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Qualitative Modifications of the Aligners' Bacterial Flora under the Effect of Tea Tree Essential Oil

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Abstract

Objectives: The aim of our study is to look for the different Gram + and Gram - germs present in the biofilm of thermoplastic appliances and to note the qualitative modifications of the aligners' bacterial flora after the use of the tea tree essential oil. ***Materials and Methods*:** Thirty-four patients were involved in the study. They were divided into two equal groups: a control group using a toothbrush and toothpaste as cleaning means and a test group using the tea tree essential oil as a supplement. ***Results:*** The results showed that the bacterial flora of the thermoplastic device is very diverse with a predominance of the genus Staphylococcus. It was isolated in more than half of the devices used by the control group. In terms of species, Enterobacter cloacae complex and Staphylococcus aureus were the most represented. In addition to the bacteria, usually found in the oral cavity, several other non-oral strains were frequently isolated, underscoring the importance of hand hygiene. The tea tree essential oil showed its effectiveness on Staphylococcus, which was significantly less encountered in the test group ($p = 0.031$). ***Conclusion*:** Thermoplastic appliances contain a diverse bacterial flora; Tea tree essential oil can be used for disinfecting purpose.

Keywords: Aligner, bacteria, hygiene, tea tree essential oil, thermoplastic appliance.

INTRODUCTION

Orthodontic brackets act as plaque traps, altering the quantity and the quality of oral biofilm (Dallel *et al*., 2019) and increasing the risk of white spots (Buschang *et al*., 2019) and periodontal diseases (Azaripour *et al*., 2015) compared to the treatment with aligners. In fact, as a removable device, thermoplastic aligners guarantee better oral hygiene and control bacterial changes both in the oral cavity and in the appliance itself. However, they remain a foreign body that can be colonized by different bacterial strains (Low *et al*., 2011) which requires rigorous hygiene during the treatment period in order to limit the presence of pathogenic bacteria. Maintaining a reduced bacterial amount will ensure the good progress of the treatment and preserve the patient's dental and periodontal health. Consequently, the first step is to learn about the bacterial strains that colonize thermoplastic aligners in order to recommend effective hygiene measures to patients that can maintain a reduced level of plaque during orthodontic aligner treatment. Knowing the germs present in the bacterial biofilm of this type of devices would make it possible to choose the appropriate antiseptics based on their spectra of activity.

Different chemical products are available in the market (Akgün *et al*., 2019; Albanna *et al*., 2017; Chang *et al*., 2014; Ismah *et al*., 2019; Levrini *et al*., 2015, 2016; Lombardo *et al*., 2017; Meto *et al*., 2019; Shpack *et al*., 2014). However, more and more patients are looking for natural remedies to avoid the harmful side effects of chemicals. Phytotherapy is the chosen alternative for many people, the use of medicinal plants still occupies an important place in certain civilizations such as the Chinese, Arabs and Indians (Létard *et al*., 2015).

In this experimental in vivo study, we investigated the contamination of orthodontic thermoplastic aligners worn for 2 weeks, by several bacteria and evaluated the efficacy of disinfection with Tea Tree essential oil.

MATERIALS AND METHODS

50 patients aged more than 18, of both sexes, under orthodontic treatment by aligners, from the department of orthodontics, dental clinic of Monastir, Tunisia, were invited to participate in the study. All patients had a clinical interview, a review of dental and medical history, and a clinical examination, and those with good general health condition, good oral hygiene, were considered as eligible participants.

Pregnant and breastfeeding women, smokers, patient with systemic disease, with periodontal disease, untreated decay, who had braces in the past or those who had used antibiotics within the previous month were excluded from the study. 42 patients met the criteria for the study; they were randomly divided into two groups: A control group and a study group. 8 patients were excluded during the study because of poor wear or loss of aligners and contaminated swab. Which left us with 34 patients, 17 in each group.

Control group have worn their aligners for 14 days, the cleaning protocol involves using a standard toothbrush and toothpaste once a day. The group includes 3 men (17.6%) and 14 women (82.4%) whose average age is 25.88 years with a standard deviation of 4.12.

The test group has worn their aligners for the same period; they used, once a day, pure tea tree essential oil as an adjunct to toothbrush and toothpaste. In fact, the cleaning protocol for this group is to apply a few drops of the essential oil for 5 minutes before brushing the appliance with standard toothpaste to remove all traces of oil.

The group is made up of 4 men (23.5%) and 13 women (76.5%) whose average age is 24.35 years with a standard deviation of 1.83.

The experimental protocol was approved by the medical ethics committee of the faculty of medicine of Monastir (IORG 0009738 N°69/ OMB 0990-0279). Each participant signed an informed consent after being informed about the course of the study.

After 14 days of wearing, the aligners were removed from the oral cavity and placed directly into a sterile test tube containing liquid culture broth **(Fig1)**. It is then labeled and incubated in a laboratory oven at 37 ° C.

