sciendo

# The effect of orthodontic appliances on the Oral *Candida* colonisation: a systematic review

Alessandra Campobasso,* Eleonora Lo Muzio,† Giovanni Battista,* Vito Carlo Alberto Caponio,* Domenico Ciavarella* and Lorenzo Lo Muzio*

Department of Clinical and Experimental Medicine, University of Foggia, Clinica Odontoiatrica Via Rovelli 50, Foggia 71122, Italy*

Department of Translational Medicine and for Romagna, School of Orthodontics, University of Ferrara, Via Luigi Borsari 46, Ferrara 44121, Italy†

*Objectives:* To evaluate the influence of Fixed (FOA) and Removable Orthodontic Appliances (ROA) on oral *Candida* colonisation.

*Methods:* A search for articles published in the English language until September 2021, was carried out using Pubmed, Scopus and Web of Knowledge databases and by applying the search terms "orthodontic" OR "orthodontics" OR "fixed appliance" OR "removable appliance" OR "bracket" OR "removable aligner" AND "Candida" OR "Candidiasis" OR "Candidosis" to identify all potentially relevant human studies. After the removal of duplicate articles and data extraction according to the PICOS scheme, the methodological quality of the included papers was assessed by applying the Swedish Council on Technology Assessment in Health Care Criteria for Grading Assessed Studies (SBU).

*Results:* The initial search identified 533 articles, 157 of which were selected by title and abstract. After full-text reading, sixteen articles were selected. The evidence quality for all the studies was moderate.

*Conclusions:* ROA induced a temporary increase of *Candida* counts from the early stage of treatment but which returned to the pre-treatment level after ROA removal. Contrasting results were reported for FOA treatment which promoted the oral colonisation of non-*albicans* species, although the most prevalent species was *Candida albicans* in both groups.

This review should be interpreted with caution because of the number, quality, and heterogeneity of the included studies.

(Aust Orthod J 2022; 38: 51 - 62. DOI: 10.2478/aoj-2022-0006)

Received for publication: November, 2021

Accepted: January, 2022

Alessandra Campobasso: alessandra.campobasso@unifg.it; Eleonora Lo Muzio: eleonoralomuzio@gmail.com; Giovanni Battista: drgiovannibattista@gmail.com; Vito Carlo Alberto Caponio: vitocarlo.caponio@unifg.it; Domenico Ciavarella: domenico.ciavarella@unifg.it; Lorenzo Lo Muzio: lorenzo.lomuzio@unifg.it

## Introduction

### *Background*

*Candida* species is a commensal yeast that colonises the oropharyngeal region of more than 60% of healthy subjects without resulting in clinical symptoms of infection.[1] Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of the *Candida* species, the most common being *Candida albicans*.[2] The ability of *Candida* to become a pathogenic microorganism is determined by risk factors, including systemic diseases (diabetes or infection) and local factors (orthodontic appliances, removable dentures and poor oral hygiene).[2] The onset of candidiasis represents a serious clinical problem, especially in immune-compromised patients,[3] because the infection can spread via the vascular route or upper gastrointestinal tract and lead to severe systemic infection.[2] Due to an increase in the use of corticosteroid and immuno-suppressive therapies as well as the improved survival of certain diseases (such as AIDS), an increasing number of immune-compromised

patients present for orthodontic treatment, along with their healthy peers.[3] Moreover, the number of children diagnosed with cancer is increasing and the greater efficacy of children's oncological treatment has globally increased the number of cancer survivors.[4] Therefore, the number of oncological children or adolescents seeking orthodontic treatment is also increasing[3] and attention must be paid to the possible complications that orthodontic treatment could induce in immuno-compromised patients[5] as it has been shown that orthodontic appliances (fixed and removable) could promote changes in the oral microbiota.[6] A Fixed Orthodontic Appliance (FOA) is the most common treatment method used in contemporary orthodontics.[7] Its complex design based on the fixed placement of brackets and bands can affect oral hygiene practices, thereby promoting the accumulation of dental plaque and altering saliva properties and microbial counts.[8] A Removable Orthodontic Appliance (ROA) is another common device used to move or retain teeth during or after orthodontic treatment.[9] Because these acrylic appliances cover a large area of mucosa for extended periods, the prolonged wear of an ROA reduces salivary flow and pH levels, protects the microbiome from the natural flow of saliva and the mechanical removal effects of the oral musculature.[9] Published literature has shown that these variables would possibly lead to pathogenic *Candida* colonisation, especially if there are favourable conditions and a reduction in immune function.[3] However, the effect of orthodontic treatment by fixed and/or removable appliances on *Candida* colonisation has not been assessed in an evidence-based manner. The only existing systematic review of treatment-induced *Candida* changes assessed few databases and found a limited number of studies.[3]

Therefore, the evaluation of the effects of orthodontic treatment on oral *Candida* status is helpful for clinicians to decide the most appropriate and individualised treatment based on the patient's clinical conditions, especially in susceptible patients who might have a high risk of local or systemic complications.[10]

Therefore, the aim of this review was to evaluate if orthodontic appliances induce changes in *Candida* colonisation in order to answer the following questions:

1. Does an orthodontic appliance affect the number and the composition of *Candida* colonies in the oral cavity?
2. Are there any differences in *Candida* populations related to FOA and ROA?

## Material and methods

### *Protocol*

This systematic review was performed according to the PRISMA statement.[11]

### *Eligibility criteria*

According to Participants-Intervention-Comparison-Outcome-Study design schema (PICOS), the inclusion and exclusion criteria are summarised in Table I.

### *Information sources and literature search*

The search for articles was carried out using four electronic databases (Pubmed, Scopus, Web of Knowledge, CENTRAL), and included publications in the English language from inception up to September 2021. Human studies which featured the keywords "orthodontic" OR "orthodontics" OR "fixed appliance" OR "removable appliance" OR "bracket" OR "removable aligner" AND "Candida" OR "Candidiasis" OR "Candidosis", were identified. In addition, the reference and citation lists of the included trials and relevant reviews were manually searched.

