobacillus actinomycetemcomitans*.[8] Colonies of different characteristics

were isolated and identified using various methods .Identification in this study carried out according to Bergey's manual of determinative bacteriology. [9-11] which based on Gram stain reaction ,colony morphology on anaerobic blood agar , ,hemolytic reaction on anaerobic blood agar ,biochemical tests and enzymatic activities including; catalase , oxidase ,gelatin hydrolysis ,Bile solubility ,fermentation of glucose and lactose ,fluorescence under UV light (360nm) using fluorescent microscope , sensitivity to different antibiotics.

RESULTS

The correct diagnosis of periodontal condition is the key to a treatment plan, prognosis and maintenance of periodontal health. Traditional procedures such as probing and radiographic assessment provide information of what had already occurred, but not for what is occurring or what will occur with disease progression .[12] The study was consist of (97)sample ,from which (76)sample were collected from periodontal pockets at specific depth for each patient suffering from periodontitis and(21) control sample were collected from normal gingival sulcus of healthy subjects. Tables (1,2)

Table (1): ANOVA The Effect of Pocket Depth on the Total Count of Anaerobic Bacteria

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.66	2	1.33	33.177	.000
Within Groups	3.77	94	4012922151.9		
Total	6.43	96			

Table (2): Duncan's Multiple Ranges Tests The Effect of Pocket Depth on the Total Count of Anaerobic Bacteria

Depth In mm	Samples No.	Mean ± S.D.*	*Duncan's Grouping	atients No.	Controls No.
< 3	23	3368.70±1814.365	A	2	21
3-5	34	23043.53±7554.462	A	34	0
> 5	40	120473.00±98091.918	B	40	0
Total	97	58555.46±81872.078		76	21

Revealed that the total count of anaerobic bacteria (C.F.U/ml)isolated from the sub gingival plaque samples and cultivated on freshly prepared blood agar which were incubated in anaerobic jar with gas generating kit(3368.70)in samples with pockets depth less than 3mm ,which were increased to(120473.00) with increasing the depth of pocket, a marked difference between the total count of the samples appeared between the first and the third range of depth. [13] demonstrated that the total level of microbial load (log 10 CFU/ml) of all isolated microbes varied from zero to 8.4 log, whereas sterile samples occurred rarely (4 cases per 104). The median of colonization after treatment was 5.5 log CFU/ml indicating the substantial effect of instrumentation on the viable count of microbes so scaling and root planning alone reduced the number of microbes in the sub-gingival area. [14] in their study show a high prevalence of anaerobic bacteria in patients with deep pockets. Tables (3,4,5, and 6).

Table (3): ANOVA for the Effect of Pocket Depth on the Total Number of Gram-negative Anaerobic Bacteria in Subgingival Plaque Samples

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	99.511	2	49.756	79.711	.000
Within Groups	58.674	94	.624		
Total	158.186	96			

Table (4): Duncan's Multiple Range Tests for the Effect of Pocket Depth on the Total Number of Gram-negative Anaerobic Bacteria in Subgingival Plaque Samples

Pocket Depth (mm)	Patients No.	No. of G-ve	Mean± S.D.	*Duncan's Test
< 3	23	17	0.74±0.752	A
3-5	34	97	2.85±0.821	B
> 5	40	131	3.28±0.784	C
Total	97	245	2.53±1.284	

* Different letters mean significant difference, S.D. standard deviation, The level of significance α=0.05, p=0.05

Table (5): ANOVA for the Effect of Pocket Depth on the Total Number of Gram-Positive Anaerobic Bacteria in Sub gingival Plaque Samples

	Sum of Squares	Df	Mean Square	F	Sig.
Between groups	22.367	2	11.184	52.879	.000
Within groups	19.880	94	.211		
Total	42.247	96			

Table (6): Duncan's Multiple Range Tests for the Effect of Pocket Depth on the Total Number of Gram-Positive Anaerobic Bacteria in Sub gingival Plaque Samples

Pocket Depth (mm)	Patients No.	No. of G+ve	Mean± St.d.	*Duncan's test
< 3	23	26	1.26±0.689	c
3-5	34	18	0.53±0.507	b
> 5	40	1	0.3±0.158	a
Total	97	45	0.49±0.663	

* Different letters mean significant difference, St.d. standard deviation
The level of significance α=0.05, p=0.05

ANOVA and Duncan's Multiple Ranges test showed that there was significant effect of pocket depth on the total number of gram negative and positive anaerobic bacteria respectively. The total number of anaerobic bacterial isolates in our study were (290) including both Gram-positive and Gram-negative anaerobic isolates. The total number of Gram-positive bacterial isolates alone were (45) and their distribution vary in samples of variable pockets depth including (26) isolates were found in the first group sample (pocket depth <3mm), (18) isolates in the second group samples (pocket depth 3-5mm) and only one Gram-positive isolate in the third group (pocket depth >5mm). While, for Gram-negative isolates the total number were (245) isolates from which (17) isolates from the first group samples (pocket depth <3mm), (97) isolates from the second group samples (pocket depth 3-5mm), and (131) isolate from the third group (Pocket depth>5mm). Tables (7).