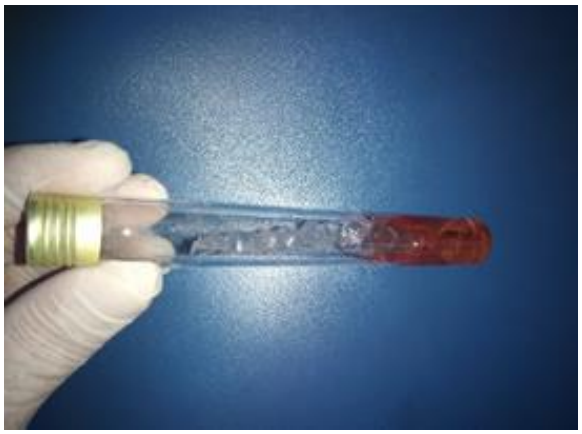

Fig-1: Samples collection

The bacteriological examination was carried out by the team of the microbiology laboratory of Fattouma Bourguiba Hospital in Monastir using the compact vitek®2 automaton from bioMérieux exploring the enzymatic profile of bacteria. SPSS version 22 software was used for statistics, no significant difference was found between the two groups regarding age ($p = 0.18$) neither sex ($p = 0.67$), the two groups thereby are statistically comparable.

RESULTS

After bacteria's culturing in the control group, 33 germs distributed among 25 different bacterial species were isolated with a frequency that varies between one and 3 aligners. In fact, *Enterobacter cloacae complex* and *Staphylococcus aureus* were detected in 3 samples, while *Serratia marcescens, Staphylococcus epidermis, Staphylococcus hominis and Staphylococcus warneri* were isolated in 2 ones. The frequency of the rest of the species is a single aligner. These species can be classified into 13 different genera with predominance of *Staphylococcus* which were detected in 9 aligners, followed by *Enterobacter* and *Streptococcus* isolated in 4 aligners, while *Kocuria* and *Serratia* were found in 2 samples. Other genera were present in only one aligner **(Fig2)**.

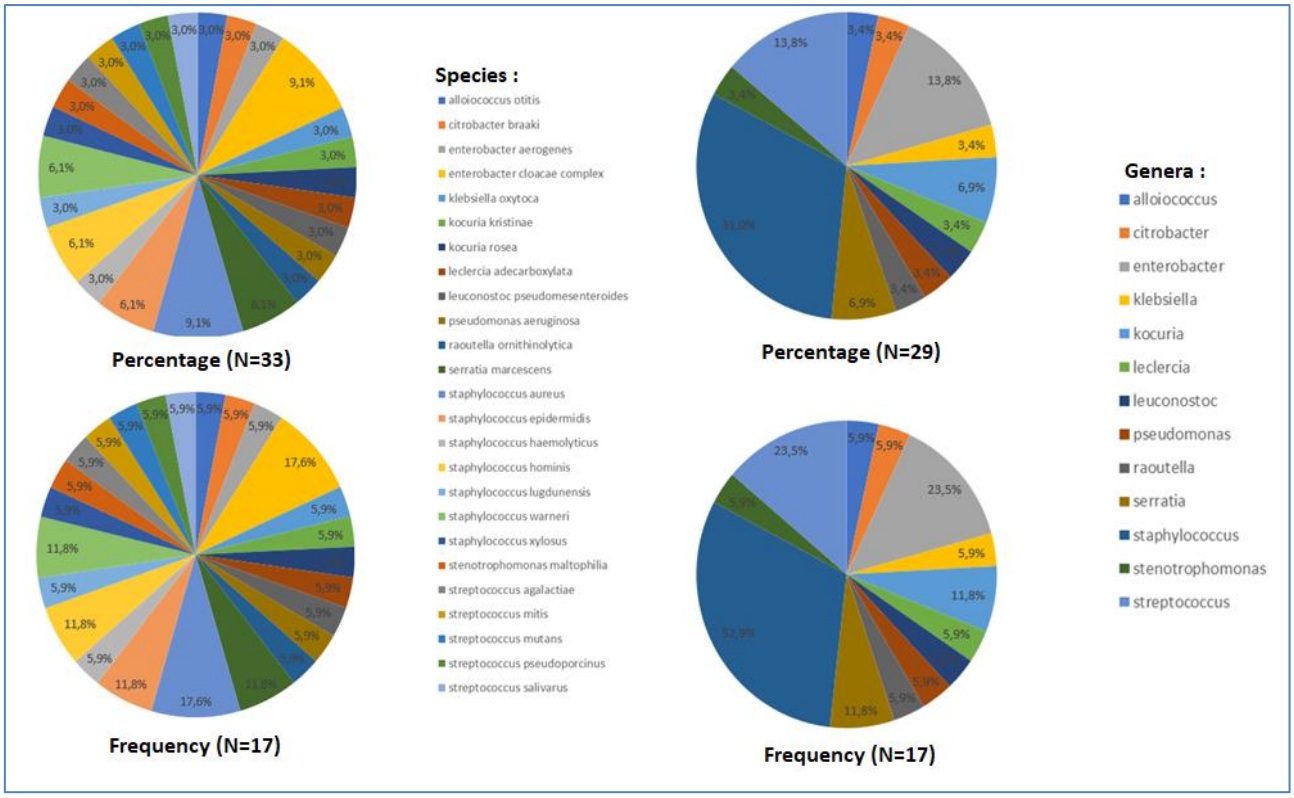

Fig-2: Control group's germs distribution in terms of species and genus

For the Gram stain, 21 Gram + bacteria (63.6%) and 12 Gram - bacteria (36.3%) were isolated, Gram + bacteria were found in 76.5% of the aligners while Gram - bacteria were isolated in 64.7 % of samples **(Fig 3)**.

