Candida prevalence and oral hygiene due to orthodontic therapy with conventional brackets

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Abstract

**Background**: Conventional brackets are often used during orthodontic therapy of patients with malocclusion. The complex construction of such brackets greatly inhibits oral hygiene, which predisposes to increased carriage of microbiota. Orthodontic brackets could act as reservoir of yeast and predispose to oral candidosis. The aim of this study was to assess *Candida* prevalence and the role of oral hygiene during fixed appliance therapy. A further aim was to characterize the isolated yeasts according to their ability to form biofilms.

**Methods**: Seventeen participants (average age 17 ± 7 years) were monitored by taking oral rinses and elastomeric ligature samples, and by evaluating the approximal plaque index (API) and gingival bleeding index (GBI) before and after placement of the orthodontic conventional brackets for twelve weeks. Isolated yeasts were counted and biofilm formation was evaluated.

**Results**: One hundred and sixteen samples (67 oral rinses and 49 orthodontic elastomers) were collected. Ten patients (58.8% subjects) were *Candida*-carriers (two were colonized after bracket placement) and *C. albicans* was the most common species. The average number of yeasts in the oral cavity showed some fluctuation during the study, but in general had an upward trend (adj. R² = 0.7967, p = 0.07025). A correlation was found between median number of yeasts and the periodontal indices (API, GBI). The average API values decreased in the *Candida*-carriers (adj. R² = 0.95; p = 0.01709), while average GBI values increased in the noncarriers (adj. R² = 0.92; p = 0.0256).

**Conclusions**: Treatment with orthodontic appliances promotes *Candida* yeast colonization, which is variable over time in terms of strain and species, with dominance of *C. albicans*, and without increased biofilm-forming activity. The API value decreases over time in carriers, and the GBI value increases in uncolonized patients, which may have predictive significance for the development of oral candidiasis during orthodontic treatment.

**Background**

Conventional brackets are often used during orthodontic therapy of patients with malocclusion. Their complicated construction greatly inhibits oral hygiene, which predisposes to increased carriage of bacteria and yeasts (1,2). An increased amount of fungi in oral cavity and the occurrence of predisposing factors can lead to the development of oral mycosis. To estimate the number of fungi, the count of yeast colonies (colony-forming unit, CFU) calculated on ml of washings collected from the patient's mouth is used. This method allows the determination of both the number (quantitative assessment) and species composition (qualitative assessment) of isolated colonies as opposed to swabs, which are less reliable for technical reasons (they require sampling from the same place, e.g. the palate, from a specific surface (e.g. 1 cm²) with a high risk of contamination by touching other anatomical structures and errors related to adherence and recovery of colonies from a swab (3). Also, the state of oral hygiene can be expressed in numbers – this is what periodontological indexes are used for. One of the most popular dental
indicators is API (Approximal Plaque Index). It expresses in percentage the amount of uncleaned interdental spaces. API at the level of 25-39% is the indicator of good hygiene, and < 25% of optimal hygiene. Permanent lack of good oral hygiene causes inflammation of gums, the first symptom of which is their bleeding. The GBI (Gingival Bleeding Index) is used to assess gum inflammation. GBI < 10% indicates clinically healthy periodontium.

One of the elements of conventional orthodontic brackets are elastomeric rings, which are used to connect bracket with orthodontic wires. Irregularity and roughness of their surface are favourable to microorganism's colonization and biofilm formation (4,5). Biofilms are usually multispecies assemblages of microorganisms, encased in a matrix. It is mode of life common to most microorganisms in natural and medical systems (also components of orthodontic appliances) that allows survival in hostile environments. Biofilm can become a reservoir of pathogens and with the occurrence of predisposing factors contribute to thrush and other forms of oropharyngeal candidosis. One of the factors of Candida virulence is the ability of yeasts to form biofilm and it would seem that strains isolated from patients colonized and using orthodontic appliances will form a biofilm well (6,7).

