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 patients with malocclusion. Nevertheless, their complicated construction greatly inhibits oral hygiene, which predisposes to the increased carriage of the microbiota. It seems that orthodontic brackets could be a reservoir of yeast and predisposing to develop oral candidosis. Objectives The aim of this study was to assess changes in Candida species prevalence and periodontal parameters after orthodontic brackets placement in patients who received oral hygiene instruction; to determine role of elastic ligatures in those changes; to characterize isolated yeasts according to their ability to biofilm formation.

Patients/Methods 17 participants (average age 17±7 years) have been monitored by taking oral rinses, elastomeric ligatures samples and evaluation of API and GBI Indexes before and after placement of orthodontic conventional brackets for 12 weeks. Isolated yeasts was counted and used to the biofilm formation assay.

Results 116 samples (67 oral rinses and 49 orthodontic elastomers) were collected. 51% of patients were carriers of Candida in which C. albicans was the most common species. The average number of colonies (CFU/ml) obtained from oral rinses showed an upward trend depending on duration of the study and some correlation with periodontal indexes (API, GBI) was found. One third of the analysed strains have shown ability to form greater biofilm than control strain.

Conclusions Orthodontic ligatures surface permit biofilms creation and orthodontic brackets change dynamic oral microbiota. Maintaining proper oral hygiene is crucial for every orthodontic patient.

Background

Conventional brackets are often used during orthodontic therapy patients with malocclusion. Their complicated construction greatly inhibits oral hygiene, which predisposes to the increased carriage of the microbiota (1). Elastomeric rings bond orthodontic archwires with brackets slots. Elastomerics have been manufactured since the 1960s and performed with different polymers. Irregularity and roughness of their surface are favourable to microorganisms colonization and biofilm creation (2, 3).

Biofilm occurs on different elements of fixed appliances (e.g. brackets, archwires, elastomers), which can become of pathogens reservoir and with predisposing factors contributed to candidosis. Candida infection is the most common oral mucosa disease, especially in immunodeficiency patients (4, 5). The most common etiological agent is still *Candida albicans*, the other *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei* also sometimes occur with high prevalence especially in susceptible patients such as diabetic (6–8). These species have the great ability to form biofilm especially with oral Gram + bacteria. However, *Candida* are normal oral commensals found in 17–75% human population (4–7).

It seems that foreign bodies such as brackets could be a reservoir of yeast and predisposing to develop oral candidosis. Only very few studies have compared *Candida* prevalence and *Candida* density in

orthodontic patients before, during, and after treatment (1, 8). The small number of papers related to this topic and the connection between orthodontic elastomeric rings and oral *Candida* growth led to this study. The purpose of this study was to explore changes in oral microbiota, especially *Candida* species and periodontal parameters during fixed appliance therapy; characteristics isolated *Candida* species according to biofilm formation and to determine the role of orthodontic elastomeric rings in *Candida* species growth.

Methods

Patients and samples

17 patients (11 females, 6 males, aged 11–30 years old, average age 17 ± 7 years old, median 14 years old) of Clinic of Orthodontics at Jagiellonian Dental Hospital (Kraków, Poland) were selected. The study group included volunteers who, due to an occlusion defect, wanted to undergo orthodontic treatment using conventional brackets, regardless of gender and age. The following inclusion criteria were considered in this study: healthy individuals, no systematic disease, no immunodeficiency, no oral mucosa disease, no smoking, no use of antibiotics, corticosteroids or any hormone medication within 3 months prior the study. All patients were informed that the use of antimicrobial mouthwashes is prohibited. No patients were pregnant or during breastfeeding.

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 had unicolor elastic rings (colour Glow Blue, catalogue index OCLGB, Orthodontic Design and Production, Inc., USA) and metal brackets Cannon Ultra System (Orthodontic Design and Production, Inc., 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) - after a night's sleep, in the morning, on an empty stomach (minimum 6 hours after taking the last meal), before morning brushing teeth and other oral hygiene procedures, if possible after discontinuation of medications and vitamin preparation (after consulting your doctor). 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. Patients under the supervision of the orthodontist rinsed the mouth for 60 seconds with 10 ml 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, oral rinses and also 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 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). 100 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 RPMI 1640 medium with L-glutamine without bicarbonate (Sigma Aldrich) supplemented with 2% glucose (Avantor Performance Materials, Gliwice, Poland) and buffered with MOPS (Sigma Aldrich). Plates were incubated for 1.5 hours at 37 °C for the adherence phase. Then washed twice with sterile 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 min 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 *Candida* CFU across multiple test 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 (9, 10), and a p-value < 0.05 was considered significant.