### *Study selection*

All titles identified from the literature were screened and selected by two independent authors (A.C.; E.L.M.). Duplicate studies were eliminated. The abstracts were examined and full texts were obtained if additional data were needed to fulfil the eligibility criteria. Conflicts were resolved by discussion with a third author (L.L.M.).

### *Data collection*

The characteristics of the included studies (study design, patients, age, orthodontic appliance, sample site, timing, analysis method, outcome, additional measures, quality of the study) were independently extracted by two authors (A.C.; E.L.M.). For further clarification, missing or unclear information was directly requested of the respective authors.

### *Methodological quality assessment*

The methodological quality of the included studies was assessed according to the "Swedish Council on

Table I. List of inclusion and exclusion criteria.

| Field | Inclusion | Exclusion |
|---|---|---|
| Patients | Children, adolescents or young adults (<25 years) of any sex, ethnicity and malocclusion, in general good health | Adults (>25 years)<br>In vitro studies<br>Animal studies |
| Intervention (exposure) | Orthodontic treatment with any vestibular fixed appliance (metal or ceramic, conventionally-ligated or self-ligated) or any removable appliances | Patients not receiving orthodontic treatment<br>Patients receiving orthodontic treatment without specific descriptions of the materials and applied technique<br>Patients receiving partial appliances<br>Patients receiving or having received systemic antibiotic treatment less than a month before or during orthodontic treatment<br>Smoking patients |
| Comparison | A. No comparison (For the descriptive analysis of Candida changes in treated patients)<br>B. Ortho-tx vs no-tx (Comparison between treated and non treated patients)<br>C. Ortho-tx vs ortho-tx (Comparison between ROA and FOA) | |
| Outcome | Quantitative and qualitative analysis of *Candida* colonies, from intra-oral mucosal sites, saliva or supra/sub-gingival plaque.<br>All available time-points will be included and categorized into pre-treatment, short-term (< 3 months) treatment, mid-term (3–6 months) treatment and long-term (< 6 months) treatment, post-treatment | No clear mention of the analysis or time-point |
| Study design | Randomized clinical trials or non-randomized, prospective or retrospective, cohort studies | |

Note: Tx, treatment; ROA, removable orthodontic appliance; FOA, fixed orthodontic appliance.

Technology Assessment in Health Care Criteria for Grading Assessed Studies" (SBU) method.[12] Articles were ranked into three levels (A, B, C) of evidence (Table II) and, based on the score assigned to each study, the review level of available evidence was further scored into four grades (1,2,3,4) (Table III).

Table II. Swedish council on technology assessment in health-care (SBU) criteria for grading assessed studies.

| **SBU criteria for grading assessed studies** |
|---|
| **Grade A** (High level of evidence) |
| Randomized clinical study or prospective study with a well-defined control group, defined diagnosis and endpoints, diagnostic reliability tests and reproducibility tests described |
| **Grade B** (Moderate level of evidence) |
| Cohort study or retrospective case series with defined control or reference group, defined diagnosis and endpoints, diagnostic reliability tests and reproducibility tests described |
| **Grade C** (Low level of evidence) |
| Large attrition, unclear diagnosis and endpoints, poorly defined patient material |

Table III. Definitions of evidence level.

| Level | Evidence | Definition |
|---|---|---|
| 1 | Strong | At least two studies assessed with level "A" |
| 2 | Moderate | One study with level "A" and at least two studies with level "B" |
| 3 | Limited | At least two studies with level "B" |
| 4 | Inconclusive | Fewer than two studies with level "B" |

### *Data synthesis*

Due to the lack of homogeneity in the study setting (study design, sample site, sample collection time and methods), only a systematic review could be conducted rather than a meta-analysis.

## Results

### *Study selection*

The initial search identified 533 articles from Pubmed, Scopus and Web of Knowledge. After eliminating duplicates and ineligible studies by title and abstract, a total of 157 full texts were screened. Finally, a total of sixteen papers were identified according to the eligibility criteria.

The flow chart of the selection of eligible studies for this review is summarised in Figure 1.