Even though Candida is a part of the normal oral microbiota found in 17 - 75% human population, it can often be the cause of oral mycoses, especially in immunodeficiency patients (8,9). The most common etiological factor of oral candidosis is Candida albicans. C. tropicalis, C. glabrata, C. parapsilosis, C. krusei sometimes occur with high prevalence, especially in susceptible patients such as diabetic (10–12). All these species have the great ability to form biofilm, especially with oral Gram + bacteria (8–11).

Only very few studies have compared Candida prevalence and Candida growth in orthodontic patients before, during, and after treatment (1,12). The small number of papers related to this topic and the correlation between orthodontic elastomeric rings and oral Candida growth led to this study.

The purpose of this study was to investigate occurrence of Candida species and role of oral hygiene measured by periodontal parameters during fixed appliance therapy. Further aim was to characterize isolated Candida species according to their ability to biofilm formation as a virulence factor in development of candidosis related to orthodontic therapy.

**Methods**

**Patients and samples**

Seventeen patients (11 females, 6 males, aged 11 - 30 years old, average age 17 ± 7 years old, median 14 years old) of Department of Orthodontics at University Dental Clinic (Kraków, Poland) were randomly selected. All subjects due to an occlusion defect, required orthodontic treatment using conventional brackets and gave their written consent to participate in the study. The following inclusion criteria were considered: healthy individuals, both sexes, aged ≤ 30 years old. The exclusion criteria were: oral mucosa disease, smoking, use of antibiotics, corticosteroids or any hormone medication within 3 months prior to
the study, pregnancy or breastfeeding. All patients were informed that the use of antimicrobial mouthwashes is prohibited during the study.

The research project included the following visit schedule: T0 – appointment before bonding brackets, T1 – approx. 2, T2 - approx. 6 and T3 - approx. 12 weeks after bonding brackets.

The patients during the study used the same type elastic rings (colour Glow Blue, catalogue index OCLGB, Orthodontic Design and Production, Inc., USA), metal brackets Cannon Ultra System (Orthodontic Design and Production, Inc., USA) and NiTi wires (Fairfield Orthodontics, USA).

All participants were thoroughly instructed by one of the authors how to properly care for oral hygiene during orthodontic treatment. Patients reported for all appointments properly prepared (in accordance with previously received written recommendations) – in the morning, on an empty stomach (minimum 6 hours after taking the last meal), before morning brushing teeth and other oral hygiene procedures. Patients were also asked to limit exercise and use a mixed diet on the day preceding the study.

During T0 visit (before bonding brackets) oral hygiene was assessed using periodontal indices: Approximal Plaque Index (API) according to Lange and Gingival Bleeding Index (GBI) according to Ainamo and Bay and oral rinses were collected.

To measure API a periodontal probe was gently guided through the approximal spaces of the first and third quadrants from the oral aspect and of the second and fourth quadrants from the buccal aspect. The presence of plaque deposits was recorded as positive results. The percentage of sites with positive results was counted.

The GBI was performed through gentle probing of the orifice of the gingival crevice. If bleeding occurred within 10 seconds a positive finding was recorded. The number of positive sites was recorded and then expressed as a percentage of the number of sites examined.

Collection of mycological material was performed through oral rinses. Patients under the supervision of the orthodontist rinsed the mouth for 60 seconds with 10 ml isotonic saline (0.9% NaCl) at room temperature, and then spit out the rinses into a sterile container, which was immediately delivered to the mycological laboratory.

During visits T1, T2, T3, API and GBI indices were re-evaluated, as well as oral rinses and elastomeric rings were collected. Orthodontic ligatures were put into Eppendorf tubes filled with saline solution. Elastomers were collected using a sterile dental kit in aseptic conditions to prevent material contamination.

**Microbiological analysis**

**Total Candida and Mean Candida Carriage**
The mouth washings samples were vigorously shaken for 90 seconds with a Vortex shaker and then quantitatively plated on Sabouraud's chloramphenicol agar (Biocorp) and incubated at 35 ± 2 °C for 72 hours. Collected elastomeric ligatures were inoculated directly onto the medium. The grown colonies were identified based on classical mycological methods i.e. colony morphology, Dalmau plate technique and commercial assimilation test – API 20C AUX (Biomerieux).

