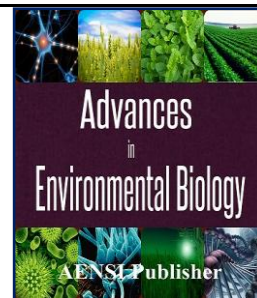




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## Isolation and identification of bacteria and parasite from teeth caries and periodontal.

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### ABSTRACT

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. The association between oral diseases and the oral micro biota is well established. 200 samples collected from 100 patient (children, teenager and adult) 70 female and 30 male with periodontal disease. Rate infection in female with parasite 87.1% more than male, while infected with bacteria, ratio in male 80% more than female 74.2%. The result shows that 80% from the sample was parasite infection and 76% with bacterial infection. The ratio of infection with parasite increased when the age of patient increased but no relationship between the age and infection with bacteria. The type of bacteria isolated belong to in gram positive, gram negative, aerobic and anaerobic bacteria. the most bacteria we found was the aerobic bacteria (10 strains) including *Bacillus* sp., *Staphylococcus* sp., *Streptococcus mutans*, *Micrococcus* spp., *Proteus vulgaris*, *Klebsiella* spp., *Echerichia coli* and *Pseudomonas aeruginosa* compared to anaerobic bacteria (4 strains) which include *Lactobacillus fermentum*, *Actinomyces naeslundii*, *Peptostreptococcus* spp. and *Bacteroides ovatus*. And 6 strains of parasite was *Entamoeba gingivalis*, *Trichomonas tenax*, *Entamoeba coli*, *Iodamoeba butschlii*, *Chilomastix mesnili* and *Giardia lamblia*.

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## INTRODUCTION

Oral cavity of human supports life for many different species of microorganisms, but bacterial infection happened when these microorganisms permeate into the tissues or in case of lower host immunity. The display of bacterial infection of dental descent is permanently changing and is a measurable reflexing of new evolution of oral flora.[1]

The studies of bacteriology have shown differences in acute microbiological dental infections. According to Rautemaa *et al.*,[2] surely this difference reflects variations in the bacteriological knock used for their isolation, identification, and gradual changes in flora due to injudicious use and not commitment to taking of antimicrobials.

About 700 or more different types of bacteria include complex microbial groups that live in the mouth, it is important to preserving healthy oral. The mouth gives an environment to growth diverse groups of microorganisms comprising lactic acid bacteria belonging to the genera *Lactobacilli*, *Streptococcus*, members of the genus *Actinomyces* and two kinds of parasite: *Entamoeba gingivalis* and *Trichomonas tenax*, these kinds of parasite attacked to periodontal. *Trichomonas tenax* anaerobic parasite found in oral cavity which responsible for chronic periodontitis parasite cans transmission by kissing and droplet spray *Entamoeba gingivalis* parasite found in mouth [3].

More recently, studies are suggesting that in addition to dental caries and periodontal disease, oral micro biota can have negative impacts on other aspects of our health [4]. This research was aimed to isolation and identification of microorganisms causes teeth decay and periodontal.

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## MATERIAL AND METHODS

### A/ sample collection:

200 samples collected from 100 patient (children, teenager and adult) 70 female and 30 male with periodontal disease, 100 of these samples taken from teeth decay and the other 100 samples taken from periodontal to detect microorganism.

### B/ Isolation procedures

#### a / examine under microscope:

To detect parasite used wet mount, normal saline and eosin stain then prepared slid and finely examined directly under microscope. [5].

Direct smears were prepared and stained with gram stain to identification of bacteria isolated after incubated in culture. All morphological arrangement and its reaction with the stain were studied by using light microscope.

#### b/ used culture:

Each sample was incubated into three blood agar plates; one of them was incubated aerobically. While the others were incubated anaerobically by using two methods of incubations; anaerobic jar with candle jar incubation. MaConkeys agar was also used and incubated aerobically. Sabouraud glucose agar was used for culture *Candida* spp. All plates were incubated at 37°C for 24 hours, with further 24 hours incubation if there was no growth. Cultures for anaerobes were incubated for 48-72 hours [6].