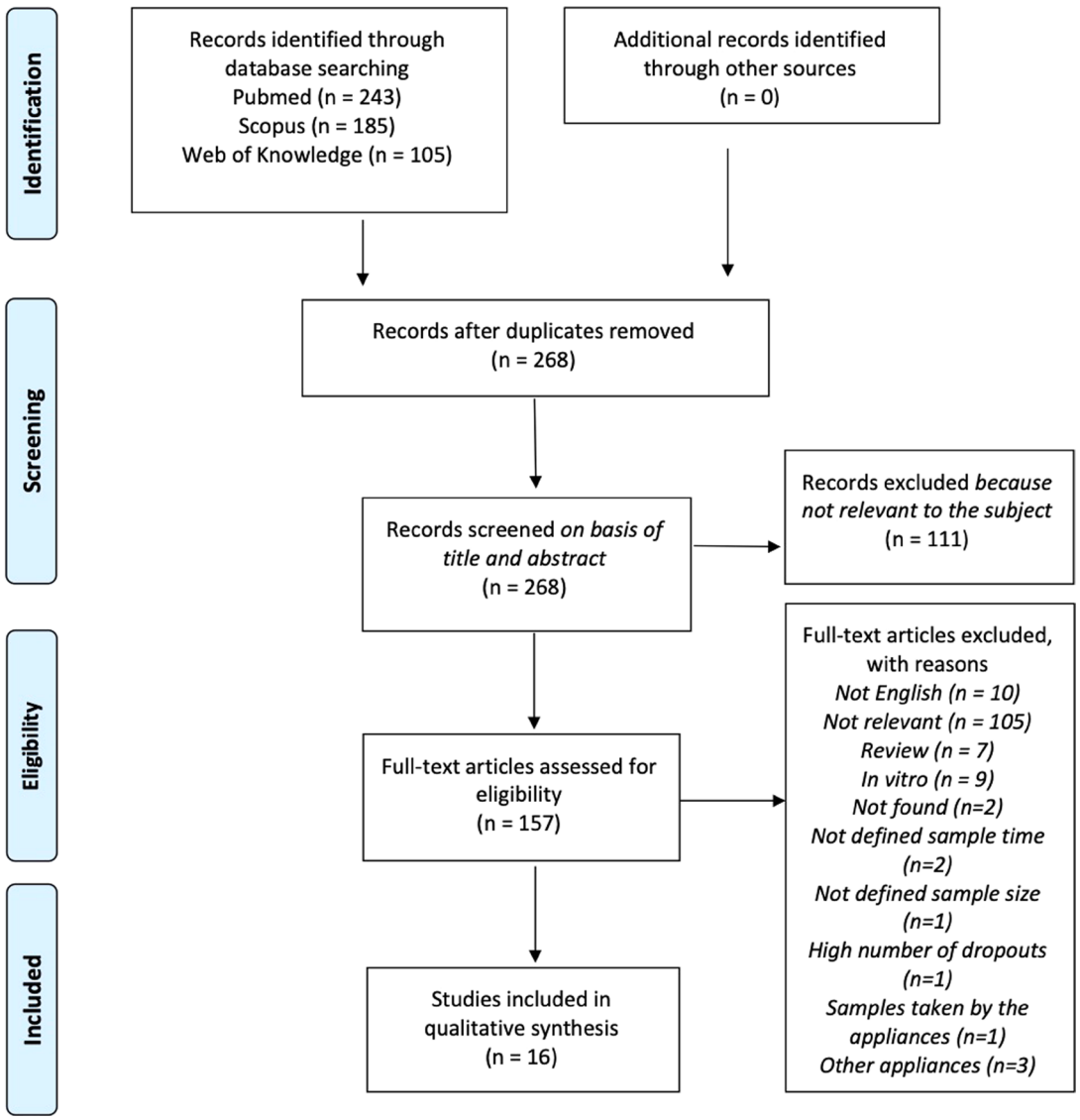


Figure 1. Flow diagram of the included studies according to the PRISMA.

### *Assessment of methodological quality*

According to the SBU tool, the quality of evidence for nine studies was moderate (grade B) and for seven studies was low (grade C). As a result, the level of evidence for the conclusions of this review was considered limited (level 3).

### *Study characteristics*

The characteristics of the studies are presented in Table IV. Of the 16 included studies, all were prospective in nature and included four reports which described the changes in *Candida* in patients treated using a ROA[13,17,20,26] and nine studies which described those treated with FOA.[8,14–16,21–24,27] Three studies analysed and compared treated and non-treated patients, two of which involved a ROA[17,18] and one used FOA[25]. An additional untreated group served as a control. Only one study compared the changes in candida between ROA and FOA therapies.[19]

### *Results of individual studies*

The results are summarised in Figure 1.

#### *Primary outcome*

### *Short-term (<3 months) Candida changes*

Two studies[20,26] described the short-term changes occurring during ROA treatment; eight studies[8,14,15,21–24,27] analysed the effects related to FOA therapy. From baseline to one month of ROA therapy, a significant increase ($p < 0.001$) in the number of *Candida albicans* counts was observed in saliva[20] and on the oral mucosa.[26] During the early stages of FOA, Hägg et al.[14] found a significant *Candida* increase on the dorsum of the tongue ($p < 0.001$), but not in saliva and plaque samples. Arslan et al.[15] reported an increase in the number of colony-forming units (CFU) was statistically significant ($p < 0.001$) both in saliva and on tooth surfaces. This was confirmed by the salivary results ($p < 0.001$) of Arab et al.[8] and by a plaque analysis ($p < 0.05$) conducted by Shukla et al.[22] Zheng et al.[21] also reported a significant increase of *Candida* counts ($p < 0.001$) in gargled samples, finding a higher ($p < 0.05$) percentage of *Candida* carriers after 2 months of FOA, compared to pre-treatment. Different results were reported by Lee et al.[16] and Grzegocka et al.,[23] who determined a non-significant increase of *Candida*, after analysing saliva samples. In addition, Soler et al.[24] found no significant differences at the vestibular level, while Kouvelis et al.[27] reported that *Candida* was not identified in any sample before and after 4 weeks of FOA.

### *Mid-term (3–6 months) Candida changes*

One study[20] reported the mid-term effects of ROA treatment and found a significant increase ($p < 0.001$) in the *Candida* counts in saliva after 3 months. Seven studies[8,14,16,21–23,27] analysed the mid-term effects after FOA placement. Hägg et al.[14] found a significant increase in candida on the dorsum of the tongue, but not in saliva and plaque samples. However, Lee et al.[16] reported a significant increase in the presence of candida in saliva. Zheng et al.[21] also showed that the presence of *Candida* was significantly higher after 3 months of FOA treatment compared to baseline, finding a significant increase ($p < 0.05$) of *Candida* counts in a gargled sample. The increase was confirmed by Arab et al.[8] and Shukla et al.[22] who analysed saliva and dental plaque, respectively. Grzegocka et al.[23] showed a non-significant upward trend of yeast numbers in saliva after 12 weeks of FOA treatment. Only one study[25] analysed the differences in candida between FOA and untreated patients suggesting that, 3–6 months after FOA placement, *Candida* was a frequently isolated species (12%) in orthodontic patients compared to a control group, and that, in the mid-term, the frequency of *Candida* significantly increased in FOA patients, compared to untreated cases.