Results

Candida carriage

116 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 during the study. The average number of yeast colonies in *Candida*-carriers at T0, T1, T2, T3 was 4.4×10^2, 8.8×10^2, 8×10^2, 1.9×10^3 CFU/ml, respectively. The highest CFU value 8.5×10^3 CFU/ml was found in one patient (P.M) at T3 stage. Table 1 summarizes the average colony count for all tested patients, API and GBI Indexes.

Table 1
Average value of *Candida* sp. (CFU/ml), API and GBI Indexes (%) during study.

	T0	T1	T2	T3	Statistic
Average count CFU/ ml +/- SD (range)	2.6×10^2 +/-4.3×10^2 (0-1.3×10^3)	5.2×10^2 +/-9.4×10^2 (0–3.0×10^3)	4.7×10^2 +/-7.6×10^2 (0–3.0×10^3)	10.7×10^2 +/-23.1×10^2 (0-8.5×10^3)	NS
Average API value [%] +/- SD (range)	40.9 +/- 22 (13-94.8)	43.4+/-22.6 (13.6–88.2)	38.3+/-17.4 (10-66.7)	39.8+/-23 (4.5–100)	NS
Average GBI value [%] +/- SD (range)	10.3+/-9 (0–25)	9.4+/-8.8 (0-31.2)	19.23+/-24.8 (0-93.8)	10.6+/-8.5 (0-29.5)	NS
Legend: API: Approximal Plaque Index; GBI: Gingival Bleeding Index; SD: standard deviation; NS: not significant; CFU: colony-forming units.					

The average number of colonies (CFU/ml) obtained from oral rinses showed an upward trend depending on duration of the study, however, statistical analysis did not show differences between individual stages for all patients (Skillings-Mack Statistic = 0.909212, df = 3, p-value = 0.823204), as well as within *Candida*-carriers themselves (Skillings-Mack Statistic = 1.564971, p-value = 0.667358) (Fig. 1).

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 in average indices values were observed. In *Candida*-carries average API values decreased, while in non *Candida*-carriers average GBI values increased (Fig. 2AB). However, statistical analysis did not show differences in indices values between stages of study: API values in *Candida*-carriers (Friedman chi-squared = 2.1383, df = 3, p-value = 0.5442) and in non *Candida*-carriers (Friedman chi-squared = 2.1383, df = 3, p-value = 0.5442) and GBI values in *Candida*-carriers (Friedman chi-squared = 4.9875, df = 3, p-value = 0.1727) and in non *Candida*-carriers (Friedman chi-squared = 4, df = 3, p-value = 0.2615).

Biofilm formation - SEM results

Topography assessment of pure elastic ring under an electron microscope showed a clean surface, free of microorganisms (Fig. 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) (Fig. 3BCD).

Ability to biofilm formation

Most of the analysed strains have created biofilm (Fig. 4). Pairwise comparisons with control (10) shows the difference in biofilm production for 7 strains (Dunn's *post hoc* test after Kruskall-Wallis' test; $p < 0,05$). These strains (59, 28, 65, 25, 30, 52, 38) produce significantly more biofilm biomass than *Candida albicans* ATCC 90028.

Discussion

The amount of *Candida* prevalence before starting orthodontic treatment differ among published data. Differences may result from population variability, methodological differences and confounding factors. A study of oral swabs of 654 healthy individuals in age from 7 to 45 years old affirmed *Candida* carrier-state in the Polish population on average 30.6% (11). Similar result 33.5% of *Candida* prevalence were

found by Tooyama et al. (12) in Japanese patients by analysed of oral swabs (N = 200, average age 47.2 years old), while using the same group and concentrated rinse method, colonization of *Candida* sp. was much higher (52%). Moreover, Tooyama et al. (12) determined reference ranges of *Candida* colony for healthy commensal carriages for 0–5 CFU/swab and 0-670 CFU/ml for concentrated rinse method. The authors did not provide patient preparation procedures, i.e. brushing teeth. In the study of Lee et al. (13) (N = 112, average age 17.7 years old) based on oral rinse analysis of Chinese patients, an increase in *Candida*-carriers was noted - from initial 32% (T0 - before bonding the brackets) to the maximum 50% (T5 - around 5 months after bonding), 15% turned into *Candida*-carriers. Unfortunately, the study did not include control of indices of oral hygiene and periodontal conditions. Zheng et al. (14) 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. The underestimated result may be the effect of brushing teeth prior to the sampling. In contrast, a study by Arslan et al. (15) on the Turkish population (N = 72, average age 19.6 years old) on the basis of saliva samples and oral swabs, reveals high *Candida*-carriage – 58.5%.