Strains were collected and stored frozen for further biofilm formation studies.

**In vivo biofilm — Scanning Electron Microscope (SEM)**

Several randomly selected ligatures from patients (present in their oral cavity for about 4 weeks) with known *Candida* growth were subjected to a scanning electron microscope analysis to assess the biofilm produced *in vivo*. The topography of pure elastic ligature was also examined as a control. Analysis of the prepared samples was performed using a JEOL JSM-35CF scanning microscope (JSM-35CF; JEOL Vacuum Evaporator) in Laboratory of the Otolaryngology Clinic, University Hospital, Krakow.

**Candida biofilm formation**

The biofilm formation assay was performed as follows: the overnight culture of investigated strains was transferred to sterile saline and the fungal suspension was adjusted to 1 on McFarland scale with a densitometer (DEN1 Biosan, Lithuania). One hundred µL of standardized suspensions (8 wells per strains) were added to each well of sterile 96-well flat-bottom polystyrene plates filled previously (100 µL per well) with 2-fold concentrated Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine without bicarbonate (Sigma Aldrich) supplemented with 2% glucose (Avantor Performance Materials, Gliwice, Poland) and buffered with 3-morpholinopropane-1-sulfonic acid (MOPS, Sigma Aldrich). Plates were incubated for 1.5 hours at 37 °C for the adherence phase. Then washed twice with sterile Phosphate Buffered Saline (PBS) to remove not adherent cells and each well was filled with new RPMI medium and incubated without shaking for 72 hours 37 °C. After that time plates were washed with PBS, dried on the air for 45 minutes and stained with 125 µL per well of 0.1% crystal violet solution (Avantor Performance Materials, Gliwice, Poland) for 45 minutes at room temperature. The microtiter dish was then washed and then dried. A 150 µL volume of 95% ethanol (Avantor Performance Materials, Gliwice, Poland) was added to each well, and then plates were covered and incubated for 45 minutes at room temperature. A 100 µL sample of the resulting ethanol-crystal violet solution was then transferred from each well to a new microtiter plate, and optical density (OD) was determined at a wavelength of 570 nm (Infinite 200 Pro Tecan Männedorf, Switzerland). The experiment was repeated – each strain was analysed in 16 replicates.

The study included 27 isolates from 10 patients and one reference strain of *C. albicans* (ATCC 90028).

**Statistical Analysis**

To detect differences in GBI, API and number of *Candida* colony-forming unit (CFU) across multiple tests attempt the one-way repeated measures analysis of variance by ranks (Friedman test and Skillings-Mack
test) were used. The differences of biofilm formation among Candida strains were evaluated with Kruskal-Wallis test with Dunn's post hoc analysis. All statistical analyses were carried out using R software (13,14), and a p-value < 0.05 was considered significant.

**Results**

*Candida carriage*

One hundred sixteen samples were collected from 17 patients (67 oral rinses and 49 orthodontic ligatures samples). Positive Candida growth was obtained in 52 samples (34 oral rinses - 51% and 18 orthodontic ligatures samples - 37%) and C. albicans was the most isolated species (91.1%) followed by C. tropicalis (4.5%) and C. guilliermondii (4.5%).

Analysis of Candida carriage among oral rinses before bonding brackets shown that 8 patients (47%) had Candida in an average number of yeasts $5.5 \times 10^2 \pm 4.8 \times 10^2$ CFU/ml, 2 more patients (11.8%) were colonized during the study. In total 10 patients (58.8%) were Candida-carriers – out of which 6 (35%) were consistent carriers (resulted positive throughout the whole study) and 4 (23.5%) were inconsistent carriers (resulted negative at least one in the study). The average number of yeast colonies fluctuate during study time with little decline at T2 stage. In Candida-carriers at T0, T1, T2, T3 was $4.4 \times 10^2$, $8.8 \times 10^2$, $8 \times 10^2$, $190 \times 10^2$ CFU/ml, respectively (Table 1). The highest CFU value $8.5 \times 10^3$ CFU/ml was found in one patient (P.M.) at T3 stage. The average number of colonies (CFU/ml) obtained from oral rinses showed an upward trend depending on duration of the study (adjusted $R^2 = 0.7967$) but without statistical significance (p = 0.07025) (Figure 1).