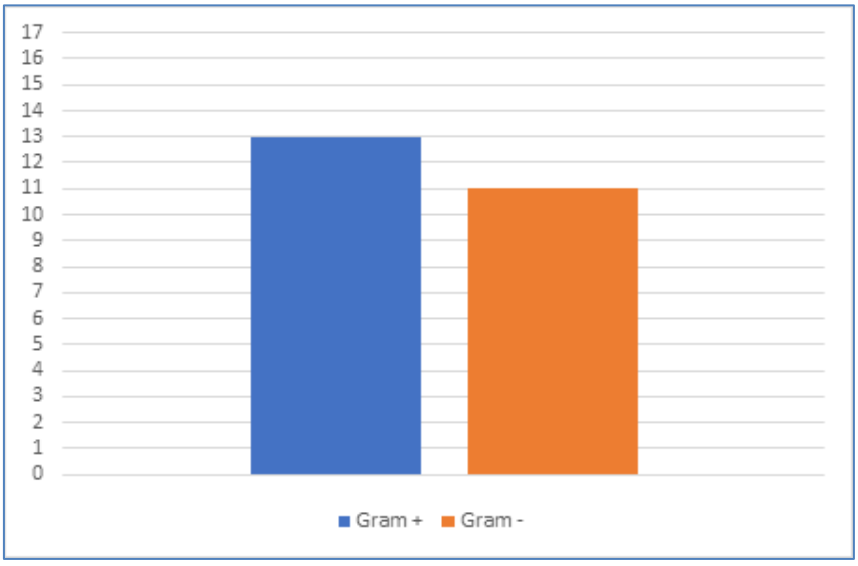

Fig-3: Frequency of bacteria in the control group according to the Gram stain

Among the 33 bacteria isolated, only 7 belong to the oral flora (21.2%), they were found in 41.2% of samples, while the 26 (78.8%) other germs have another origin and were found in 88.2% of samples **(fig4)**.

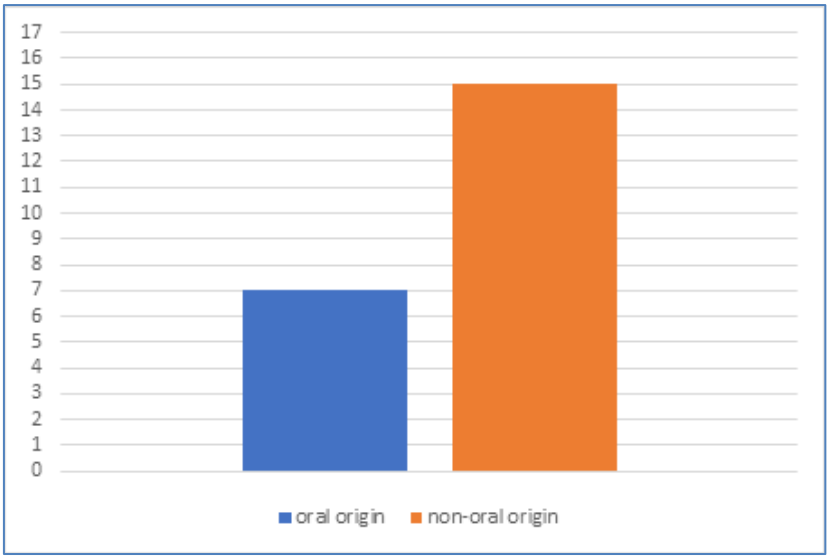

Fig-4: Frequency of bacteria in the control group according to habitat

Concerning the test group, the culture of the bacteria in the 17 aligners gave 24 germs classified into 16 different species. *Enterobacter cloacae complex*, *Staphylococcus aureus* and *Streptococcus pseudoporcinus* are the most common strains, they were identified in 3 aligners. In the second place, we find *Serratia marcescens* and *streptococcus sanguinis* with a frequency of 2 aligners. For the rest of the species, they were represented by a single sample. Germs isolated in this group can be classified into 11 different genera with predominance of *streptococcus* (28.6%) which were found in 6 aligners, they are followed by *Enterobacter* and *Staphylococcus* (14.3%) isolated in 3 samples. Then we find the *Serratia* (9.5%) which were detected in two different aligners. For the rest of types, they have a frequency equal to one aligner **(fig-5)**.