### *Long-term (> = 6 months) Candida changes*

One study[20] investigated the long-term *Candida* changes in patients treated with ROA, and observed a significant increase of *Candida* counts ($p < 0.001$) in saliva after 6 months. Two studies[17,18] compared *Candida* counts between ROA and control groups, and found conflicting results. Mahmoudababi et al.[17] reported the prevalence of the salivary colonisation of *Candida* spp. was significantly higher ($p < 0.001$) in ROA patients, compared to untreated subjects. Gonçalves et al.[18] observed no statistically significant differences in saliva yeast counts between the ROA and a control group. A further study[19] compared the differences between two orthodontic groups, one treated with ROA and one with FOA, through an

Table IV. Characteristics of the studies.

| No. | Study ID/ | Design | Patients (M/F) | Age[a] | Appliance | Sample site | Timing | Analysis method | Outcome | Additional measures | Quality of the study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Arendorf 1985[13] | Prospective | Exp: 33 (15/18) | 8–17 y | ROA | Six mucosal site (ant and post palate, ant and post tongue, r and l cheek) | T0 = before AppIns<br>T1 = during therapy<br>T2 = after AppRem (after average 9 mos) | Imprint Culture (*Arendorf and Walker technique*) | Prevalence (%) Density | PI + saliv pH | C |
| 2 | Hägg 2004[14] | Prospective | Exp: 27 (13/14) | 15.5 +- 2.3 y | FOA | Rinse Dorsum of the tongue Supra and subgingival plaque | T0=before AppIns<br>T1 = 1 mos after T0<br>T2 = 2 mos after T0<br>T3 = 3 mos after T0 | Oral Rinse (*Samaranayake technique*)<br>Imprint Culture (*Arendorf and Walker technique*)<br>(*SDA, Gram stain, germ tube test, API 20 C AUX*)<br>Pooled Plaque | Prevalence (%)<br>Density (CFU)<br>Species composition | PI + count of *Enterobacteriaceae* + total bacterial count | B |
| 3 | Arslan 2008[15] | Prospective | Exp 1: 72<br>Exp 2: 42<br>(19/23)<br>*Candida* carriers out of 72 subjects, that were treated | 19.8 y | FOA (metal brackets) | Dorsum of the tongue (only for T0)<br>Mid-palate (only for T0)<br>Saliva<br>U5/L5<br>U1/L1 | T0 = before AppIns<br>T1 = 1 mos after T0<br>T2 = 6 mos after T0<br>T3 = 12 mos after T0 | Swab Culture<br>*(Kleinegger method, SDA)*<br>Salivary culture<br>*(SDA, Gram staining, germ-tube test, chlamydo-spore, API 20C AUX system))*<br>Pooled plaque<br>*(SDA)* | Prevalence (%)<br>Density (CFU)<br>Species composition | None | B |
| 4 | Lee 2008[16] | Prospective | Exp: 97 (38/59) | 17.7 y | FOA | Rinse | From T0 = before AppIns to T10 = 12 mos after T0 | Oral rinse technique (*Samaranayake technique*, SDA)<br>Phenotypic methods *(germ-tube test; API ID 32C)*<br>Genotypic methods *(RAPD analysis)*<br>Dendogram analysis | Prevalence (%)<br>Species composition | None | C |
| 5 | Mahmoudadabi 2009[17] | Prospective | Exp 1: 34<br>Exp 2: 34 | 13 y (Exp 1)<br>12.5 y (Exp 2) | ROA (upper) Cr | Saliva<br>*Surface of upper appliance (not considered)* | T0 = before AppIns<br>T1 = over 8 mos after T0 | Culture (Arendorf and Walker, Davenport techiques) (*CHROMagar; germ-tube test)* | Prevalence (%)<br>Count (CFU)<br>Species composition | None | C |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | Gonçalves e Silva 2014[18] | Prospective | Exp 1: 30<br>Exp 2: 30 | 9.1+-1.7 (Exp 1)<br>7.7+-1.5 (Exp 2) | ROA<br>Cr | Cheek and lateral surface of the tongue<br>Saliva | T0 = before AppIns<br>T1 = at least 6 mos after T0 | Culture (*SDA; CHROMagar*)<br>Phenotypic methods<br>Exfoliative cytology | Prevalence (%)<br>Count (CFU)<br>Species composition | Counts of Anti-C.*albicans* IgA | B |
| 7 | Arab 2016[8] | Prospective | Exp 1: 30 (6/24) | 12–18 y | FOA | Saliva | T0 = before AppIns<br>T1 = 6 we after T0<br>T2 = 12 we after T0<br>T3 = 18 we after T0 | Culture *(SDA)* | Count (CFU) | Salivary flow and pH<br>Microbial counts (S.*mutans*/ L.*acidophilus*) | C |
| 8 | Khanpayeh 2014[19] | Prospective | Exp 1: 40<br>Exp 2: 40 (35/45) | 7–18 y | FOA *(metal)*<br>ROA | *Unstimulated* saliva | T0 = before AppIns<br>T1 = 6 mos after T0 | Culture *(SDA; Germ-tube test; corn meal agar)*<br>Biochemical tests *(API 20C method)* | Frequency (%)<br>Species composition | None | B |
| 9 | Kundu 2016[20] | Prospective | Exp 1: 10<br>Exp 2: 10 *(not considered)* | 6–15 y | ROA Fixed space maintainers (nc) | *Unstimulated* saliva | T0 = before AppIns<br>T1 = 1 mo after T0<br>T2 = 3 mos after T0<br>T3 = 6 mos after T0 | Culture *(SDA)* | Count (CFU) | Bacterial count (*S. mutans* and *Lactobacillus* sp.) | B |
| 10 | Zheng 2016[21] | Prospective | Exp 1: 50 (23/27) | 10–18 (13.6 y) | FOA | Gargle | T0 = before AppIns<br>T1 = 1 mo after T0<br>T2 = 2 mos after T0<br>T3 = 3 mos after T0<br>T4 = 6 mos after T0 | Culture *(CHROMagar)*<br>PCR (*Tiangen Biotech)* | Incidence (%)<br>Count (CFU)<br>Species composition | None | B |
| 11 | Shukla 2017[22] | Prospective | Exp 1: 60 | 16–18 y | FOA | Buccal and labial Plaque of anterior teeth and U6 + L6 | T0 = before AppIns<br>T1 = 2 mo after T0<br>T2 = 3 mos after T0 | Swab Culture (*SDA; Gram stain; germ tube test, counts in CFU*) | Count (CFU) | *S.mutans* | B |
| 12 | Grzegocka 2020[23] | Prospective | Exp 1: 17 (6M/11) | 17+-7 y | FOA | Oral rinse Elastomeric rings (nc) | T0 = before AppIns<br>T1 = 2 we after T0<br>T2 = 6 we after T0<br>T3 = 12 we after T0 | Culture *(Dalmau plate technique)*<br>Biochemical tests *(API 20C AUX)* | Prevalence (%)<br>Count (CFU)<br>Species composition | API<br>GBI<br>Biofilm formation | C |
| 13 | Sanz-Orrio-Soler 2020[24] | Prospective Controlled Trial | Exp 1: 124 (43/80) | 19.5 y | FOA (metal or ceramic) | U and L vestibule | T0 = before AppIns<br>T1 = 1 mo after T0<br>T2 = 6 mos after T0<br>T3 = 12 mos after T0<br>T4 = 6 mos after AppRem | Swab Culture *(CHROMagar plates, Becton Dickinson)* | Frequency (%)<br>Species composition | Questionnaire about hygiene habits | B |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Pellissari 2021[25] | Prospective | Exp 1: 23 (7/10) Exp 2: 6 (2/4) | 20.7+ 8.7 y (Exp 1) 19.6+1.3 y (Exp 2) | FOA Cr | Biofilm around bck | From 3 to 6 mos after ApplIns | Culture Biochemical tests (*VITEK 2 compact system*) | Prevalence (%) Species composition | Fungal strains and resistance to Antifungals Bacterial strains and resistance to Antimicrobials | B |
| 15 | Rodríguez-Renteria 2021[26] | Prospective | Exp 1: 55 (34/21) | 8.4 y | ROA | Support oral mucosa Surface of ROA (not considered) | T0 = before ApplIns T1 = 4 we after T0 | Chromogenic culture (*ID 32 C AUX system*) | Frequency (%) Species composition | Microbial species (*Str.aureus, P.aeruginosa*) | C |
| 16 | Kouvelis 2021[27] | Prospective | Exp 1: 30 (17/13) | 13.97 +- 2.07 | FOA | Saliva | T0 = before ApplIns T1 = 4 we after T0 T1 = 12 we after T0 | Culture | Count | Salivary pH, flow rate, buffering capacity Other microbial species | C |