Biochemical reactions, sugar fermentation tests, bile solubility, starch hydrolysis, gelatin digestion, catalase, lecithinase, indole production and nitrate reduction test, which are the most characteristics tests to differentiate between them [7].

### C / Statistical analysis:

Statistical analysis was performed using SPSS statistical computer software.

### Result:

The result show that 80% from the sample was parasite infection and 76% with bacterial infection and present 87.1% female infected with parasite and 74.2% with bacteria from Table(1) Statistical analysis show there was  $p < 0.05$  significant difference .

Table (2, 3) show the ratio of infection with parasite and bacteria increased when the age of patient increased with  $p < 0.05$  significant difference in parasite only.

The type of bacteria isolated belong to in gram positive, gram negative, aerobic and anaerobic bacteria as shown in table (4) the most bacteria which found were the aerobic bacteria (10 strains) including *Bacillus* sp. , *Staphylococcus* sp. , *Streptococcus mutans* , *Micrococcus* spp. , *Proteus vulgaris*, *Klebsiella* spp. , *Echerichia coli* and *Pseudomonas aeruginosae* compared to anaerobic bacteria (4 strains) which include *Lactobacillus fermentum*, *Actinomyces naeslundii* , *Peptostreptococcus* spp. and *Bacteroides ovatus* . and 6 type of parasite was *Entamoeba gingivalis*, *Trichomonas tenax* , *Entamoeba coli* , *Iodamoeba butschlii*, *Chilomastix mesnili* and *Giardia lamblia* and yeast. (Table3).

**Table 1:** The number and percentage of positive and negative samples isolated from female and male

Total	Number of samples contain parasite				Number of samples contain bacteria				Nature of samples
	Female		Male		Female		Male		
	N.(%)	P.(%)	N.(%)	P.(%)	N.(%)	P.(%)	N.(%)	P.(%)	
100	3(4.2)	34(48.5)	7(10)	5(16.6)	9(12.8)	24(34.2)	4(13.3)	14(46.6)	Teeth decay
100	6(8.5)	27(38.5)	4(13.3)	14(46.6)	9(12.8)	28(40)	2(6.6)	10(33.3)	Periodontal
200	9(12.8)	61(87.1)	11(36.6)	19(63.3)	18(25.7)	52(74.2)	6(20)	24(80)	Total

$p < 0.05$  significant difference

**Table 2:** relationships between parasite infection and age

Age	No. of sample	<i>Entamoeba gingivalis</i>		<i>Trichomonas tenax</i>	
		No.	%	No.	%
1-10	14	5	35.7	4	28.5
11-20	46	25	54.3	9	36
21-30	30	15	50	10	66.6
31-40	5	4	80	4	100
41+	5	4	80	0	0
Total	100	53		27	

$p < 0.05$  significant difference

**Table 3:** relationships between bacteria infection and age

Age	No. of sample	No. of positive sample (%)
1-10	14	9(64.3)
11-20	46	32(69.5)
21-30	30	24(80)
31-40	5	4(80)
41<	5	5(100)
Total	100	76

p>0.05 no significant difference

**Table 4:** Number and type of microorganism isolated from periodontal disease

Type of microorganism	Number of microorganism
1- bacteria	
a-aerobic	
1-Grams positive	
<i>Bacillus cereus</i>	12
<i>Bacillus subtilis</i>	15
<i>Staphylococcus aureus</i>	25
<i>Streptococcus mutans</i>	30
<i>Micrococcus spp.</i>	5
<i>Proteus vulgaris</i>	17
<i>Staphylococcus epidermidis</i>	12
2-Grams negative	
<i>Klebsiella spp.</i>	9
<i>Escherichia coli</i>	8
<i>Pseudomonas aeruginosae</i>	7
b-Anearobic	
1- Grams positive	
<i>Lactobacillus fermentum</i>	10
<i>Actinomyces naeslundii</i>	4
<i>Peptostreptococcus spp.</i>	16
2- Grams negative	
<i>Bacteroides ovatus</i>	3
2- parasite	
<i>Entamoeba gingivalis</i>	53
<i>Trichomonas tenax</i>	27
<i>Entamoeba coli</i>	2
<i>Iodamoeba butschlii</i>	1
<i>Chilomastix mesnili</i>	1
<i>Giardia lamblia</i>	1
3- Yeast	
<i>Candida albicans</i>	34

