Effect of fixed orthodontic appliances on the change of the oral bacteria and *Candida* species in AL-Samawah city, Iraq

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Abstract

A total of 75 samples from fixed orthodontic appliances of at many ages (16-30) years and for both genders (50 male and 25 female). The patients were selected from a larger sample of orthodontic patients who were clinically examined once to obtain baseline data before active treatment. Thereafter, the experimental group was examined three times after 3 and 6 months and over6 months follow-up period after insertion of the orthodontic appliance. The whole mouth plaque score was obtained, and the oral cavity was then sampled for *Candida* species and bacterial species by culture.

The results of this study showed that *Staphylococcus aureus* was present in higher percentages than other microorganisms (43.2%), followed by *Streptococcus* species (15.8 %), then *Pseudomonas aeruginosa* (13.4), *Klebsiella* sp. (16.4) and *Enterobacter* species(10.9) respectively .Females showed higher bacterial growth compared with males, and higher mixed growth over single growth in a female while male showed the reverse. Number of bacterial growth after 4 months period was higher than 1 month's period and both periods were higher than the period of 6 months, with maximum bacterial growth. Generally, fixed appliances showed more bacterial growth compared with before treatment for all isolated microorganism types. While, *Candida albicans* were present in higher percentages than other microorganisms (35%), followed by *Candida tropicals* (23.7 %), then *C. parapsilosis* and *Candida krusei* respectively. *Candida* species growth after 6 months period was higher than 5 months period and both periods were higher than the period of 4,2,1 months, respectively. Results also revealed than antibacterial and antifungal resistance were *Staphylococcus aureus*, *Streptococcus species, Pseudomonas aeruginosa, Klebsiella* sp. and *Enterobacter* species for the all four tested antibiotics, followed by *Candida* species.

Key words: orthodontic appliances, Candida, antibiotic, microflora.

INTRODUCTION

The orthodontic appliances are placed in the subjects undergoing orthodontic treatment provokes adverse changes in gingival bacteria with the development of gingivitis and consequently periodontitis (1, 2).

Several microbiological studies demonstrate that, in the absence of good oral hygiene, the placement of orthodontic bands results in the formation of increased pocket probing depths concomitantly with the quantitative increase and shifts of a microbial composition of the sub-gingival plaque which resembles the plaque usually found in periodontal diseases, where *Tannarella forsythia*, *Porphyromonas gingivalis* ,and *Treponema denticola* are prevalent (3, 4, 5, 6, 7).

Candida is a pathogenic fungus. The pathogenicity of *Candida* isolated from human mouths can be classified into eight strains: *Candida albicans, Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida krusei, Candida kefyr, Candida stellatoidea,* and *Candida dubliniensis. C. albicans* accounts for 45-75% of the total incidence of candidiasis, whereas *C. tropicalis* and *C. parapsilosis* account for about 7% of all cases (8). Fixed orthodontic appliances are artificial devices in the mouth that can greatly affect oral health and allow plaque and food scraps to accumulate. Fixed orthodontic appliances can also bring about an increased number of microorganisms and amalgamated infections in the mouth, including caries of the teeth, lips, buccal surfaces, and tongue. Fixed orthodontic appliances can also cause an increase in the number of Gram-positive bacteria in the mouth (9, 10). Increased levels of dental plaque are related to the development of gingivitis. Patients with gingivitis are prone to periodontal disease and loss of periodontal support (11, 12).

The aim of this study was:
1. To explore changes in oral bacterial and Candida strains among healthy before and after treatment with fixed orthodontic appliances.
2. To assess quantitative and qualitative alterations in the carrier rate of the bacteria and *Candida* spp. after placement of fixed orthodontic appliances on the permanent dentition.
3. To evaluate of antibacterial and antifungals combination by microdilution techniques.

MATERIALS AND METHODS

Swabs were 75 collected from patients (25males and 50 females) with mean age (16-30) years were analyzed to evaluate gender differences .after that patients ready to receive orthodontic therapy, after insertion of fixed appliances. All samples were subjected to bacteriological diagnosis, and the microorganisms isolation and identification were depending on (13, 14).

Plaque samples were collected from buccal and labial aspects of the anterior teeth and four first molars to determine oral carriage of bacterial isolation and Candida species of these patients. Second, third, fourth, fifth, sixth and more than 6 months after the placement of appliances, plaque samples were collected again from the same site before treatment.