In the current study based on oral rinses, the percentage of *Candida*-carriers before orthodontic treatment was high – 47%, moreover, it increased during orthodontic treatment to almost 59% (nearly 12% subjects turned into carriers). The number of colonies in the most intensively colonized patient exceeded 34×10^3 CFU/ml.

Results of trials are also inconsistent with yeast escalation during orthodontic treatment. Hägg et al. (16) using the oral rinse, pooled plaque and imprint technique demonstrated that in orthodontics patients (N = 27, average age 15 years old) the overall candida prevalence increases immediately after bonding brackets and then remains relatively constant during 3 months of observation. A decrease in the amount of *Candida* sp. was found in the Bergamo publication (17) (N = 15, mean age 17.53 ± 8.0 years) based on a 90-day observation after braces application. Contrarily, mentioned above Zheng et al. (14) study showed that the CFU/ml increases in users of fixed orthodontic appliances after two months of treatment. Lee et al. (13) 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. (15) on the basis of 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.

In our research, we found no statistically significant differences in the number of *Candida* sp. during 12 weeks of study; however, the obtained results suggest a positive correlation between the average number of colonies and the duration of the experiment. A lack of statistical significance may be associated with a small research sample and high initial oral *Candida* carrier in the studied group or proper oral hygiene during study. The amount of oral *Candida* fluctuates due to hygiene habits, food consumed and yeast biology. This is demonstrated by the large dispersion of individual results. There are also no clear criteria to separate colonization from the development of candidiasis.

The species profile obtained in the current study is similar to the results by Hägg and al. (16) and Lee at al. (13) - *Candida albicans* dominates and *C. guilliermondii* and *C. tropicalis* were isolated in small percentage. An interesting phenomenon, not noted by the other authors, were changed in colonizing species in one patient during the study.

Conventional orthodontic brackets have many retention places that impede proper oral hygiene and thus can lead to greater plaque build-up. Hägg et al. found a statistically significant increase in PI (Plaque Index) during the second and third visit after starting orthodontic treatment with fixed braces (16). Differently - no differences in PI (Plaque Index), GI (Gingival Index) and GBI (Gingival Bleeding Index) coefficients during the use of fixed orthodontic appliances were observed by Bergamo et al. (17).

In our study, the mean number of *Candida* CFUs has upward tendency in the time of study; however, API (Approximal Plaque Index) and GBI indexes do not show significantly change. Moreover, statistically significant fungal growth was demonstrated in patients whose API decreased (i.e., oral hygiene improved) during subsequent visits and patients with increasing GBI are less 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. However, this was not the subject of this study.

Only a few studies determine the ability of microorganisms to multiply on orthodontic elastomers (3, 18). Casaccica et al. (2) 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 ligature taken by the author of this publication also confirmed the absence of microorganisms. The surface of elastic ligatures enables the creation of architecturally and species-rich biofilms and it is rapidly colonized during orthodontic treatment. However, the culture of elastic ligatures is not a reliable representation of the number of *Candida* sp. in the oral cavity.

Conclusions

Conventional orthodontic brackets with elastic ligatures create dynamic change in oral microbiota. For every orthodontic patients it is very important to follow the instructions of the attending orthodontist and maintain proper oral hygiene. Elastic ligatures should be changed regularly.

Abbreviations

API: Approximal Plaque Index; GBI: Gingival Bleeding Index; SD: standard deviation; NS: not significant; CFU: colony-forming units

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 age16 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

GK 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, financing from own grant. KP execution of tests, data analysis and interpretation, statistical analysis, manuscript writing, review of selected literature, critical revision of the article. TĆK execution of a part of the tests, performance of scanning electron microscope photography, data analysis and interpretation, critical revision of the article. LBW study design, critical revision of the article. All the authors have read and approved the final manuscript.

