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## ORIGINAL ARTICLE

# Influence of fixed orthodontic appliances on the change in oral *Candida* strains among adolescents

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**KEYWORDS**

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**Abstract** *Background/purpose:* The aim of this study was to explore the presence and variability of oral *Candida* in adolescents before and during treatment with fixed orthodontic appliances.

*Materials and methods:* A total of 50 patients aged 10–18 years old were randomly selected for this study. Microorganism samples were obtained prior to and after orthodontic treatment and identified by culture methods. Molecular biology techniques were used to investigate the samples further and the effect of the orthodontic appliance on oral pathogenic yeasts was studied longitudinally.

*Results:* The percentage of patients with candidiasis and the total number of colony-forming units significantly increased 2 months after orthodontic treatment. Changes in the type of oral candidiasis prior to and after treatment were significant.

*Conclusion:* Fixed orthodontic appliances can influence the growth of oral pathogenic yeasts among adolescents.

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## Introduction

*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.<sup>1–5</sup> Many internal and

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external factors, such as systemic disease and impaired immune function, can result in environmental changes in the oral cavity. These changes affect the kinds of microorganisms found in the oral cavity as well as their metabolic and pathogenic activities.<sup>6</sup> *Candida* is often detected in the oral cavity of patients with denture stomatitis, especially middle-aged and elderly people with false teeth.<sup>7</sup>

Fixed orthodontic appliances (FOAs) are artificial devices in the mouth that can greatly affect oral health and allow plaque and food scraps to accumulate. FOAs can also bring about an increased number of microorganisms and amalgamated infections in the mouth,<sup>8</sup> including caries of the teeth, lips, buccal surfaces, and tongue. FOAs can also cause an increase in the number of Gram-positive bacteria in the mouth.<sup>9,10</sup> Increased levels of dental plaque are related to the development of gingivitis.<sup>11</sup> Patients with gingivitis are prone to periodontal disease<sup>12</sup> and loss of periodontal support.<sup>13</sup>

Few studies have been published about fungal colonization in patients with FOAs. Among 60 patients treated with an FOA, oral *Candida* flora were found in 15 (25%) patients, 14 of whom were aged between 16 years and 18 years. Removable orthodontic appliances can temporarily affect *Candida* colonization.<sup>14–16</sup> No study on the type, number, and pathogenic changes in oral *Candida* caused by orthodontic appliances has yet been published. The aim of this study was to explore changes in oral *Candida* strains among healthy adolescents prior to and after treatment with FOAs.

## Materials and methods

### Patients and samples

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lanzhou University, Lanzhou, China. Written informed consent was obtained from all participants. Fifty patients with FOAs were randomly selected. Of these patients, 23 were male adolescents and 27 were female adolescents. The average age was 13.6 years. No patient had a systemic, oral mucosal, or periodontal disease. The administration of antibiotic drugs to patients was stopped 2 weeks prior to sampling. The participants carefully brushed their teeth after breakfast and samples were taken 2 hours after food consumption. Microbiological samples were obtained via the gargle method 1 month, 2 months, 3 months, and 6 months prior to and after installation of the FOAs. The patients were required to gargle with 10 mL of sterile Phosphate Buffered Saline (PBS) for 1 minute. The resulting gargle was sent to the laboratory within 2 hours.

### Identification and culture

Microbiological samples were centrifuged and 50  $\mu$ L of the supernatant were cultured in CHROMagar *Candida* identification Petri dishes (CHROMagar, Paris, France) at 37°C 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, tropical *Candida* is blue, *C.*

*krusei* is pink, and other unidentified fungi in the culture medium are white. Colonies that did not grow within 7 days were not included in the count.