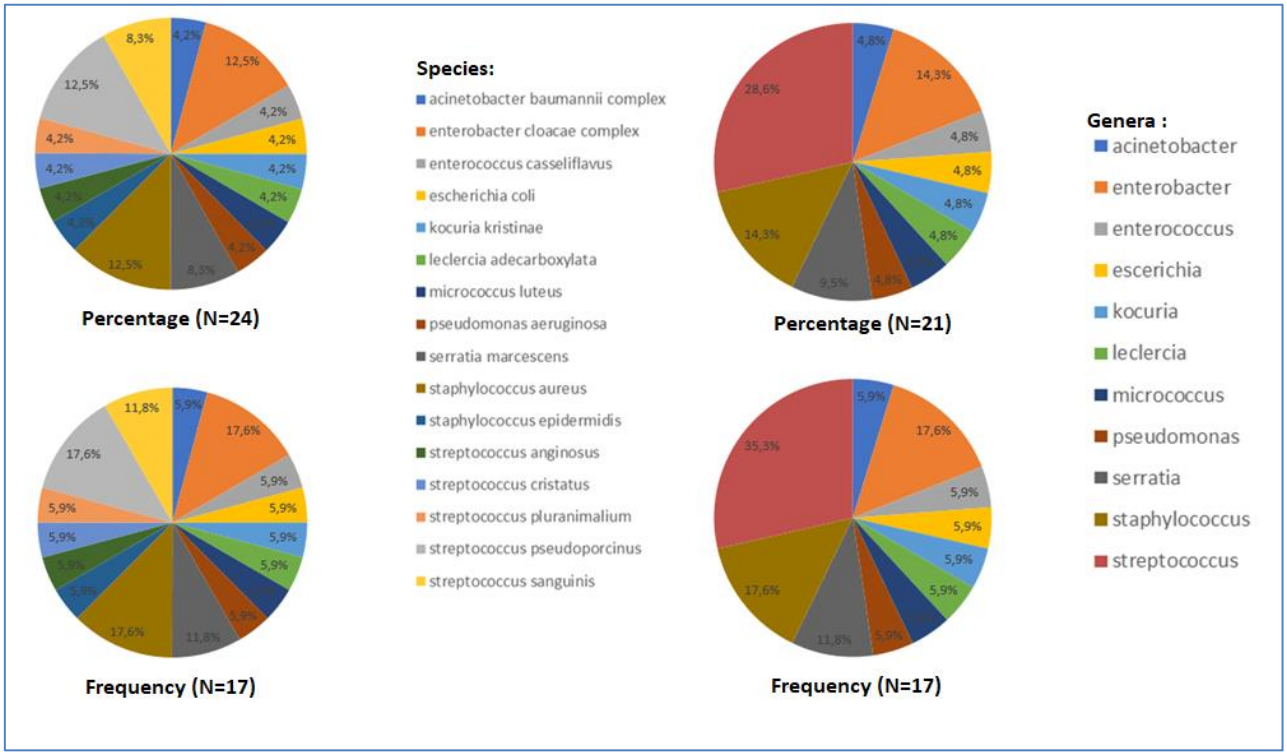

Fig-5: Test group's germs distribution in terms of species and genus

15 Gram + bacteria (62.5%) and 9 Gram- bacteria (37.5%) were isolated; Gram + strains were identified in 64.7% of the aligners while the Gram- strains were found in 47.1% of the aligners **(fig-6)**.

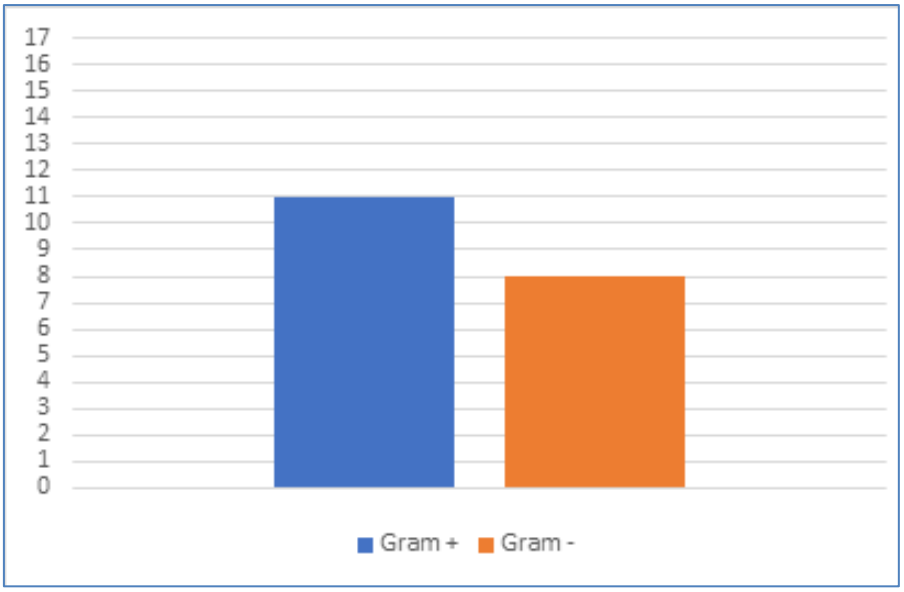

Fig-6: Frequency of bacteria in the test group according to the Gram stain

The number of strains which belong to the oral flora is less than the number of strains having another origin. In fact, we have only detected 7 oral bacteria (29.2%) distributed over 35.3% of the aligners against 17 non-oral bacteria (70.8%) found in 70.6% of the samples **(fig-7)**.