| Table 1 Average value of Candida sp. (CFU/ml), API and GBI Indexes (%) during study for all investigated patients. |
### Differences between stages

| *Candida* colonization [CFU/ml count] | T0                  | T1                  | T2                  | T3                  | Differences between stages |
|--------------------------------------|----------------------|----------------------|----------------------|----------------------|-----------------------------|
| Average                              | 2.6×10^2             | 5.2×10^2             | 4.7 × 10^2           | 10.7 × 10^2          | p = 0.9092 (Skillings-Mack Statistic) |
| +/- SD                               | +/-4.3×10^2          | +/-9.4×10^2          | +/-7.6×10^2          | +/-23.1×10^2         |                             |
| (range)                              | (0-1.3×10^3)        | (0-3.0×10^3)        | (0-3.0×10^3)         | (0-8.5×10^3)         |                             |
| Median                               | 0×10^2              | 1×10^2              | 2×10^2              | 1×10^2              |                             |

| API value [%]                        | Average              | 40.9                | 43.4                | 38.3                | 39.8                        |
|--------------------------------------|----------------------|----------------------|----------------------|----------------------|-----------------------------|
| +/- SD                               | +/- 22               | +/-22.6              | +/-17.4              | +/-23               | p = 0.7968 (Friedman rank sum test; chi-squared) |
| (range)                              | (13-94.8)            | (13.6-88.2)          | (10-66.7)            | (4.5-100)           |                             |
| Median                               | 42.3                 | 38.0                 | 35.3                 | 35.0                 |                             |

| GBI value [%]                        | Average              | 10.3                | 9.4                 | 19.23               | 10.6                        |
|--------------------------------------|----------------------|----------------------|----------------------|----------------------|-----------------------------|
| +/- SD                               | +/- -9               | +/-8.8               | +/-24.8              | +/-8.5               | p = 0.4929 (Friedman rank sum test; chi-squared) |
| (range)                              | (0-25)               | (0-31.2)             | (0-93.8)             | (0-29.5)             |                             |
| Median                               | 8.3                  | 7.5                  | 11.8                 | 12.5                 |                             |

Legend: API: Approximal Plaque Index; GBI: Gingival Bleeding Index; SD: standard deviation; CFU: colony-forming unit.

### API and GBI results

The mean API and GBI values at T0 stage were 41% +/- 22 and 10% +/- 9, respectively. There were no differences in the distribution of GBI values for all patients between stages of study (Friedman rank sum test chi-squared = 2.4041, df = 3, p-value = 0.4929) or differences in API values (Friedman rank sum test; chi-squared = 1.0185, df = 3, p-value = 0.7968).

When the study groups were divided into two subgroups: (1) non *Candida*-carriers - 41%, (2) *Candida*-carriers - 59% (patients in whom yeast growth was found at any stage of sampling) some tendencies of changes in median indices values were observed. In *Candida*-carries medians of API values decreased (adjusted R-squared coefficient = 0.94; p = 0.01709), while in non *Candida*-carriers medians of GBI values increased (adjusted R-squared coefficient = 0.92; p = 0.0256) (Figure 2AB).

### Biofilm formation - SEM results

Topography assessment of pure elastic ring under an electron microscope showed a clean surface, free of microorganisms (Figure 3A).
On the surface of colonized orthodontic elastic ligatures, microorganisms have created a multicellular, an architecturally complicated structure with the presence of various types of bacteria (cocci, bacilli, rods) and yeast (both early and late stage of biofilm formation) (Figure 3BCD).