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Figures

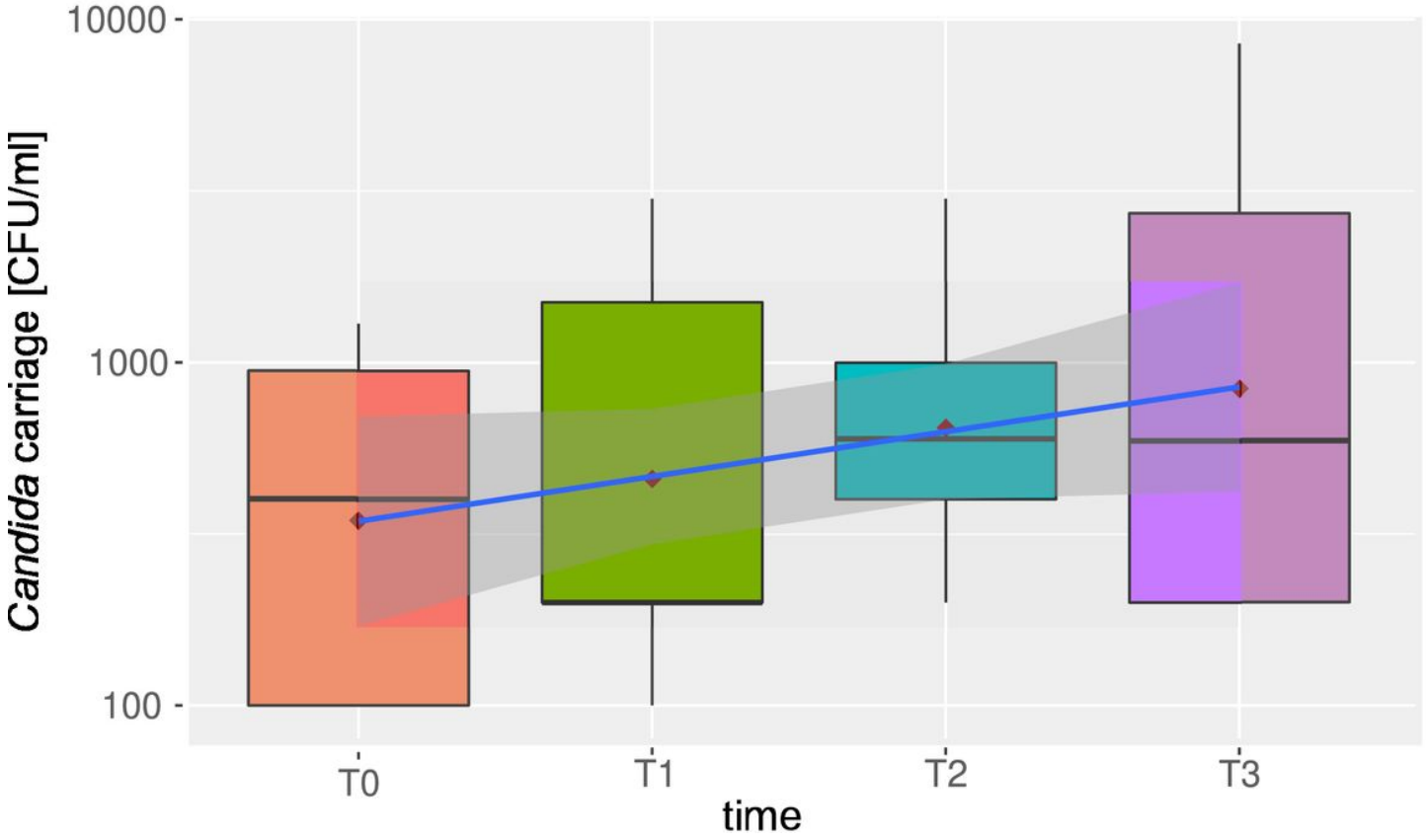

Figure 1

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 (♦) shows an upward trend, grey area is the confidence interval for the mean.