### Polymerase chain reaction identification

DNA was extracted according to the instructions provided in the kit used (Tiangen Biotech, Beijing, China). A specific sequence of a wild strain of phage M13 microsatellites was used as a single primer for polymerase chain reaction (PCR) amplification. The primer sequence was 5'-GAGGGTGGCGTTCT-3'.<sup>16</sup> For the PCR reaction, 1  $\mu$ L of DNA, 2  $\mu$ L of the primer, and 10  $\mu$ L of 2  $\times$  PCR Master Mix (Tiangen Biotech, Beijing, China) were added to 20  $\mu$ L of double-distilled H<sub>2</sub>O. The reaction conditions were as follows: 95°C denaturation for 1 minute, 95°C denaturation for 30 seconds, 60°C annealing for 30 seconds, extension at 68°C for 90 seconds, 25 cycles, and finally extension at 68°C for 10 minutes. PCR products were detected using 1.5% agarose gel electrophoresis and scanned by a gel imaging camera. International standards for *C. albicans* ATCC90028 were used as positive controls and sterilized PBS buffer was used as a negative control.

## Results

### Identification and culture results

As shown in Fig. 1, several patients were carriers of pure strains of bacteria, which appeared as monochromatic colonies. The strains could be identified by colony color. Other patients were carriers of mixed bacteria, where the colonies had two or three different colors.

### PCR results

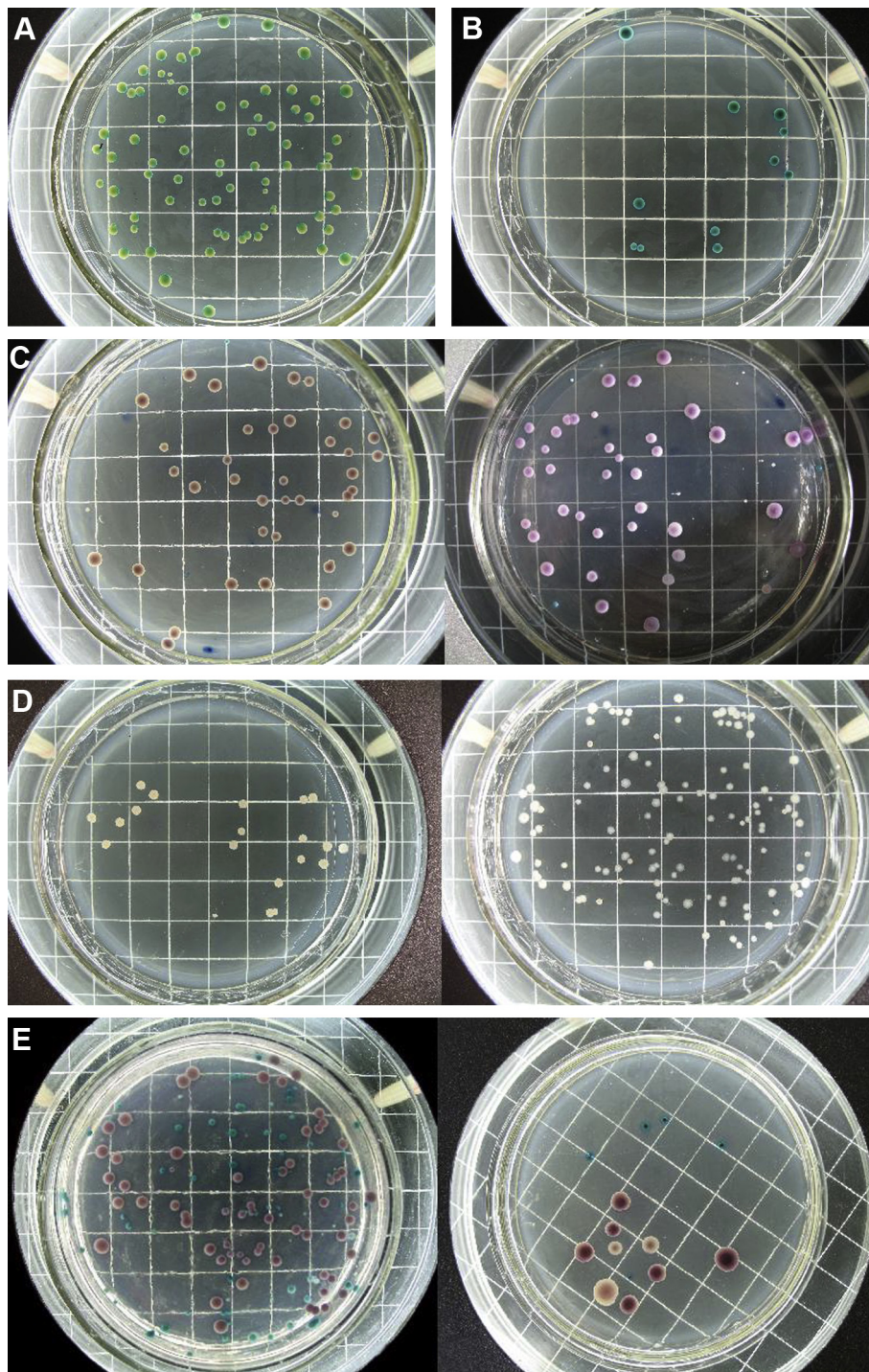
The PCR results showed that green colonies have bands similar to those of *C. albicans* ATCC90028, thus these colonies may be identified as *C. albicans*. This result further confirmed the accuracy of the CHROMagar *Candida* color culture. *C. albicans* was also detected by PCR using the international standard for *C. dubliniensis* ATCC6258 as the control. The results confirmed that the green colonies in the clinical samples were *C. dubliniensis* (Fig. 2).