**Ability to biofilm formation**

Most of the analysed strains have created biofilm (Figure 4). Pairwise comparisons with control (14) shows the difference in biofilm production for 7 strains (Dunn's *post hoc* test after Kruskal-Wallis-test; p < 0.05). These strains (59, 28, 65, 25, 30, 52, 38) produce significantly more biofilm biomass than *Candida albicans* ATCC 90028.

In the overwhelming majority of cases the biofilm formation was homogenous among strains isolated from particular patients (F. A., F. P., O. U., P. J., P. M., R. A., S. P.). In only few cases, when various species were isolated (i.e. patient D. M. 2, and K. J.) and in one case of *Candida albicans* isolation (D. M.) the biofilm formation differs during the stages of the study.

There is no correlation between biofilm forming ability with API nor GBI indexes (adjusted $R^2 = 0.02$, p = 0.2377, adj. $R^2 = 0.04$, p = 0.1824, respectively).

**Discussion**

In the current study, the percentage of *Candida*-carriers before orthodontic treatment was relatively high — 47% investigated patients. Moreover, it increased during orthodontic treatment to almost 59% (nearly 12% of subjects turned into carriers). It is in contradiction to the occurrence of *Candida* in the Polish population which was confirmed on average 30.6% healthy individuals (15). However, cited study was based on the oral swabs (N = 654; 7 - 45 years old) and lower result may be the effect of different collecting method. Tooyama et al. (3) showed that the test result is significantly dependent on sampling method. In his study of Japanese patients (N = 200, average age 47.2 years old) the result of *Candida* prevalence when analysed by oral swabs was 33.5% of patients, whereas using the same group but concentrated rinse as collecting method, colonization of *Candida* sp. was much higher 52% and comparable with our results and methodology. It shows that the method of sampling has a large impact on the results and not all studies can be directly compared.

Zheng et al. (16) determined the incidence of oral *Candida* sp. only in 14% of young Chinese adults (N = 50, average age 13.6 years old) before the application of fixed orthodontic appliance. This lower result may be the effect of brushing teeth prior to the sampling. Arslan et al. (17) on the Turkish population (N = 72, average age 19.6 years old) based on saliva samples and oral swabs, reveals high *Candida*-carriage - 58.5%. This may suggest the occurrence of large population variables, both within age and ethnic groups, as carrier of fungi depends on many factors such as diet and lifestyle.

In our investigation 2 patients (nearly 12%) turned into new *Candida*-carriers during the course of experimental phase of the study (after bracket placement). This is coherent with other publications
suggesting that treatment with orthodontic appliances promotes *Candida* yeast colonization. Hägg et al. (18) using imprint technique demonstrated that (N= 27, av. age 15 years old) the overall *Candida* prevalence increases after bonding brackets. Lee et al. (19) published that 15% subjects turned into new *Candida*-carriers.

In the study of Lee et al. (19) (N = 112, average age 17.7 years old) which was based on oral concentrated rinse analysis of Chinese patients, an increase in *Candida*-carriers was noted - from initial 32% (T0 - before bracket bonding) to the maximum 50% (T5 - around 5 months after bonding). They reported that 11% of subjects were consistent carriers of *Candida* species, 64% - inconsistent *Candida*-carriers and only 25% - consistent non *Candida*-carriers i.e. they never carried *Candida* species throughout the experimental period of 12 months. We were also able to distinguish a group of consistent (35%) and inconsistent (23.5%) *Candida*-carriers. This may indicate that in the initially tested non *Candida*-carriers group at the base line of the study are some possible false negative results. It needs to be considered that the introduction of orthodontic appliance is only one of possible variable factors influencing prevalence of *Candida* yeast in the oral cavity (others being diet, oral hygiene, lifestyle, immune system, etc). In the future studies longer observation of the study group with multiple tests is recommended to isolate the group of consistent and inconsistent carriers before the start of the experimental phase of the study.