Swabs were also collected from the same site and cultured on Sabouraud's dextrose agar (SDA) for *Candida* counts.*Candida* was identified by growth characteristics, Gram stain, and germ tube test. *Candida* samples were cultured in CHROMagar *Candida* identification Petri dishes (CHROMagar, Paris, France) at 37C for 36-48 hours. Different Candida strains were identified based on the color of the colonies. *C. albicans* exhibits green

coloration, smooth *Candida* is purple, *Candida tropicals* is blue, *C. krusei* is pink, and other unidentified fungi in the culture medium are white.
The isolates subjecting to antibiotic disc sensitivity test on Muller Hinton agar depending on (Kirby-Baure Methods).

Statistical Analysis
Chi–square test was used for statistical analysis of the data by SPSS.

RESULTS AND DISCUSSION:

The results of this study showed that *Staphylococcus aureus* was present in higher percentages than other microorganisms (43.2%), followed by *Streptococcus* species (15.8 %), then *Pseudomonas aeruginosa , Klebsiella* sp. and *Enterobacter* species respectively. Bacterial growth after 4 months period was higher than 1 months period and both periods were higher than the period of 6 months, with maximum bacterial growth. The results of the statistical analysis test showed significant differences (P<0.05) as shown in the table (1).Generally, fixed appliances showed more bacterial growth compared with before treatment for all isolated microorganism types.

This study, which investigated microflora during fixed orthodontic therapy, indicates that the wearing such appliances lead to the increased carriage and considerable changes in the oral bacterial population, possibly due to the appliance-induced ecological alterations within the oral cavity. Low plague index and total bacterial count for the baseline patient was not elevated and this associated with good oral hygiene prior to the experiment. After insertion fixed and removable appliances a significant increase in plaque index and bacterial counts according to the type of appliance and time of insertion also, the difference very clear according to gender group (15, 16, 17, 18).

This study showed a higher colonization of *Candida* species with fixed orthodontic appliances in comparison with the normal flora. *Candida albicans* were present in higher percentages than other microorganisms (35%), followed by *Candida tropicals* (23.7 %), then *C. parapsilosis* and *Candida krusei* respectively. *Candida* species growth after 6 months period was higher than 5 months period and both periods were higher than the period of 4,2,1 months, respectively. The results of the statistical analysis test showed non-significant differences (P>0.05) between the percentage of *Candida* isolates (table 2). The installation of fixed orthodontic appliances within 6 months, the rate of pathogenesis and number of colonies of oral Candida significantly increased compared with those prior to treatment, particularly at 5 months after fixed orthodontic appliances installation; these values then gradually decreased over time. These findings may be due to the fixed orthodontic appliances resulting in a lowering of the local defense mechanism of oral mucosal cells. Oral mucosal cells, which act as mechanical barriers, and metabolism play important roles in increasing the resistance of the mouth to infection. Thus Candida can easily adhere to any damage in the oral epithelia. Lip buccal mucosal damage was observed in many patients shortly after the application of fixed orthodontic appliances. Varying degrees of oral ulcers or gum inflammation, both of which can decrease local defense mechanisms, were also observed (19).

C. albicans increase in the patients due to differences in the living conditions of other Candida strains. After the application of fixed orthodontic appliances, the pH of plaque and the strain and number of microorganisms in the oral cavity changed. In addition to *C. albicans*, other *Candida* strains aggregate or adhere more easily to fixed orthodontic appliances. The adhesive force of *C. krusei* to the surface of nonliving substrates is very strong. *C. krusei* can also attach to and reproduce on the surface of nonliving substrates via cell surface hydrophobicity (20).

Table (1): Showing number of bacteria isolated according to period of wearing orthodontic appliance.

Period of treatment	*Staphylococcus aureus*	%	*Streptococcus Sp.*	%	*pseudomonas aeruginosa*	%	*Klebsiella sp.*	%	*Enterobacter sp.*	%	Total (%)
1months	10	14	3	11.5	2	9	7	25.9	6	33.3	28(17)
2months	7	9.8	5	19.2	1	4.5	1	3.7	0	0	14(8.5)
3months	5	7	6	23	5	22.7	1	3.7	1	5.5	18(10.9)
4months	9	12.6	4	15.3	3	13.6	9	33.3	4	22.2	29(17.6)
5months	13	18.3	2	7.6	1	4.5	2	7.4	3	16.6	21(12.8)
6moths	8	11.2	1	3.8	6	27.2	5	18.5	2	11.1	22(13.4)
More than 6 months	15	21.1	2	7.6	2	9	1	3.7	1	5.5	21(12.8)
Before treatment	4	5.6	3	11.5	2	9	1	3.7	1	5.5	11(6.7)
Total (%)	71(43.2)	100	26(15.8)	100	22(13.4)	100	27(16.4)	100	18(10.9)	100	164