Note: [a]Patient ages are reported as means (one value) or if no mean is available as range (two values in parentheses). Exp, experimental group; Cr, control group; ROA, removable orthodontic appliance; FOA, fixed orthodontic; nc, not considered in this study; %, percentage; CFU, colony forming unit; PI, plaque index; saliv pH, salivary pH; API, Approximal Plaque Index; GBI, Gingival Bleeding Index; We, week; mo, month; y, years; ApplIns, appliance insertion; AppRem, appliance removal; bck, brackets; Ant, anterior; post, posterior; r, right; l, left; U, upper; L, lower; U5, upper second premolar; L5, lower second premolar; U1, upper central incisors; L1, lower central incisors; U6, upper first molars; L6, lower first molars; SDA, Sabouraud's dextrose agar; PCR, Polimerase Chain Reaction; RAPD, Random Amplification of Polymorphic DNA.

analysis of the salivary samples of 80 subjects (40 for each group). A statistical significance ($p < 0.001$) was found in an increased colonisation of *Candida* in patients treated using FOA, compared to those treated with a ROA.[19] Four studies[15,16,21,24] analysed the alteration in candida counts in patients treated with FOA. In comparing pre-treatment and long-term values, Arslan et al.[15] found a significant increase ($p < 0.001$) of *Candida* in saliva and tooth samples, although the increase was not significant during the 6 to 12 month period. Lee et al.[16] also observed significant differences ($p < 0.005$) in the presence of oral *Candida* in the saliva of FOA patients, at long-term follow-up. Alternative results were reported by Zheng et al.[21] in which, after 6 months, the candida levels were comparable with those prior to treatment. In addition, Sanz-Orrio-Soler et al.[24] observed no statistical difference in the frequency of *Candida* over the long-term.

### *Candida changes after orthodontic appliance removal*

Two studies evaluated the differences in *Candida* counts before and after ROA[13] and FOA[24] treatment. Arendorf et al.[13] observed a significant decrease in candida ($p < 0.001$) to baseline levels after ROA removal, although a transient significant increase ($p < 0.001$) occurred during therapy, especially on the posterior (63.6%) and anterior palate (60.6%). Sanz-Orrio-Soler et al.[23], reported no statistically significant increase in *Candida* colonisation during FOA treatment. The slight increase in *Candida* levels from pre-treatment (T0 = 3.2%) to post-treatment (T4 = 4.8%) was not significant. Moreover, no significant differences in the presence of *Candida* were found between the two different analysed fixed appliances (metal or ceramic brackets).[24]

### *Candida species changes during orthodontic treatment*

Eleven studies described the changes in the frequency of the different candida strains during orthodontic treatment using a ROA[17–19,26] and FOA.[14–16,21,23–25] Mahmoudabi et al.[17] observed that *C. albicans* was the most prevalent species isolated from saliva in ROA patients (35.3%) and in control patients (26.5%), but a wider variety of *Candida* species were associated with ROA (30.8%), compared to controls