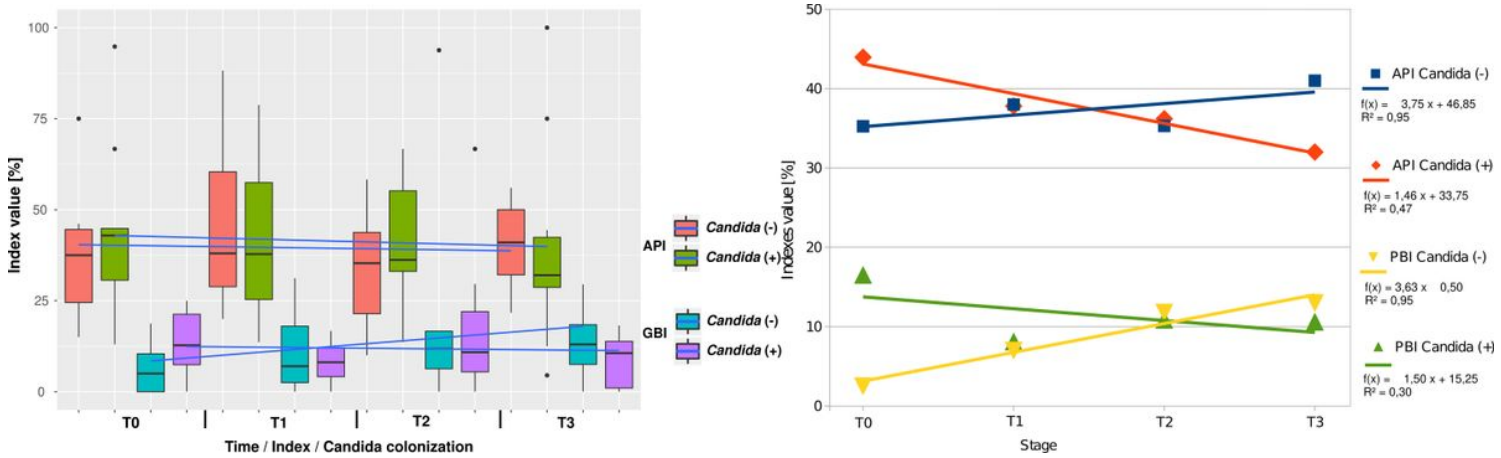

Figure 2

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

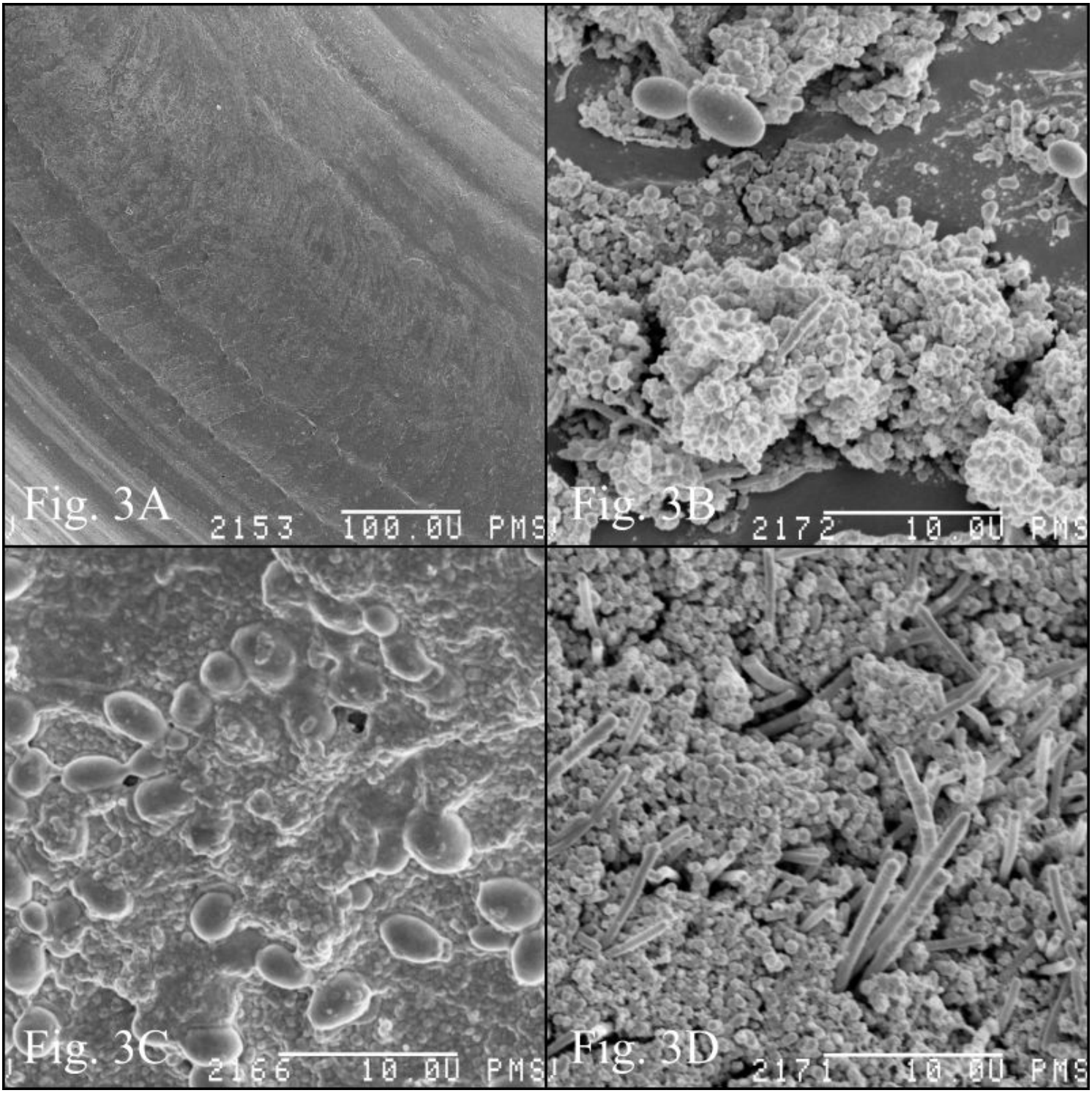

Figure 3