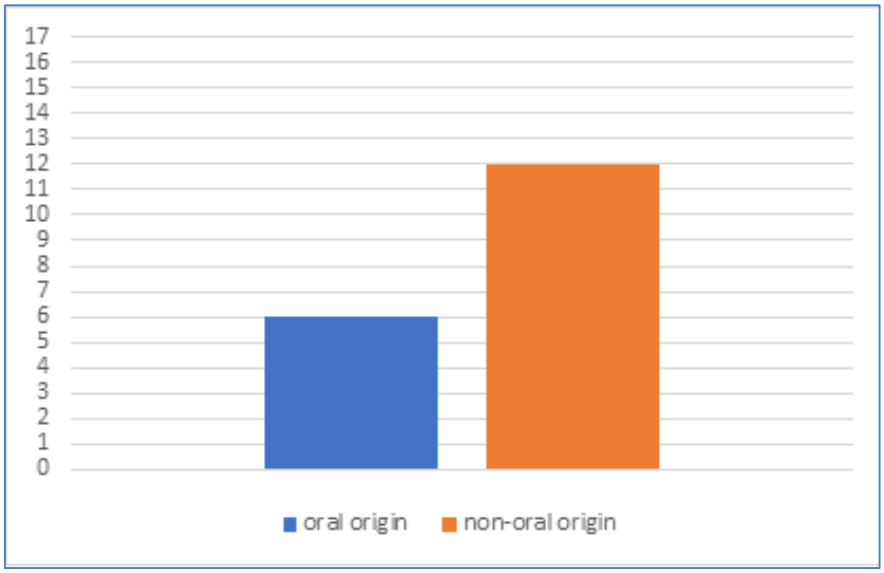

Fig-7: frequency bacteria in the test group according to habitat

The comparison between the species isolated from the two groups shows that the *Enterobacter cloacae complex*, the *kocuria kristinae*, the *Leclercia adecarboxylata*, the *Pseudomonas aeruginosa*, the *Serratia marcescens* and the *Staphylococcus aureus* are distributed equally but with different frequencies. For *Staphylococcus epidermis*, they were twice as frequent in the control group as in the test group, while *Streptococcus pseudoporcinus* were 3 times more frequent in the test group. There are specific species to the control group and others that were isolated exclusively from the test group. It should be noted that bacteria were not found in two samples from the group using tea tree essential oil, they only showed fungi. The difference between the two groups in terms of species is not significant according to the chi-square test ($p > 0.05$) **(fig-8)**.

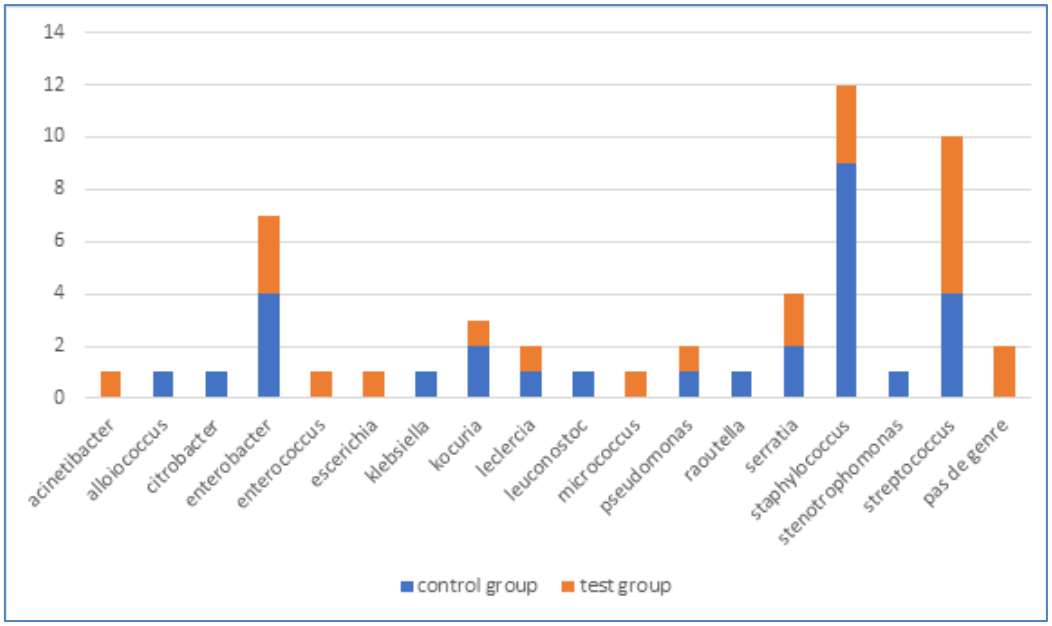

Fig-8: Comparison between the genera isolated in the control group and the test group

Regarding genus distribution, *Enterobacter*, *Leclercia*, *Pseudomonas* and *Serratia* were found equally in both groups. However, *kocuria* and, even more, *Staphylococci* were more prevalent in the control group, while *Streptococci* were more frequently encountered in the test group. *Alloiococcus*, *Citrobacter*, *Klebsiella*, *Leuconostoc*, *Raoutella* and *Stenotrophomonas* are specific types of the control group while the *Acinetibacter*, *Enterococcus*, *Escerichia* and *Micrococcus* were exclusively isolated from the tea tree group with two negative samples. Only *Staphylococcus* showed a statistically significant difference (p = 0.031) (fig-9).