(9.1%). Six yeast species *(C. parapsilosis, famata, sake, glabrata, dubliniensis, S. cerevisiae, P.etchellsii)* were isolated only in the ROA group. Gonçalves et al.[18] also found a higher incidence of non-*albicans Candida* in the ROA group (55.2%) compared to a control group (42.9%), such as *C*. Iusitaniae (10.3%/4.8%), *C*. krusei (10.3%/0), *C*. Tropicalis (13.3%/9.5%), *C.* parapsilosis (6.9%/4.8%). Rodríguez-Rentería et al.[26] noted that, after 4 weeks of ROA treatment, *C. albicans* and *C. glabrata* were the most prevalent species. Hägg et al.[14] found that the predominant *Candida* species isolated during the first stages of FOA treatment was *C. albicans* (83–87%), while *C. parapsilosis*, *C. tropicalis* and *C. guillermondii* were less common. Arslan et al.[15] found that the 58.5% (42 of the 72 patients) of an initial FOA group were *Candida* carriers and the most common species identified was *Candida albicans* (73.8%), followed by *C.tropicalis, C.krusei* and *C. kefyr* (7.14%) and by *C. parapsilosis* (4.76%). No long-term qualitative evaluation was carried out. Lee et al.[16] observed that *C. albicans* was the most isolated species, while the non-*albicans* species identified were: *C. tropicalis* (4 isolates), *C. parapsilosis* (2 isolates), S. *cerevisiae* (2 isolates), *C. globosa* (1 isolate). Zheng et al.[21] evaluated the *Candida* strains in a long-term follow-up, and determined that the presence of *C. albicans* was 85.7% of that at T0, which subsequently further decreased during treatment in favour of an increase in other strains, specifically *C. parapsilosis, C. krusei* and *C. tropicalis*. Grzegocka et al.[23] identified that 58.8% of subjects were *Candida*-carriers (two were colonised after bracket placement), with a predominant colonisation of *C. albicans* (91.1%), followed by *C. tropicalis* (4.5%) and *C. guillermondii* (4.5%). Soler et al.[24] reported that the most isolated candida strains in FOA patients were *C. albicans*, while *C. glabrata* and *C. krusei* were each found in one patient out of 124, respectively. Pellisari et al.[25] observed that, in patients treated with FOA, the isolated fungal strains were *C. albicans* and *C. krusei,* compared to untreated subjects. Khanpayeh et al.[19] noted a higher frequency of salivary *Candida* carriers ($p = 0.0001$) and a higher colonisation of non-*albicans Candida* species ($p = 0.001$) in a FOA group compared to a ROA sample ($p = 0.0001$). The negative saliva culture was 22.5% in ROA patients but only 5% in FOA patients. The most frequent species in the ROA group was *C. albicans* (62.5%), while in the FOA group, the frequency of *C. albicans* was lower (45%). The frequencies of other species were also higher in the FOA than the ROA group (*C. tropicalis* (FOA/ROA = 20%/7.5%), *C. parapsilosis* (15%/5%), *C. Krusei* (10%/2.5%), *C. Kefyr* (5%/0%)).

## Discussion

*Candida* yeasts are able to form a biofilm on abiotic surfaces, such as the brackets of FOA or the acrylic surfaces of ROA, leading to an increased oral *Candida* presence to produce pathogenic oral mycoses, especially in immunodeficient patients.[23] A recent review[28] revealed a strong relationship between orthodontic treatment and the oral colonisation of *Candida* species.

### *Candida counts and orthodontic treatment: summary of evidence*

Arendorf et al.[13] suggested that ROA may initiate a *Candida* carrier state by inducing a significant, although transient, increase in *Candida* colonisation, especially on the palate. According to several studies, an incremental change was found in the *Candida* counts during ROA therapy, from short[20,26] to a long-term period.[20] These results agree with previous studies confirming that ROA wear alters oral microbiological homeostasis due to the presence of new retentive surfaces, the ROA design, and the duration of ROA use, all of which favour bacterial adhesion and biofilm formation.[26,29,30] Mahmoudabi et al.[17] also reported a significant increase in the prevalence of oral colonisation by *Candida* spp. at a long-term period in ROA patients, compared to untreated controls. Alternative results were reported by Gonçalves et al.[18] who suggested that, although orthodontic treatment may favour the adherence of *Candida* to epithelial cells, ROA did not influence the presence of yeasts in saliva.

Several studies[8,15,21,22] reported a significant increase in candida colonies during the early stages of FOA treatment, compared to pre-treatment levels. However, contrasting results were reported by earlier studies[16,23,24] in which FOA did not increase the number of *Candida* carriers during the first few months, while Kouvelis et al.[27] failed to identify *Candida* albicans during FOA therapy. Hägg et al.[14] reported a considerable individual variation in candida counts during the short and the mid-time periods after FOA insertion. A significant increase in candida

density on the dorsum of the tongue was found when an imprint culture was used, although the overall prevalence rates of candida obtained using oral saliva and pooled plaque techniques did not demonstrate a change. In contrast, Lee et al.[16] found a significant increase in *Candida* counts in oral saliva after 5 months of FOA. In a mid-term follow-up, a statistical increase in *Candida* counts was found in patients with FOA in gargled samples[21] and dental plaque,[22,25] although Grzegocka et al.[23] reported a non-significant upward trend. Limited studies reported the long-term effect of FOA on *Candida* density in contrast with two studies which reported a significant increase[15,16] and two other studies reporting non-significant changes.[15,16,21,24] Only one study by Soler et al.[24] investigated the candida effects after FOA removal, finding that FOA (both metal and ceramic appliances) did not influence the presence of *Candida* albicans. Khanpayeh et al.[19] compared the salivary sample of 80 orthodontic subjects, treated with ROA and FOA by dividing subjects into two groups matched by gender and age. A higher frequency of *Candida* colonisation was found in the FOA group, compared to the ROA group.