Table (7): The Effect of Pocket Depth on the Number of Different Species of Anaerobic Bacteria Isolated from Subgingival Plaque Sample

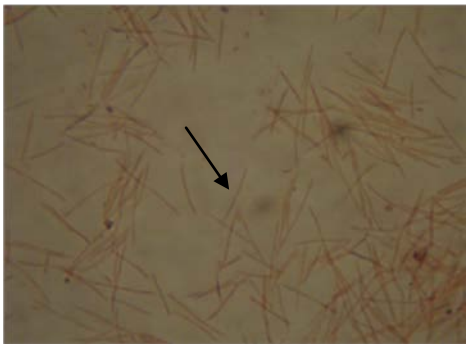
Bacterial species	Ranges of Depth (mm)			Total
	< 3	3-5	> 5	
Actinomyces spp.	20 (58.8%)	13 (38.2%)	1 (2.9%)	34
Peptostreptococcus spp.	2 (33.3%)	4 (66.7%)	0	6
Oral streptococci	4 (100%)	0	0	4
Veillonella spp.	9 (21.4%)	18 (42.9%)	15 (35.7%)	42
Prevotella spp.	4(9.8%)	14 (34.1%)	23 (56.1%)	41
Fuosobacterium spp.	2 (4.3%)	18 (38.3%)	27 (57.4%)	47
Porphyromonas spp.	1 (1.6%)	27 (44.3%)	33 (54.1%)	61
A. actiomycetemcomitans	0	10 (35.7%)	18 (64.3%)	28
Bacteroid spp.	1 (3.8%)	10 (38.5%)	15 (57.5%)	26
Anaerobic Gram +ve rod	0	1 (100%)	0	1
	43	115	132	290

Revealed a positive correlation between the pocket depths and the number of Gram-negative isolates (r^2=0.716) and negative correlation (r^2= -0.671) between pocket depth and the number of Gram-positive isolates.[15] studied the microbiological diagnosis of the sever chronic periodontitis in which anaerobic microbiology was completed for 27 patients and found that Gram-negative anaerobic bacteria were isolated in 92.6% (25 of 27 patients), involving 90.9% of untreated patients (20 of 22 cases) and all the five treated patients. Manual instrumentation decreases the population of Gram-negative bacteria and allows for an increase in the population of Gram-positive microbes. This shift is usually associated with an improvement in clinical parameters, such as decreased pocket depth (PD)or bleeding on probing (BOP).[16] Table (8) .

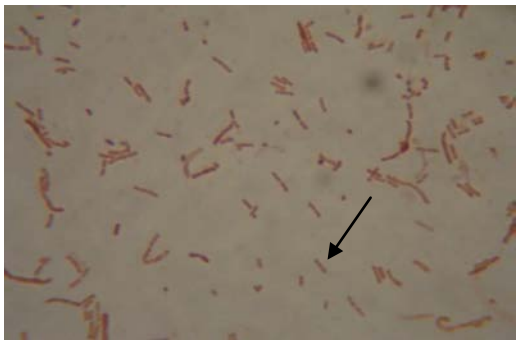
Table (8): The Effect of Pocket Depth on the Number of Different Species of Anaerobic Bacteria Isolated from Sub-Gingival Plaque Sample

Bacterial species	Ranges of Depth (mm)			Total
	< 3	3-5	> 5	
Actinomyces spp.	20 (58.8%)	13 (38.2%)	1 (2.9%)	34
Peptostreptococcus spp.	2 (33.3%)	4 (66.7%)	0	6
Oral streptococci	4 (100%)	0	0	4
Veillonella spp.	9 (21.4%)	18 (42.9%)	15 (35.7%)	42
Prevotella spp.	4(9.8%)	14 (34.1%)	23 (56.1%)	41
Fuosobacterium spp.	2 (4.3%)	18 (38.3%)	27 (57.4%)	47
Porphyromonas spp.	1 (1.6%)	27 (44.3%)	33 (54.1%)	61
A. actiomycetemcomitans	0	10 (35.7%)	18 (64.3%)	28
Bacteroid spp.	1 (3.8%)	10 (38.5%)	15 (57.5%)	26
Anaerobic Gram+ve rod	0	1 (100%)	0	1
Total	43	115	132	290