In our study a quantitative analysis of *Candida* colonies in carriers group was carried out as the number of yeast colonies indicates development of oral yeast infection during orthodontic treatment. Tooyama et al. (3) determined reference ranges of *Candida* colony for healthy commensal carriages at 0-5 CFU/swab and 0-670 CFU/ml for concentrated rinse method. In our study, five patients have an average colony count higher than 670 CFU/ml but clinically they didn't show any symptoms that can suggest that *Candida* grew on the abiotic surface of dental appliances, not on the mucosa. Analysis of quantitative data shows that the number of colonies differs in time and particular patients. However, in our research, we found no statistically significant differences in the number of *Candida* sp. during 12 weeks of study. Despite this the obtained results suggest a positive correlation between the average number of colonies and the duration of study. The amount of oral *Candida* could fluctuate, and a lack of statistical significance may be affected due to hygiene habits of patients, consumed food and yeast biology, a small research sample, and high initial oral *Candida*-carrier in the studied group.

Our findings regarding upward trend of yeast growth during orthodontic treatment are consistent with the data presented in the literature (16–19). Zheng et al. (16) study showed that the CFU/ml increases in users of fixed orthodontic appliances after two months of treatment. Lee et al. (19) in long-term research (almost 12 months of observation) showed that amount of *Candida albicans* increases after bonding brackets and reaches its peak in the 5th month of treatment, then slightly decreases and approaches the maximum again at the end of the first year of treatment. Arslan et al. (17) based on one-year observation of a group of orthodontic patients stated that a statistically significant increase in the *Candida* population occurs in the first month of orthodontic treatment. A decrease in the amount of *Candida* DNA in wash mouth samples was only found in the Bergamo et al. publication (20) (N = 15, mean age 17.53 ± 8.0 years) based on a 90-day observation after brackets application. The study group was heterogenous.
– it consisted of 14 women and one male. Unfortunately, the authors of the above mentioned studies did not describe any patient preparation procedures, i.e. brushing teeth. Only Zheng et al. (16) admitted patients were after breakfast and their regular morning oral hygiene routine. Our recommendations were typical for laboratory tests - patients reported on an empty stomach, without having brushed teeth. This is one of the methodology variable factors that may greatly influence results of the study.

The species profile obtained in our study is similar to the results by Hägg and al. (18) and Lee at al. (19) - *Candida albicans* dominates and *C. guilliermondii* and *C. tropicalis* were isolated in small percentage. This may be a guideline for choosing antibiotic therapy for a patient affected by oral mycosis. An interesting phenomenon, not noted by other authors, were changes of colonizing species in one patient during the study (K. J. *C. albicans* replaced with *C. tropicalis*, and D. M. occurrence of high biofilm formed strains of *C. guillermondii* – Figure 4). This may need further research to understand better intraspecies relationships of *Candida* yeast.

Conventional orthodontic brackets have many retention areas that impede proper oral hygiene and thus can lead to greater plaque build-up. In our study, the mean number of *Candida* CFUs has a growing tendency. However, API (Approximal Plaque Index) and GBI (Gingival Bleeding Index) do not show significant change. Interestingly, statistically significant fungal growth was demonstrated in patients, whose API decreased (oral hygiene improved) during subsequent visits. Whereas patients with increasing GBI (periodontal inflammation) are in a less percentage carriers of yeasts. This leads to the conclusion that *Candida* sp. was less proliferating in patients with a changed periodontium, i.e. probably more colonized by bacteria. Similarly, no differences in PI (Plaque Index), GI (Gingival Index) and GBI (Gingival Bleeding Index) during the use of fixed orthodontic appliances were observed by Bergamo et al. (20). However, Hägg et al. (18) found a statistically significant increase in PI (Plaque Index) during the second and third visit after starting orthodontic treatment with brackets.

One of the factors of *Candida* virulence is the ability of yeasts to form biofilm on abiotic surfaces - also components of orthodontic appliances. The formation of a biofilm protects the cells forming it against the adverse effects of the environment, including antimicrobial drugs. Few studies have taken up the topic of microorganisms’ multiplication on orthodontic elastomers (5,21). Casaccica et al. (4) prove that elastomers used in orthodontics are manufactured with due care and do not pose a biological threat. SEM images of the topography of uninfected elastic ligatures presented in this publication also confirm the absence of microorganisms. The surface of elastic ligatures enables creation of architecturally and species-rich biofilms and it is rapidly colonized during orthodontic treatment.