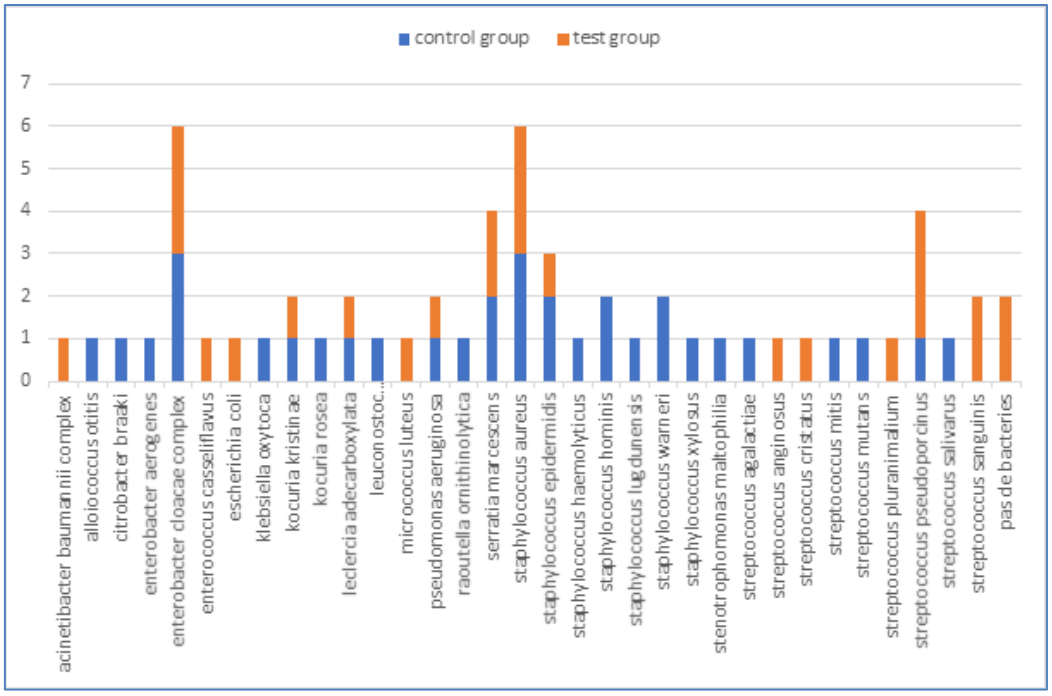

Fig-9: Comparison between the species isolated in the two groups

Concerning the Gram stain, for both groups we have a predominance of Gram + bacteria. In fact, the mean of Gram + bacteria in the control group is equal to 1.24 ± 1 and that of Gram- bacteria is 0.71. ± 0.8. For the test group, 0.88 ± 0.9 Gram+ bacteria were detected per aligner and 0.53 ± 0.6 Gram- bacteria per aligner. Thus, the group of patients who used only toothpaste brushing had more Gram + and Gram - bacteria. However, the difference control

group, while in the test group an average score of 1 ± 0.9 was found. For the oral strains, they are less present with a similar average score in the two groups (0.41). Comparison between the two groups gave no significant difference regarding the origin of the bacteria.

DISCUSSION

Knowing the bacterial strains that colonize thermoplastic appliances is essential for the establishment of effective hygiene measures during orthodontic treatment with aligners. In this context, our qualitative study carried out on 34 patients found a very diverse bacterial flora in the thermoplastic appliances worn for two weeks with a predominance of *Staphylococcus* which were isolated in more than half of the samples of the control group. In terms of species, *Enterobacter cloacae complex* and *Staphylococcus aureus* were the most common. Another very interesting result revealed is the abundance of non-oral bacterial strains, which highlights the need for good hand hygiene before touching the aligner.

However, it appears that the qualitative approach and the untargeted identification of bacteria are specific to our work. In fact, following a literary search, it was found that almost all of the articles dealt with this subject from a quantitative point of view, either by evaluating the abundance of the biofilm with or without identification of the bacteria, or by looking for few predefined strains. Therefore, Gujar (Gujar *et al*., 2020) quantified 8 periodontopathogen species (*Campylobacter rectus, Fusobacterium nucleatum, Fusobacterium periodontium, Prevotella intermedia, Prevotella melaninogenica, Porphyromonas gingivalis, Tannerella forsythia* and *Tannerella denticola*) which he found in invisalign® aligners with a varied distribution from one germ to another. Low (Low *et al*., 2011), for his part, limited himself to the morphological description of bacteria. He noted the presence of filamentous germs, bacilli and Cocci grouped in clusters or chains. Meto (Meto *et al*., 2019) used only two aligners from the same patient and identified 8 different species: *Haemophilus parainfluenzae, Neisseria (mucosa* and *sicca), Staphylococcus aureus, Streptococcus (mitis, oralis* and *sanguinis)* and *Rothia aeria*. She studied the bacteriological variations of the biofilm of the aligners according to the seasons (winter and summer) and found a predominance of *Streptococcus mitis* and *oralis* in both periods with a slight decrease in the overall amount of biofilm during the summer. Levels of Staphylococcus *aureus, Haemophilus parainfluenzae* and *Neisseria (mucosa* and *sicca)* were noted to increase slightly during winter due to the frequent presence of these germs in the upper airways during this season. Albanna (Albanna *et al*., 2017) also used the vitek2® technique to identify bacteria and confirmed the predominance of *Staphylococcus* and *Streptococcus* we found in our study. She also reported that *Acinobacter* and *Pseudomonas* were the most abundant Gram +.

Many chemical products are used for aligners disinfection but we preferred to use a natural one: the tea tree essential oil. We studied his qualitative effect on the bacterial flora of the aligners to show that the once per day use of few drops of this oil as an adjuvant to brushing with toothpaste of thermoplastic aligners reduced the rate of certain genera such as *Enterobacter*, *Kocuria* and *Staphylococcus*. Only the last genus described a statistically significant decrease ($p = 0.031$). These results remain to be confirmed by a quantitative study.