### *Candida species and orthodontic treatment: summary of evidence*

It is accepted that the most common aetiological contributor of oral candidiasis is *Candida albicans*, which causes 45–75% of the total incidence of candidiasis, whereas C. *tropicalis*, C. *glabrata*, C. *parapsilosis*, C. *krusei* account for about 7% of all cases.[21]

The assessment of candida colonies in orthodontic patients compared to untreated controls showed that *Candida albicans* was the most prevalent species isolated in both groups, although the method of sampling and analysis differed.[17,18,25]

The analysis of the *Candida* species showed that the most prevalent species in orthodontic patients was *C. albicans*, while other yeast species were less common during ROA[26] and FOA[14–16,21,23,24] treatment. Varying levels of *Candida* strains were reported among the analysed studies, likely due to individual variability and to the different collection methods.

Furthermore, differences in the oral yeasts of patients with or without orthodontic appliances have demonstrated a higher *Candida* diversity in the orthodontic group. The wearing of orthodontic appliances significantly increased the tendency for colonisation by multiple yeast species, especially non-*albicans* species (as *C. parapsilosis, C. famata, C. sake, C. glabrata)*.[14–19,21,23–26]

A higher colonisation of non-*albicans Candida* species was also seen in FOA subjects compared with ROA subjects ($P = 0.001$).[19] The most frequent species in the ROA group was *C. albicans* (62.5%), while in the FOA group, the frequency of *C. albicans* was lower (45%) and the frequencies of other species (*C. tropicalis, C. parapsilosis, C. Krusei, C. Kefyr*) was higher than in the ROA group. Therefore, FOA seemed to promote an increase in the presence of salivary *Candida*, particularly non-*albicans Candida* species compared to ROA patients.[19] All of these yeast species have a great ability to form biofilms[23,28] in patients undergoing orthodontic therapy,[6] mainly in FOA cases.[19,28] *Candida* strains aggregate or adhere more easily to orthodontic fixed appliances.[16]

The increase in *Candida* species other than *C.* albicans in FOA patients, may be due to the different environmental conditions of non-*albicans Candida* strains. After FOA placement, the pH of plaque, the strains and number of micro-organisms in the oral cavity are altered,[8,14,22,23,27] which allows non-*albicans* strains to proliferate and adhere more easily to FOA.[16] Moreover, the increased risk of *Candida* colonisation in orthodontic patients could be attributed to a varying degree of gingival inflammation and mucosal damage that is often seen during orthodontic therapy, and which could have decreased the local defense mechanisms.[20,21] Recent literature[28] reported that other local factors, such as mucosal barriers, contributed to the formation of *Candida* colonies. The first line of defense against the *Candida* species is an intact mucosa,[28] and therefore, there will be an increased risk of infection if there are oral lesions due to local trauma associated with orthodontic appliances.[3,24] It is important to consider that the presence of oral appliances does not appear to increase the clinical signs of candida in individuals who are healthy carriers.[31] However, Goncalves et al.[18] and Zheng et al.[21], respectively, reported that the presence of microtrauma of the oral mucosa in orthodontic patients, did not produce candidiasis in the studied patients, despite *Candida* colonisation.

This situation may be explained by the opportunistic pathogenic character of these micro-organisms, that may cause infection in cases of immuno-suppression. Therefore, clinicians should be cautious when providing orthodontic treatment in immuno-compromised

children because of an increased risk of candida infection. This is especially valid during FOA treatment because traumatic mucositis often occurs to the oral mucosa due to FOA irritation throughout treatment.[15]

Additional host-dependent variables, such as sialometric variations,[8,13] immuno-deficiency, a diet rich in sugar and deficient oral hygiene,[14,23] should also be considered as contributors to the formation of a *Candida* spp. biofilm.[28]

The oral prevention, correct hygiene habits and a greater awareness of children under orthodontic treatment and their parents, not only guarantees the success of treatment, but can also decrease the risk of systemic and/or local diseases, especially in immuno-compromised patients.[14,19,21–24,26]

## Limitations

Considering the clinical heterogeneity of the reviewed studies, as well as the differences between the sample sites, the analytical methods and, in the quantitative assessment (the number composition was expressed as counts of CFU or as a percentage of frequency), the present review reflects only the changing trend in the colonisation of oral *Candida* during orthodontic treatment. Further high-quality randomised clinical trials are needed to increase the quality of evidence regarding the changes in the candida population during orthodontic treatment.

## Conclusions

According to the SBU tool, the present review may draw conclusions reflecting a limited level of evidence.

1. ROA induced a temporary increase of *Candida* counts from an early stage of treatment, back to a pre-treatment level after ROA removal.
2. Contrasting and conflicting results have been reported for FOA treatment.
3. FOA therapy seemed to increase the frequency of *Candida* carriers, compared to ROA.
4. Orthodontic treatment (especially with FOA) promoted oral *Candida* colonisation of non-*albicans* species, although the most prevalent species was *Candida albicans* in both groups.

## Conflict of Interest

The authors declare that there is no conflict of interest.

## Corresponding author

Alessandra Campobasso
Department of Clinical and Experimental Medicine
University of Foggia, Clinica Odontoiatrica Via Rovelli 50, 71122, Foggia, Italy

Email: alessandra.campobasso@unifg.it
ORCID 0000-0003-4726-8816

## Acknowledgments

This research received no support from funding agencies in the public, commercial, or not-for-profit sectors.