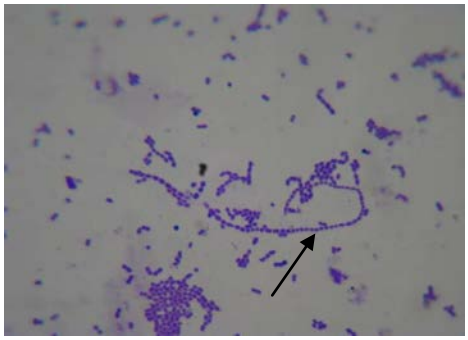
Revealed that in healthy gingival sulcus with depth less than three millimeters mainly (*Actinomyces* spp. and oral streptococci were isolated, and only small percentage of some type species of strict anaerobic bacteria like(*Veillonella* spp. , *Prevotella* spp., *Fusobacterium* spp. , *Porphromonas* spp., and *Bacteriods* spp.) were isolated from the first range of pocket depth . As the depth was increased the percentage of *Actinomyces* spp. and oral streptococci Were reduced compared with increase in the number of anaerobic periodonto-pathogens, this is particularly marked in the third range of pocket depth in which (*Veillonella* spp., *Prevotella* spp., *Fusobacterium* spp., *Porphromonas* spp., *Bacteriods* spp., *actinobacillus actinomycetemcomitans*, and anaerobic Gram-positive rod) were increased in number. Identification based on cultural characteristic and biochemical tests on Dentaid-1 *A. actinomycetemcomitans* produced white color colonies which stick to the surface of agar (Figure 1g), and Mitis Salivarius Agar for oral streptococci ,which appeared blue in color (Figure 1k) and this characteristic was sufficient to differentiated it from Gram-positive anaerobic cocci (GPAC). *Prevotella* and *Porphyromonas* Produced dark black colonies on blood agar by forming a heam-protein complex see (Figure 1 h), some species of *Actinomyces* produced colonies that had the"molar tooth" appearance (Figure1L) .Colonies of *Prevotella* and veillonella show red fluoresces ,when examined under long wave UV light (Figure1j),while *fusobacteria* fluoresces chartreuse (Figure1i). hence a very high prevalence of a complex strict anerobic Gram-negative rod in sub gingival plaque samples of deep pockets were noted in this study.[17] explained that formation colonization and progression of pocket represent an evolving process result from change from aerobic to anaerobic condition. The microbial population shift from mainly *Actinomyces* spp. and *Streptococcus* spp. toward anaerobic Gram-negative bacteria.[18] demonstrated that *Prevotella intermedia* and *A. actinomycetemcomitans* were highest in patients with pocket depth > 3 mm and attachment loss 3.1-5.[15] revealed that *P. intermedia P. gingivalis* alone or in combination were found in 31.8% of samples from (PD 3-5mm) and 40.9% in those with (PD 5-7mm) and 50% in those with (PD >7mm).



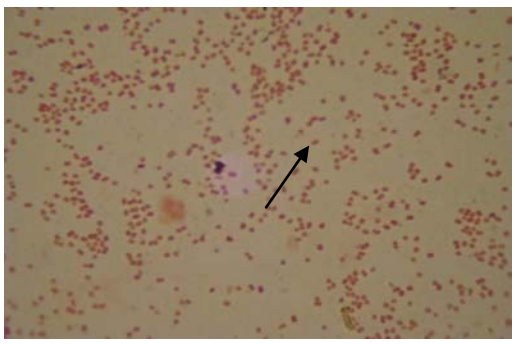
(a)



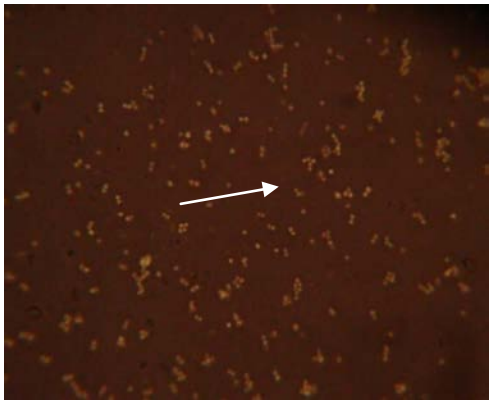
(b)