It would seem that strains isolated from patients colonized and using orthodontic appliances will form a biofilm well. For our patients, the level of colonization before treatment was high, and during the course of treatment the ability to create biofilm did not change except for sporadic cases. This indicates that while the number of yeasts including those derived from biofilms produced in the oral cavity increased, the biofilm-forming potential (virulence) of the isolated strains did not change. Hence, it can be concluded that while the oral appliances promote colonization by yeasts, they do not increase their virulence.
Therefore, correct antifungal prophylaxis should allow to control the development of oral mycosis during long-term orthodontic therapy. However, in vivo biofilm formation may not coincide with the in vitro biofilm formation capacity. The model does not take into account all factors (especially the presence of bacteria). Therefore, Candida pathogenicity (biofilm formation) described in the literature does not coincide with the pathogenicity of strains in vivo.

**Conclusions**

- Treatment with orthodontic appliances promotes Candida yeast colonization

- Yeast colonization is variable over time in terms of strain and species of fungi, with domination of Candida albicans.

- In patients who are carriers, the API value decreases over time, and in non-colonized patients the GBI value increases – which may have a predictive significance for the development of oral candidiasis during orthodontic treatment – but this requires further research to confirm these relationships and determine the cut-off point.

- Strains isolated from orthodontically treated patients do not show increased biofilm-forming activity.

**Abbreviations**

API: Approximal Plaque Index; GBI: Gingival Bleeding Index; SD: standard deviation; CFU: colony-forming unit, RPMI: Roswell Park Memorial Institute, MOPS:3-morpholinopropane-1-sulfonic acid, PBS: Phosphate Buffered Saline; PI: Plaque Index; GI: Gingival Index, ATCC: American Type Culture Collection.

**Declarations**

**Ethics approval and consent to participate**

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was approved by the Bioethics Committee of Jagiellonian University, Kraków, Poland (Process KBET/121/B/2012, 24th March 2012) and the study has taken place from October 2013 to December 2014. Before beginning work, we orally explained our study objectives and procedures to all participants and obtained their permission to have their specimens involved in our study. Meanwhile, written informed consent was signed by each participant in age 16 and over 16 years old. The written informed consent was obtained also from a parent or guardian for participants under 16 years old.

**Consent for publication**

Not applicable.
Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The funder played no role in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Authors' contributions

KG patient qualification for the study, clinical material collection, data analysis and interpretation, article design, test design, manuscript writing, review of selected literature search, critical revision of the article. PK execution of tests, data analysis and interpretation, statistical analysis, manuscript writing, review of selected literature, critical revision of the article. AHP data analysis and interpretation, critical revision of the article. JEL data analysis and interpretation, critical revision of the article. KTĆ execution of a part of the tests, performance of scanning electron microscope photography, data analysis and interpretation, critical revision of the article. BWL study design, critical revision of the article. All the authors have read and approved the final manuscript.

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Figures
Figure 1

Occurrence of Candida in oral cavity of Candida carriers throughout the study (shown as a boxplot with average number of colony). Blue line connecting the averages (red diamond) shows an upward trend, grey area is the confidence interval for the mean.

Figure 2
A. Changes of API, GBI Indexes on the duration of the study among Candida positive and Candida negative patients. Blue line connects the average value. B. The same correlation as in the figure 2, but shows correlation with median value among Candida positive and Candida negative patients.

Figure 3

(A) The electron microscopy image of clear orthodontic ligatures - no microbial contamination. (BCD) Orthodontic ligatures topography after 4 weeks presence in oral cavity. (B) Budding yeast cells. (C) Yeast cells in dense bacterial biofilm. (D) Bacterial biofilm, with rods rising straight from cocci layer.
Figure 4

Ability of biofilm formation by isolated strains with comparison to reference C. albicans strains (ATCC 90028).