## References

1. Dar-Odeh N, Shehabi A, Al-bitar Z, Al-Omari I, Badran S, Al-Omiri M, et al. Oral Candida colonization in patients with fixed orthodontic appliances: the importance of some nutritional and salivary factors. Afric J Microbio Res 2011;5:2150–4.
2. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002;78:455–9.
3. Hibino K, Wong RW, Hägg U, Samaranayake LP. The effects of orthodontic appliances on Candida in the human mouth. Int J Paediatr Dent 2009;19:301–8.
4. Reedijk AMJ, Kremer LC, Visser O, Lemmens V, Pieters R, Coebergh JWW, et al. Increasing incidence of cancer and stage migration towards advanced disease in children and young adolescents in the Netherlands, 1990-2017. Eur J Cancer 2020;134:115–26.
5. Mitus-Kenig M, Derwich M, Czochrowska E, Pawlowska E. Cancer survivors present significantly lower long-term stability of orthodontic treatment: a prospective case-control study. Eur J Orthod 2021;43:631–8.
6. Lucchese A, Bondemark L, Marcolina M, Manuelli M. Changes in oral microbiota due to orthodontic appliances: a systematic review. J Oral Microbiol 2018;10:1476645.
7. Jiang Q, Li J, Mei L, Du J, Levrini L, Abbate GM, et al. Periodontal health during orthodontic treatment with clear aligners and fixed appliances: a meta-analysis. J Am Dent Assoc 2018;149:712–20; e12.
8. Arab S, Nouhzadeh Malekshah S, Abouei Mehrizi E, Ebrahimi Khanghah A, Naseh R, Imani MM. Effect of Fixed Orthodontic Treatment on Salivary Flow, pH and Microbial Count. J Dent (Tehran) 2016;13:18–22.
9. Ghazal ARA, Idris G, Hajeer MY, Alawer K, Cannon RD. Efficacy of removing Candida albicans from orthodontic acrylic bases: an in vitro study. BMC Oral Health 2019;19:71.
10. Belibasakis GN, Bostanci N, Marsh PD, Zaura E. Applications of the oral microbiome in personalized dentistry. Arch Oral Biol 2019;104:7–12.
11. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009;62:e1–e34.
12. Bondemark L, Holm AK, Hansen K, Axelsson S, Mohlin B, Brattstrom V, et al. Long-term stability of orthodontic treatment and patient satisfaction. A systematic review. Angle Orthod 2007;77:181–91.
13. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol 1985;12:360–8.

14. Mahmoudabadi AZ, Drucker DB, Mandall N, O'Brien K, Johnson EM, Theaker ED. The oral yeast flora: Effect of upper removable orthodontic appliances. Microbial Ecol Health Dis 2002;14:149–52.
15. Kundu R, Tripathi AM, Jaiswal JN, Ghoshal U, Palit M, Khanduja S. Effect of fixed space maintainers and removable appliances on oral microflora in children: an in vivo study. J Indian Soc Pedodontics Preventive Dentistry 2016;34:3–9.
16. Rodríguez-Rentería M, Márquez-Preciado R, Ortiz-Magdaleno M, Bermeo-Escalona J, Sánchez-Vargas LO. Frequency of pathogenic microorganisms in removable orthodontic appliances and oral mucosa in children. J Clin Pediatric Dentistry 2021;45:135–9.
17. Hägg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. Eur J Orthodontics 2004;26:623–9.
18. Arslan SG, Akpolat N, Kama JD, Ozer T, Hamamci O. One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral Candida. J Oral Pathol Med 2008;37:26–9.
19. Lee W, Low BK, Samaranayake LP, Hagg U. Genotypic variation of Candida albicans during orthodontic therapy. Front Biosci 2008;13:3814–24.
20. Zheng Y, Li Z, He X. Influence of fixed orthodontic appliances on the change in oral Candida strains among adolescents. J Dental Sci 2016;11:17–22.
21. Shukla C, Maurya R, Singh V, Tijare M. Evaluation of role of fixed orthodontics in changing oral ecological flora of opportunistic microbes in children and adolescent. J Indian Soc Pedodontics Preventive Dentistry 2017;35:34–40.
22. Grzegocka K, Krzyściak P, Hille-Padalis A, Loster JE, Talaga-Ćwiertnia K, Loster BW. Candida prevalence and oral hygiene due to orthodontic therapy with conventional brackets. BMC Oral Health 2020;20:277.
23. Sanz-Orrio-Soler I, Arias de Luxán S, Sheth CC. Oral colonization by Candida species in orthodontic patients before, during and after treatment with fixed appliances: a prospective controlled trial. J Clin Exp Dent 2020;12:e1071–e7.
24. Kouvelis G, Papadimitriou A, Merakou K, Doulis I, Karapsias S, Kloukos D. A prospective cohort study assessing the impact of fixed orthodontic appliances on saliva properties and oral microbial flora. Oral Health Preventive Dentistry 2021;19:67–76.
25. Gonçalves e Silva CR, Oliveira LD, Leão MV, Jorge AO. Candida spp. adherence to oral epithelial cells and levels of IgA in children with orthodontic appliances. Braz Oral Res 2014;28:28–32.
26. Pellissari BA, Sabino GSP, de Souza Lima RN, Motta RHL, Suzuki SS, Garcez AS, et al. Antimicrobial resistance of bacterial strains in patients undergoing orthodontic treatment with and without fixed appliances. Angle Orthod 2021;91:672–79.
27. Khanpayeh E, Jafari AA, Tabatabaei Z. Comparison of salivary Candida profile in patients with fixed and removable orthodontic appliances therapy. Iranian J Microbiol 2014;6:263–8.
28. Khan I, Ahmad T, Manzoor N, Rizvi MA, Raza U, Premchandani S. Evaluating the role of local host factors in the candidal colonization of oral cavity: a review update. Natl J Maxillofac Surg 2020;11:169–75.
29. Freitas AO, Marquezan M, Nojima Mda C, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: a systematic review. Dental Press J Orthod 2014;19:46–55.
30. Guo R, Lin Y, Zheng Y, Li W. The microbial changes in subgingival plaques of orthodontic patients: a systematic review and meta-analysis of clinical trials. BMC Oral Health 2017;17:90.
31. Ronsani MM, Mores Rymovicz AU, Meira TM, Trindade Grégio AM, Guariza Filho O, Tanaka OM, et al. Virulence modulation of Candida albicans biofilms by metal ions commonly released from orthodontic devices. Microb Pathog 2011;51:421–5.