### Rate of carrying bacteria and strain analyses

Tables 1 and 2 show that the number of total and mixed carriers was higher 2–3 months after treatment than prior to treatment. After 6 months these changes were comparable with the levels prior to treatment, suggesting that the most significant changes in the number and type of *Candida* strains in the mouth may be found 2–3 months after fitting an FOA.

### Total number of colonies

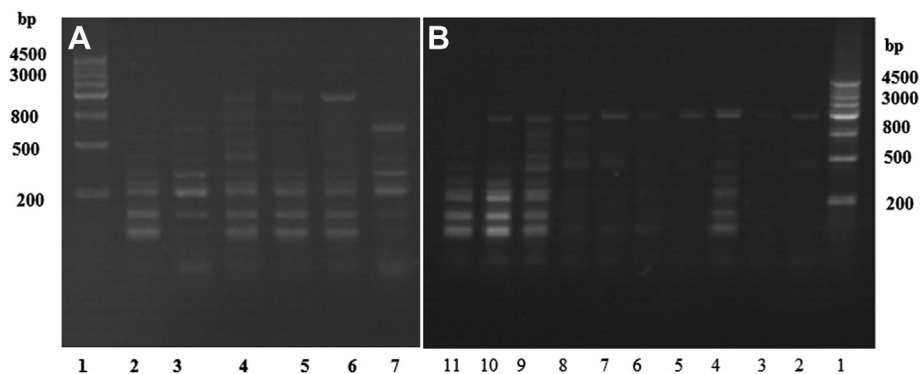
The results were analyzed using two-factor variance analysis, which revealed that the total number of colonies in different Petri dishes were not significantly different ( $P = 0.928$ ). This finding suggests that the cultivation



**Figure 1** Results obtained after 48 hours of culture of clinical samples. Different kinds of *Candida* appear as different colors. Scale 10 mm/square. (A) *Candida albicans* (green, light green). (B) *Candida tropicalis* (green, dark green). (C) *Candida glabrata* (purple; left, lamplight; right, natural light). (D) Unknown yeasts (white). (E) Fixed yeasts: *C. glabrata* (purple), *C. albicans* (green), *Candida krusei* (pink).

conditions in the Petri dishes were exactly the same. However, the total number of colonies at different stages was significantly different ( $P = 0.003$ ). This number increased during the next three periods: 2 months prior to treatment and during treatment ( $P = 0.000785$ ); 3 months prior to treatment and during treatment ( $P = 0.046811$ );

and between 1 month and 2 months after treatment ( $P = 0.002619$ ). The total number of colonies significantly decreased ( $P = 0.009289$ ) 2 months and 6 months after treatment ( $P = 0.009289$ ). The number of colonies was highest 2 months after treatment. The results are shown in [Tables 3 and 4](#).