### Discussion:

In our study patients with periodontal disease are more likely to infection with protozoa. In female rate of infection with parasite higher than male these result matches with other study [8] (Table 1). In fact, 53% were infected with *Entamoeba gingivalis* while *Trichomonas tenax* where 27% (Table 2). This result was higher than another study which refers to infection with parasite was 31.67% of *Entamoeba gingivalis* while infection with *Trichomonas tenax* was 35% higher than our study [5]. In the other study rate of *E.gingivalis* was 35% while *T. tenax* was 3.3% [8].

In another study in Babylon city rate of infection with *E. gingivalis* was 42.9 % and *T. tenax* was 20.6% which closely to our result [16].

Infection of *E.gingivalis* and *T. tenax* may be return to periodontitis and have no oral cleanliness [5],or probable these parasites are opportunists particularly lesions gingivalis and periodontal pockets [10].

In this study we found age groups (11-20), (13-40) and (41<) more likely to infected with *E.gingivalis*, while infected with *T. tenax* in age group(21-30) and (31-40)(Table 2) .these results were agreement with many studies which say infection with parasites increase with aging [8] Statistical analysis show there was p<0.05 significant difference.

It has been suggested these protozoans could affect the formation of contribute to the development and progression of periodontal disease. The other researchers state that these protozoans may be opportunistic, since they are capable of proliferating in the microenvironment of the mucobuccal fold affected by periodontal disease [11].

Therefore, if *Entamoeba gingivalis* helps the development and progression of periodontitis and gingivitis, these diseases increasingly facilitate the proliferation of these protozoa, this vicious circle could explain the increased incidence of these microorganisms in the dental plaque and saliva samples of patients with periodontitis and gingivitis. A suspension of *Entamoeba gingivalis* was spread on the gingival margins of rats'

immunosuppressed with (prednisolone acetate), leading to the development of the clinical signs and inflammatory process of periodontal disease much faster than that observed in immunocompetent rats [12].

Protozoa are not the only pathogenic found in patients with periodontal disease, in this study we found wide range of bacteria. The result show that 76% with bacterial infection and present 74.2% female and 80 % male infected with bacteria this result similar the result obtain by [13]. The ratio of infection with parasite and bacteria increased when the age of patient increased. Statistical analysis show there was  $p < 0.05$  significant difference in parasite only.

In this study we found wide range of gram positive, gram negative, aerobic and anaerobic bacteria as shown in table (3) the most bacteria we found was the aerobic bacteria (10 strains) including *Bacillus sp.*, *Staphylococcus sp.*, *Streptococcus mutans*, *Micrococcus spp.*, *Proteus vulgaris*, *Klebsiella spp.*, *Echerichia coli* and *Pseudomonas aeruginosae* compared to anaerobic bacteria (4 strains) which include *Lactobacillus fermentum*, *Actinomyces naeslundii*, *Peptostreptococcus spp.* and *Bacteroides ovatus* (gram positive bacteria more than gram negative bacteria) this results almost similar to [13]

From the 76 patients about 30 patients have *streptococcus mutans* and 37 patients had *Staphylococcus spp.* that main *Staphylococcus spp.* higher than *streptococcus mutans* which is the opposite of [14]

This wide range of bacteria and protozoa in mouth may cause lose of teeth in younger people [14] further more to that it may cause coronary disease, arteriosclerosis, myocardial infection, pneumonia pre-term births and low birth weight [15,16].

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