In literature, we have not yet found any study that tested the antiseptic effect of this oil on the bacterial flora of thermoplastic appliances. Various other products were tried but the evaluation was only quantitative. In fact, Ismah (Ismah *et al*., 2019) tested the effect of a mouthwash containing 0.1% chlorhexidine on Gram + bacteria, in particular *Streptococcus mutans*, in Essix® splints. She noted that a 10 minute, twice a week immersion is able to significantly reduce the number of Gram+ germs compared to a simple immersion in water. For Shpack (Shpack *et al.*, 2014), he found that immersion of the aligners for 15 minutes once a day, in a chlorhexidine-based mouthwash as a complement to brushing, decreases bacterial load by 16% compared to a simple toothpaste brushing. Chang (Chang *et al*., 2014), meanwhile, considers that a simple toothpaste brushing is more than enough to reduce 99% of microorganisms and recommends reserving the use of chlorhexidine for immunocompromised patients at high risk of infection. However, Akgün (Akgün *et al*., 2019) considers that brushing alone is not sufficient to maintain good hygiene of thermoplastic aligners but must be supported by the use of an antiseptic agent such as acetic acid. Indeed, she showed that the immersion of the removable orthodontic appliance in a solution of 5% of white vinegar for 5 minutes once a day enable to reduce the quantity of cariogenic bacteria in the aligners significantly compared to a simple brushing. She also tested, by applying the same protocol above, effervescent tablets based on alkaline peroxide, Corega®. The results were similar to those obtained with the white vinegar. Nevertheless, Albanna (Albanna *et al*., 2017) did not validate the antiseptic effect of alkaline peroxide products on the biofilm of thermoplastic splints. In addition to Corega®, she tested two other products containing the same active principle: Retainer Brite® and Kukis. None of the three drugs significantly reduced the amount of biofilm compared to just brushing with water. Thus, Albanna described the presence of Gram + bacteria with predominance of *Streptococcus* and *Staphylococcus*, and Gram - bacteria predominated by *Acinetobacter* and *Pseudomonas* which persisted after the use of the different cleaning protocols. She confirmed these results by the disk diffusion method which revealed only a small zone of inhibition of *Staphylococcus epidermis* by Corega®. In the same context, Ismah (Ismah *et al*., 2019) tested the

antibacterial effect of another removable dentures cleaning product on the biofilm of thermoplastic aligners, Polident® which is available on the market in the form of effervescent tablets based on enzymatic neutral peroxide. She reported that immersion, once a day for 5 minutes, of the appliance in a solution containing a lozenge of Polident® can significantly reduce the number of colony-forming units CFU compared to a simple immersion in water.

A few other studies have tested the effect of a product intended specifically to cleaning aligners and which is marketed by the company Invisalign®. It has the form of crystals based on sulfate and sodium carbonate. Levrini (Levrini *et al*., 2015) showed, through scanning electron microscopy that immersing the aligner for at least 30 minutes in a solution containing these cleaning crystals, followed by brushing with toothpaste for at least 30 seconds, is more effective in reducing the bacterial plaque adhering to the outside face of the gutters than a simple immersion in water. Levrini (Levrini *et al*., 2016) completed his microscopic observations with a quantitative study based on the ATP-metry technique which is used in molecular biology to quantify, through the principle of bioluminescence, the adenosine triphosphate ATP whose rate is proportional to the amount of bacterial biofilm. He repeated the same hygiene protocols applied in his first study and reported that the combination of toothpaste brushing with an immersion in a cleaning crystal solution was the best, it significantly reduced the number of relative light units RLU (the technique's specific unit of measurement) compared to a single brushing which was, itself, significantly more effective than a simple immersion in water. In another study, Shpack (Shpack *et al*., 2014) supported the effect of Invisalign® cleaning crystals by ultrasound, he found that disinfecting the aligner, once per day for 15 minutes, in an ultrasonic bath containing a solution of this product is capable of reducing 50% of the initial amount of plaque with a cleaning effect 3 times more effective than immersion in a chlorhexidine-based mouthwash. In the same context, Lombardo (Lombardo *et al*., 2017) studied the cleaning efficiency of sound and ultrasonic waves combined with different solutions (water, anionic detergent and cationic detergent) and found that the 5 minutes, twice a day use of a 42kHz ultrasonic bath containing a cationic solution based on benzalkonium chloride, was the most, significantly, effective method compared to other methods which gave similar results. Meto (Meto *et al*., 2019) tested an antiseptic product widely and significantly used in dentistry and, in particular, in endodontics. It is the calcium hydroxide. She used a 1.25% Cupral® solution. She did not detect any bacterial multiplication following exposure of the aligners to calcium hydroxide. In addition, she reported that the three-dimensional structure of the biofilm adhering to the appliance was deeply affected.

CONCLUSION

Thermoplastic aligners are a real niche for bacteria whose origin is twofold: oral and non-oral. A rigorous hygiene protocol is therefore necessary as it encompasses three aspects:

1. Oral hygiene which will reduce the risk of intraoral contamination of the aligners
2. Hand hygiene which will reduce the risk of extra oral contamination of the aligners.
3. The hygiene of the equipment which will maintain a reduced rate of bacteria, in order to avoid discoloration and bad breath.