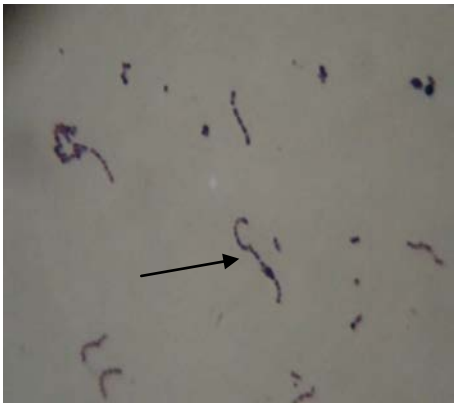
(c)



(d)

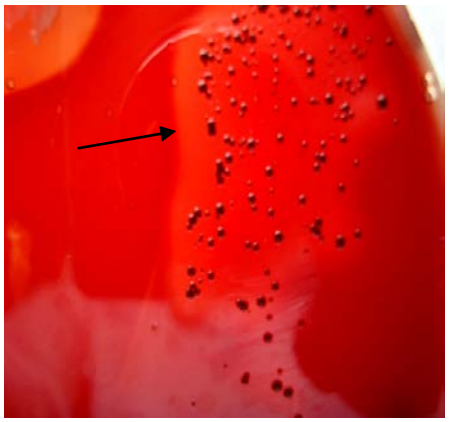


(e)

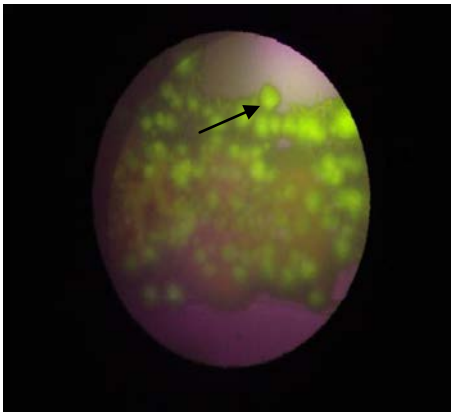


(f)

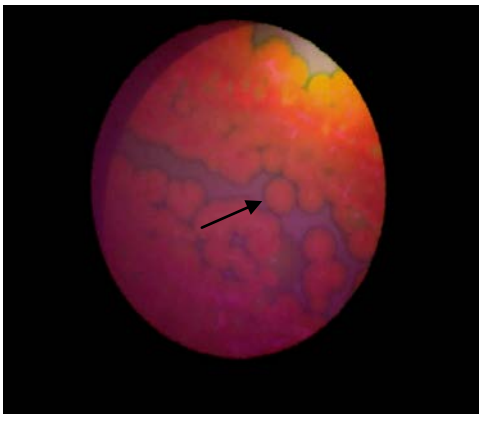
(g)



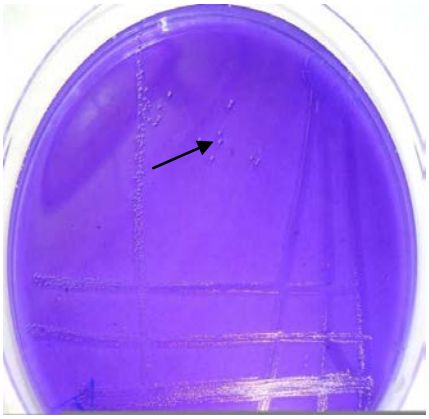
(h)



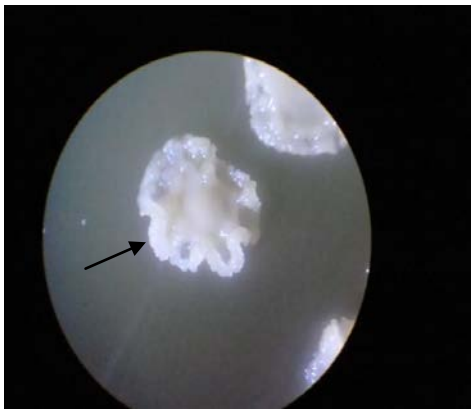
(i)



(j)



(k)



(L)

Figure (1): Microscopical and Cultural Characteristics of Different Anaerobic Bacteria Isolated from Subgingival Plaque