**Figure 2** Identification of *Candida albicans* by polymerase chain reaction. Compared with ATCC90028 *C. albicans*, the clinical culture samples which appeared green are all *C. albicans*. At the same time, ATCC6258 *Candida dubliniensis*, which has a similar gene type to *C. albicans* had also been detected by polymerase chain reaction, the results confirmed that there are no *C. dubliniensis* in the clinical samples. (A) 1 = Marker; 2 = ATCC90028 *C. albicans*; 3, 7 = ATCC6258 *C. dubliniensis*; and 4–6 = clinical samples. (B) 1 = Marker; 2–4 = three colonies of patient A; 5–7 = three colonies of patient B; 8–9 = two colonies of patient C; 10 = one colony of patient D; and 11 = ATCC90028 *C. albicans*.

## Discussion

In this study, the incidence of oral *Candida* among normal adolescents prior to the application of FOAs was 14%, lower than the reported rate of 24–40%.<sup>17</sup> This may be attributed to the fact that the patients brushed their teeth prior to sampling, thus reducing the number of microbes in the oral cavity. Regional differences may also contribute to the variations observed.

Within 3 months of the installation of FOAs, the rate of pathogenesis and number of colonies of oral *Candida* significantly increased compared with those prior to treatment, particularly at 2 months after FOA installation; these values then gradually decreased over time. These findings may be due to the FOAs 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.<sup>18</sup> Lip buccal mucosal damage was observed in many patients shortly after the application of FOAs. Varying degrees of oral ulcers or gum inflammation, both of which can decrease local defense mechanisms, were also observed.

The interaction of *Candida* and other oral bacteria, including adhesion between *C. albicans* and other microorganisms in the host cells, is an important factor in maintaining the commensalism of bacteria in the human body. *Escherichia coli*, *Streptococcus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* can restrain the pathogenicity of *Candida*. Obligate anaerobic bacteria can inhibit the proliferation and adhesion of *C. albicans* to mucosa. *S. aureus* and *Candida* have synergetic pathogenic characteristics.<sup>19</sup> Grimaudo and Neabitt<sup>20</sup> showed that the aggregation of *C. albicans* and oral *Fusobacterium* may be an important factor in colony formation in the mouth. According to oral microecological theory, an ecological balance exists between the microorganisms in the mouth and the host. When the host conditions change, the type, number, and proportion of mouth microbes also change. An FOA can change the original ecological balance of the oral cavity and oral microbial plexus. Studies have shown that the oral cavity of patients with FOAs have more *Bacteroides melaninogenicus* and intermediate *Bacillus*. Moreover, the proportion of anaerobic bacteria to facultative anaerobic bacteria also increased.<sup>21</sup> The increase in the proportion of *Bacillus* in patients with FOAs can increase the number of *Candida* colonies in the oral cavity.

Adhesion of *Candida* to parts of the FOA, during which *Candida* adheres to different dental metal material surfaces, may also affect colony formation. The extent of adhesion is dependent on the surface roughness and type of material used. The adhesion of *Candida* to the surface of metal or ceramic orthodontic appliances should be studied further.

There is also a relationship between *Candida* and the resistance of patients, where an increase in the *Candida* population may cause a temporary weakening of the resistance of the body during the adaptive phase after the application of the FOA. The amount of bacteria gradually declined 3 months after treatment, which may be due to the gradual adaptation of the patients to the new oral environment. Moreover, full recoveries of systemic and local resistance were also observed. The increase in the number of *Candida* colonies may be associated with an

**Table 1** Incidence of yeasts in different periods in orthodontic treatment.

	Before treatment	Months after treatment			
		1	2	3	6
Pure carrier	7 (14)	7 (14)	9 (18)	8 (16)	7 (14)
Mixed carrier	0 (0)	2 (4)	6 (12)*	5 (10)**	3 (6)
Total carrier	7 (14)	9 (18)	15 (30)	13 (26)	10 (20)

Data are presented as  $n$  (%);  $\alpha = 0.05$ .

\* $\chi^2 = 6.3830$ ,  $P < 0.05$ .

\*\* $\chi^2 = 5.2632$ ,  $P < 0.05$ .