X^2calculated = 44.93   d.f =28   p<0.05
X^2tabulated = 27.80   significant

Table (2): Showing number of *Candida* species according to period of wearing orthodontic appliance.

| Period of treatment	*Candida albicans*	*%*	*Candida tropicals*	*%*	*Candida krusei*	*%*	*C. parapsilosis*	*%*	Total (%)
1months	5	7.3	6	13	3	7.8	7	17	21(10.8)
2months	7	10.2	5	10.8	2	5.2	4	9.7	18(9.2)
3months	3	4.4	4	8.6	4	10.5	2	4.8	13(6.7)
4months	10	14.7	9	19.5	6	15.7	5	12.1	30(15.4)
5months	13	19.1	7	15.2	5	13.1	9	21.9	34(17.5)
6moths	17	25	11	23.9	8	21	8	19.5	44(22.6)
More than 6 months	9	13.2	2	4.3	7	18.4	6	14.6	24(12.3)
Before treatment	4	5.8	2	4.3	3	7.8	1	2.4	10(5.15)
Total (%)	68(35)	100	46(23.7)	100	38(19.5)	100	41(21.1)	100	194

X^2calculated = 12.68   d.f =21 $p> 0.05$
X^2tabulated = 6.44   Non significant

Table (3): Showing distribution of growth results between sexes.

Gender	Before treatment				After treatment			
	Bacterial isolation		Candida strains		Bacterial isolation		Candida strains	
	Pure carrier	Mixed carrier	Pure carrier	Mixed carrier	Pure carrier	Mixed carrier	Pure carrier	Mixed carrier
Female (50)	4	2	5	2	43	17	56	23
Male(25)	3	2	3	0	38	13	36	15
total	7	4	8	2	81	30	92	38

X^2calculated = 2.85   d.f =7 $p> 0.05$
X^2tabulated = 1.23   Non significant

Table (4): Showing percentage of bacterial antibiotic resistance.
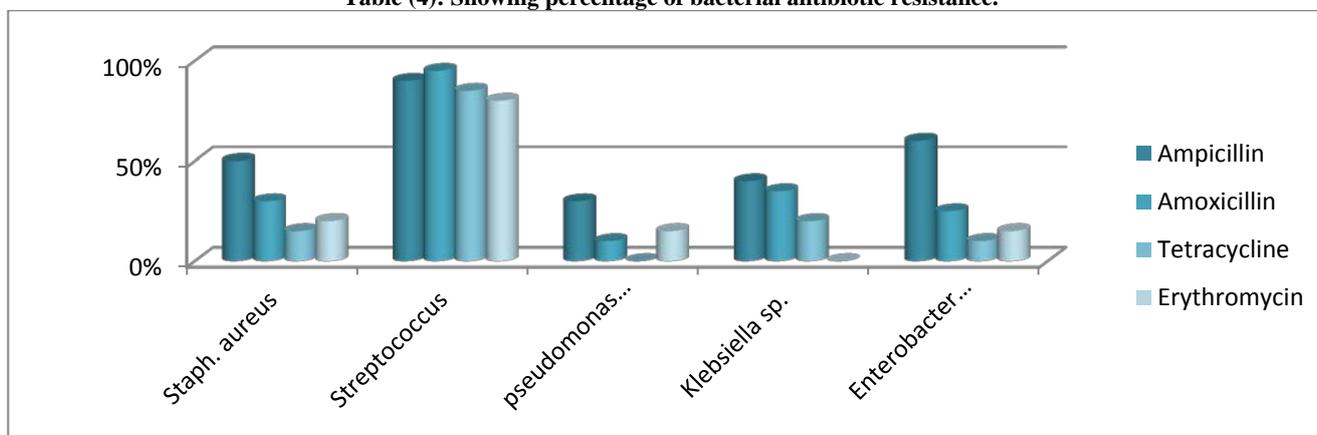
Table (5): Showing percentage of candida antifungal resistance.
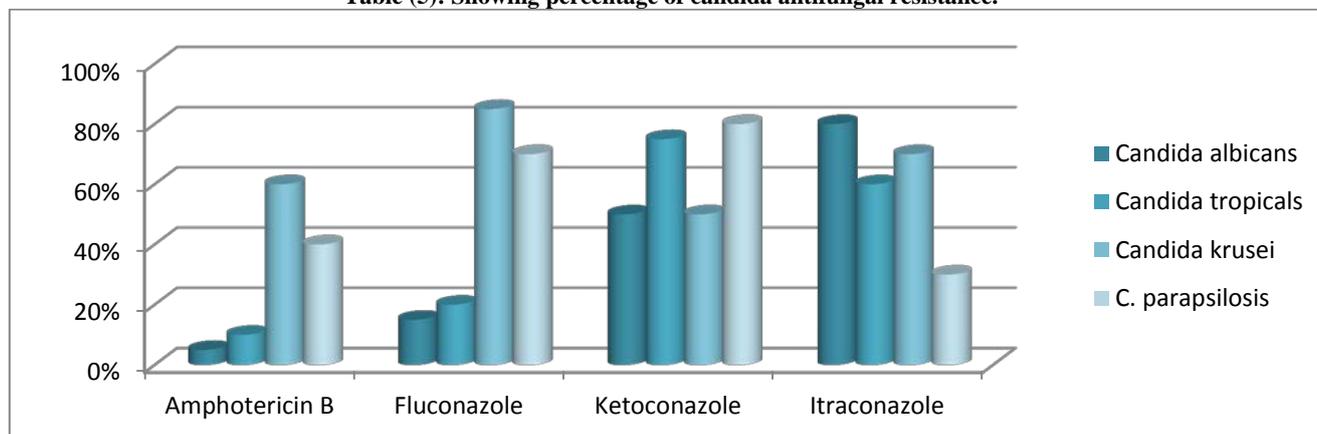
The female showed higher bacterial growth compared with male, and higher mixed growth over single growth in the female while male showed the reverse. The results of the statistical analysis test showed non-significant differences (P>0.05) between the distribution of growth according to sexes (table 3).