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.

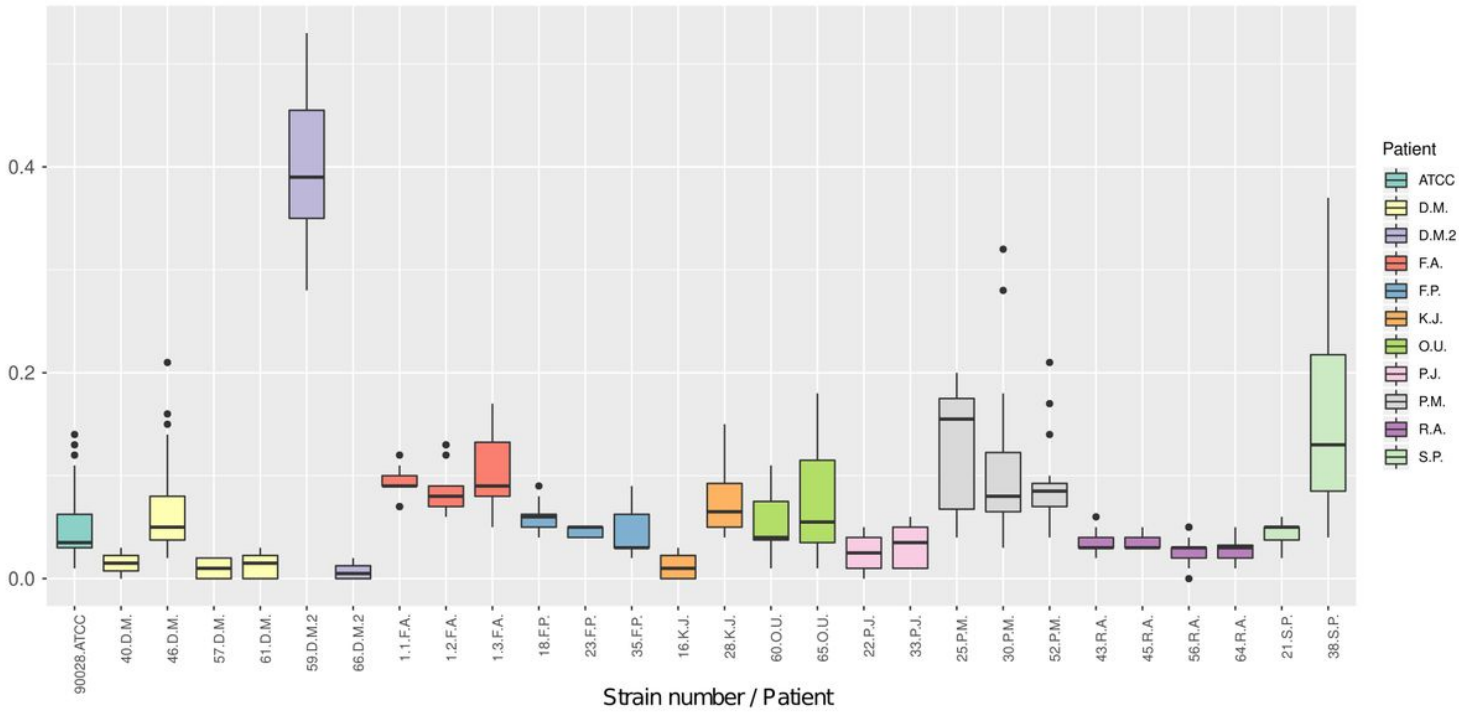

Figure 4

Ability of biofilm formation by strains isolated from the study group.