**Table 2** Occurrence of various kinds of yeasts in patients at different treatment periods.

	Before treatment	Months after treatment			
		1	2	3	6
<i>Candida albicans</i>	6 (85.7)	4 (44.4)	5 (33.3)	4 (30.8)	6 (60)
<i>Candida parapsilosis</i>	0 (0)	2 (22.2)	1 (6.67)	2 (15.4)	0 (0)
<i>Candida tropicalis</i>	0 (0)	0 (0)	1 (6.67)	1 (7.69)	0 (0)
<i>Candida krusei</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
X	1 (14.3)	1 (11.1)	2 (13.3)	1 (7.69)	1 (10)
<i>C. albicans</i> + <i>C. parapsilosis</i>	0 (0)	0 (0)	3 (20)	1 (7.69)	0 (0)
<i>C. albicans</i> + <i>C. tropicalis</i>	0 (0)	0 (0)	0 (0)	1 (7.69)	0 (0)
<i>C. albicans</i> + <i>C. krusei</i>	0 (0)	0 (0)	1 (6.67)	0 (0)	0 (0)
<i>C. albicans</i> + X	0 (0)	1 (11.1)	1 (6.67)	1 (7.69)	1 (10)
<i>C. parapsilosis</i> + <i>C. tropicalis</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
<i>C. albicans</i> + <i>C. parapsilosis</i> + <i>C. krusei</i>	0 (0)	1 (11.1)	0 (0)	1 (7.69)	1 (10)
<i>C. albicans</i> + <i>C. parapsilosis</i> + X	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>C. albicans</i> + <i>C. krusei</i> + X	0 (0)	0 (0)	1 (6.67)	1 (7.69)	0 (0)
Total	7 (100)	9 (100)	15 (100)	13 (10)	10 (100)

Data are presented as n (%).

X = unidentified by CHROM agar medium; + = two or three colonies.

**Table 3** Number of colony-forming units at different time periods.

Prior to treatment	Months after treatment			
	1	2	3	6
13.05 ± 11.294	17.30 ± 21.352	36.98 ± 37.857	27.38 ± 25.667	20.57 ± 14.258

Data are presented as mean ± SD.

increase in the amount of plaque and microbes in the oral cavity of patients shortly after FOAs start to be worn.

The increase in the population of other strains and in mixed bacteria, except *C. albicans*, may be related to differences in adhesion and interaction between bacteria and fungi.

The ratio of *Candida* to mixed bacteria is 10%; this value is about 11.3% in normal people. The mixed carrying bacterial rate of *Candida* carriers is 23.8%, higher than that of normal people. Our results reveal that the rate of carrying bacteria is significantly high. However, increases in the possibility of infection due to FOAs require further study.

The increase in the number of *Candida* colonies aside from *C. albicans* in patients may be due to differences in the living conditions of other *Candida* strains. After the application of FOAs, 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 FOAs. 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.<sup>22</sup> Similar features of this behavior in FOAs require further research.

**Table 4** Total number of colonies at different stages.

Stage I	Stage J	Mean difference (I - J)	Standard error	P	95% confidence interval	
					Lower limit	Upper limit
Prior to treatment (mo)	1	-4.24868	7.690483	0.58143	-16.9745	8.477102
	2	-23.9302	6.985228	0.000785*	-35.4889	-12.3714
	3	-14.337	7.154153	0.046811*	-26.1753	-2.49871
	6	-7.51905	7.520372	0.318954	-19.9633	4.925242
After 1 mo	After 2 mo	-19.6815	6.43432	0.002619*	-30.3286	-9.03433
	After 3 mo	-10.0883	6.617323	0.129414	-21.0383	0.861654
	After 6 mo	-3.27037	7.011637	0.64157	-14.8728	8.332093
After 2 mo	After 3 mo	9.593162	5.782633	0.099146	0.024387	19.16194
	After 6 mo	16.41111	6.230003	0.009289*	6.102052	26.72017
After 3 mo	After 6 mo	6.817949	6.418834	0.289807	-3.80358	17.43947

P < 0.05 indicates significant difference.

White colonies that were not identified by the CHROMagar *Candida* medium may be *Candida* strains other than *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, or yeast strains.

FOA can change the carrying rates of *Candida* in the oral cavities of adolescents. The carrying rates, total number of bacterial colonies, and strains of *Candida* all changed prior to and after the application of FOAs.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

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