(a) Gram negative spindle shape of *Fusobacterium* spp. under (1000x) light microscope.
(b) Gram negative short rods of *Prevotella* spp. under (1000x)light microscope.
(c) Gram positive oral streptococci under (1000x) light microscope.
(d) Gram negative cocci of *Veillonella* spp. under (1000x)
(e) Capsule stain of *A.actinomycetemcomitans* under (1000x) light microscope.
(f) Beaded appearance of *Actinomyces* spp. under (1000x) light microscope.
(g) Stickiness of *A.actinomycetemcomitans* to the surface of agar.
(h) Jet Black colonies of *porphyromonas* spp.on blood agar.
(i) Fluoresces chartreuse of *Fusobacterium* spp. under low Power fluorescent Microscope
(j) Brick-red Fluoresces of *Prevotella* spp. under low power fluorescent microscope
(k) Oral streptococci on Mitis Salivarius Agar (MSA).
(L) Molar Tooth appearance of some *Actinomyces* spp. under (400x) light microscope

CONCLUSION

Anaerobic culture used in this study provided information about the susceptibility of the individuals to develop periodontal diseases on bone.

REFERENCES

1. Spratt D. (2003). Dental plaque and bacterial colonization. In: Medical biofilms. Jass J, Surman S, Walker J, editors, John Wiley Vol. 4(1): 175-98.
2. Nield-Gehrig J. S. ,Willmann D. E. (2003). Foundation of Periodontics, for Dental Hygienist; Wolters Kluwer Company, Baltimore .
3. Baelum V., Fejerskov O., Manji F. (1988). Periodontal diseases in adult Kenyans *J. Clin. Periodontol*. Vol. 15 (7): 445-452. [Abstract[
4. Moore W. E. C., Moore L. V. H. (1994). The bacteria of periodontal disease. *Periodontology 2000* Vol. 5: 66-77.
5. Bollen C. M. L., Vandekerckhove B. N. A., Papaioannon W., Vaneldere J., Quirynen M. (1996). Full-Versus partial mouth disinfection in treatment of periodontal infections .A pilot study, long term microbiological observation. *J. Clin. Periodontol*. Vol. 23:960-970.
6. Samaranayake L. (2006). Essential Microbiology for Dentistry, Churchill Livingstone Company, London: 275.
7. Summanen, P. H. (1999). Comparison of Recovery of Anaerobic Bacteria Using the Anoxomat, Anaerobic Chamber, and Gas Pak Jar Systems *J. Anaerobe* Vol. 5: 5-9.
8. Alsina M., Olle E., Frias J., (2001). Improved. Low-Cost Selective Culture

Medium of A. actinomycetemcomitans *J Clin Microbiol* Vol. 39 : 509–513.
9. Holt J. G., Krieg N. R., Sneath P. H., Staley J. T., and Williams S. T. (1994). Bergys manual of Determinative Bacteriologu. 9th ed., Williams Wilkins Company,Baltimore.
10. Forbes B. A., Saham D. F., Weissfeld A. S. (2007). Diagnostic Microbiology. 12th ed Mosby company,St.Louis.
11. Collee J. G., Marmion B. P., Fraser A. G. and Simmons A., (1996). Practical Medical Microbiology 14th ed. Churchill Livingstone Company, London.
12. Louie H, Larjava H. (1994). A critical evaluation of diagnostic tests for periodontal disease. *J. Can Dent Assoc.*Vol. 60:1042-1049.
13. Loivukene K., Pahkla., Koppel T., Naaber P. (2005). The microbiological status of patients with periodontitis in southern Estonia after non surgical periodontal therapy, *Baltic Dental and Maxillofacial J.* Vol. 7:45-7.
14. Hussein M. S., Kadkhoda Z. (2004). Rate of cultivable sub gingival periodontopathogenic bacteria in chronic periodontitis, *J. Oral Science* Vol. 64 (3): 157-161.
15. Boyanova L., Setchanova G., Gergova T., Kostyanev D., Popova C., Kostilkov K., Mitov I. (2009). Microbiological diagnosis of severe chronic periodontitis *J. of AMAB-Annual Proceeding* (scientific paper) Book 2.
16. Haffajee, A. D., Cugini, M. A., Dibart, S., Smith, C., Kent, R. L., and Socransky, S. S. (1997). The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *J. Clinical Periodontolol.*Vol. 24:324-334.
17. Kores I., Lepp P. W., Relman D. A. (1999). Bacterial Diversity with in the human sub gingival crevice. *J. Proc Natl Acad Sci* Vol. 96:14546-14552.
18. Escalona L. A., Brito A., Almon R., Bravo I. M., Perrone M., Correnti M. (2007). Distribution of P. intemedia A.actinomycetemcomitans in Vennezuelan population with chronic periodontitis. *J. Rev Venes Inves Odonto.*Vol. l 7(1):29-37.