The results of the investigation in this study showing the significant differences in sex group after insertion of orthodontic appliances which leads to alter the ecological environment in the oral cavity by introducing new stagnant areas available for the bacterial colonization and retention of substances in tow figures as mixed and single growth. the single growth more than mixed in males and visa versa in females, this observation may be due to hormonal changes and physiological activity differences between them (10, 21).On the other hand, Arendorf and Walker (22) have shown that the presence of prosthesis or an appliance increase Candidial numbers, not only at the occluded site, but at all mucosal sites sampled. Hence, it is possible that orthodontic appliances may affect oral coliform colonization and carriage in the adults. In the recent study where the bacterial composition of dental plaque deposits on (metallic and ceramic)compared using 'Checkerboard' DNA-DNA hybridization analysis, 37 bacterial species were isolated, implying that the appliance surface may modify the oral environment and help colonize non-indigenous bacteria(23).

The presence of pathogenic and non-pathogenic bacteria resistant to different antibiotics has been reported by others authors (24, 25). In the present study *Staphylococcus aureus* was resistant to ampicillin (50%), amoxicillin (30%), tetracycline(15%) and erythromycin (20%). Streptococcus Sp. was resistant to ampicillin (90%), amoxicillin (95%), tetracycline(85%) and erythromycin (80%).While, *pseudomonas aeruginosa* was resistant to ampicillin (30%), amoxicillin (10%), tetracycline (0%) and erythromycin (15%). *Klebsiella* sp. was resistant to ampicillin (40%), amoxicillin (35%), tetracycline (20%) and erythromycin (0%) and Enterobacter sp. was resistant to ampicillin (60%), amoxicillin (25%), tetracycline (10%) and erythromycin (15%) (Table 4) .

In this study, polyenes and azoles are the most common antifungal agents used in the treatment of patients with fungal infections (12). Resistance rates to Amphotericin B in *C. albicans* were (5%), *C. tropicalis* (10%), *C. krusei* (60%), and *C. parapsilosis* (40%), whereas resistant rates to fluconazole were in *C. albicans* (15%) ,*C. krusei* (20%) and *C. parapsilosis* (70%) and Ketoconazole rates were in *C. albicans, C. tropicalis, C. krusei* and *C. parapsilosis* isolate as (50%, 75%, 50% ,and 80%) respectively (Table 5). These rates were reported in C. *albicans, C. tropicalis, C. glabrata and C. krusei* isolate as 7% (12/172), 33.3% (2/6), 15% (6/40) and 10% (6/60), respectively (11, 26).

CONCLUSION

1- Treatment with affixed appliances may alter the ecology in the oral cavity by introducing new stagnant areas available for colonization and retention of *Candida* species.

2- The result confirms this by indicating that fixed appliances have a direct effect upon bacterial count and adverse effect upon the prevalence of *Candida* and coliform carriage in this group of the adult.

3- The results of this study indicate that special efforts must be doing and attention has to be paid to control infection may be associated with fixed orthodontic appliances.

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