Tea tree essential oil is a great natural alternative for disinfecting aligners. Thanks to its wide spectrum of activity, it is able to affect various bacteria including *Staphylococcus.*

REFERENCES

- Akgün, F. A., Şenışık, N. E., & Çetin, E. S. (2019). Evaluation of the Efficacy of Different Cleaning Methods for Orthodontic Thermoplastic Retainers in terms of Bacterial Colonization. *Turkish Journal of Orthodontics*, *32*(4), 219–228. https://doi.org/10.5152/TurkJOrthod.2019.19061
- Albanna, R. H., Farawanah, H. M., & Aldrees, A. M. (2017). Microbial evaluation of the effectiveness of different methods for cleansing clear orthodontic retainers: A randomized clinical trial. *The Angle Orthodontist*, *87*(3), 460–465. https://doi.org/10.2319/072916-585.1
- Azaripour, A., Weusmann, J., Mahmoodi, B., Peppas, D., Gerhold-Ay, A., Van Noorden, C. J. F., & Willershausen, B. (2015). Braces versus Invisalign®: Gingival parameters and patients' satisfaction during treatment: a cross-sectional study. *BMC Oral Health*, *15*, 69. https://doi.org/10.1186/s12903-015-0060-4
- Buschang, P. H., Chastain, D., Keylor, C. L., Crosby, D., & Julien, K. C. (2019). Incidence of white spot lesions among patients treated with clear aligners and traditional braces. *The Angle Orthodontist*, *89*(3), 359–364. https://doi.org/10.2319/073118-553.1
- Chang, C. S., Al-Awadi, S., Ready, D., & Noar, J. (2014). An assessment of the effectiveness of mechanical and chemical cleaning of Essix orthodontic retainer. *Journal of Orthodontics*, *41*(2), 110–117. https://doi.org/10.1179/1465313313Y.0000000088
- Dallel, I., Merghni, A., Ben Tanfous, S., Tobji, S., Ben Amor, F., & Mastouri, M. (2019). The effect of orthodontic appliances on oral microflora: A case-control study. *Oral Science International*, *16*, 29–34. https://doi.org/10.1002/osi2.1005
- Gujar, A. N., Al-Hazmi, A., Raj, A. T., & Patil, S. (2020). Microbial profile in different orthodontic appliances by checkerboard DNA-DNA hybridization: An in-vivo study. *American Journal*

of Orthodontics and Dentofacial Orthopedics, *157*(1), 49–58. https://doi.org/10.1016/j.ajodo.2019.01.026

- Ismah, N., Purwanegara, M. K., & Rahmawati, T. (2019). Efficacy of disinfectants against gram-positive bacteria found on thermoplastic retainers. *International Journal of Applied Pharmaceutics*, 130–133. https://doi.org/10.22159/ijap.2019.v11s1.180
- Létard, J.-C., Canard, J.-M., Costil, V., Dalbiès, P., & Grunberg, B. (2015). *Phytothérapie – Principes généraux*. *5*, 7.
- Levrini, L., Mangano, A., Margherini, S., Tenconi, C., Vigetti, D., Muollo, R., & Marco Abbate, G. (2016). ATP Bioluminometers Analysis on the Surfaces of Removable Orthodontic Aligners after the Use of Different Cleaning Methods. *International Journal of Dentistry*, *2016*, 5926941. https://doi.org/10.1155/2016/5926941
- Levrini, L., Novara, F., Margherini, S., Tenconi, C., & Raspanti, M. (2015). Scanning electron microscopy analysis of the growth of dental plaque on the surfaces of removable orthodontic aligners after the use of different cleaning methods. *Clinical, Cosmetic and Investigational Dentistry*, *7*, 125–131. https://doi.org/10.2147/CCIDE.S95814
- Lombardo, L., Martini, M., Cervinara, F., Spedicato, G. A., Oliverio, T., & Siciliani, G. (2017). Comparative SEM analysis of nine F22 aligner cleaning strategies. *Progress in Orthodontics*, *18*(1), 26. https://doi.org/10.1186/s40510-017-0178-9
- Low, B., Lee, W., Seneviratne, C. J., Samaranayake, L. P., & Hägg, U. (2011). Ultrastructure and morphology of biofilms on thermoplastic orthodontic appliances in 'fast' and 'slow' plaque formers. *European Journal of Orthodontics*, *33*(5), 577–583. https://doi.org/10.1093/ejo/cjq126
- Meto, A., Colombari, B., Castagnoli, A., Sarti, M., Denti, L., & Blasi, E. (2019). Efficacy of a Copper–Calcium–Hydroxide Solution in Reducing Microbial Plaque on Orthodontic Clear Aligners: A Case Report. *European Journal of Dentistry*, *13*(3), 478–484. https://doi.org/10.1055/s-0039-1695653
- Shpack, N., Greenstein, R. B.-N., Gazit, D., Sarig, R., & Vardimon, A. D. (2014). Efficacy of three hygienic protocols in reducing biofilm adherence to removable thermoplastic appliance. *The Angle Orthodontist*, *84*(1), 161–170. https://doi.org/10.2319/012413-75.1