ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

Isolation of Microorganisms in Used Orthodontic Aligners Using Culture Media in the South Indian Population – An Observational Study

Dr. M. S. Suwethaa[1]; Dr. Purushotaman Deenadayalan[2]; Dr. C. Deepak[3]; Dr. Akshay T.[4]; Dr. Nidhi. A.[5]; Dr. Praveen[6]; Dr. Reshma Mohan[7]

[1-7] Department of Orthodontics and Dentofacial Orthopaedics
SRM Kattankulathur Dental College & Hospital, SRM Institute of Science and Technology, Chengalpattu District, Tamil Nadu State, India.

Corresponding Author: Dr. Purushotaman Deenadayalan*
Associate Professor, Department of Orthodontics and Dentofacial Orthopaedics, SRM Kattankulathur Dental College & Hospital, SRM Institute of Science and Technology, Chengalpattu district, Tamil Nadu State, India.

Publication Date: 2025/08/30

Abstract: Background: Clear aligners have transformed orthodontic treatment by offering a removable, esthetic alternative to fixed appliances. However, prolonged intraoral wear makes them susceptible to microbial colonisation. Aim: To isolate and identify microorganisms present on used orthodontic aligners and assess the effect of different oral hygiene practices on microbial load. Materials & Methods: Fifteen patients undergoing clear aligner therapy were grouped based on oral hygiene practices: once-daily brushing, twice-daily brushing, and twice-daily brushing with mouthwash. Used aligners (worn for two weeks) were sampled using sterile swabs, cultured on blood agar and Sabouraud dextrose agar, and analysed using Gram staining and biochemical tests. Statistical analysis included ANOVA, Tukey's post hoc, and Chi-square tests. Results: All samples exhibited bacterial growth, predominantly *Streptococcus mutans*, with no fungal growth. Mean colony-forming units (CFU) were significantly lower in patients using both brushing and mouthwash compared to other groups ($p < 0.001$). Conclusion: Oral hygiene significantly influences bacterial colonisation on aligners. Twice-daily brushing with adjunctive mouthwash is effective in reducing microbial load.

Keywords: *Clear Aligners; Orthodontic Appliances; Microbial Colonisation; Oral Hygiene Practices.*

How to Cite: Dr. M. S. Suwethaa; Dr. Purushotaman Deenadayalan; Dr. C. Deepak; Dr. Akshay T.; Dr. Nidhi. A.; Dr. Praveen; Dr. Reshma Mohan (2025) Isolation of Microorganisms in Used Orthodontic Aligners Using Culture Media in the South Indian Population – An Observational Study. *International Journal of Innovative Science and Research Technology*, 10(8), 1666-1671. https://doi.org/10.38124/ijisrt/25aug1062

I. INTRODUCTION

Clear aligners have become increasingly popular in contemporary orthodontic practice due to their discreet appearance, comfort, and removability (Rossini et al., 2015).[1] In comparison to fixed appliances, removable aligners facilitate better oral hygiene and are associated with lower plaque accumulation, especially in the early months of treatment.[2] However, aligners remain in continuous contact with the oral environment—saliva, food debris, and resident microbes—and can become substrates for biofilm formation.

Dental biofilm forms on both hard and prosthetic surfaces, initiating with pellicle formation followed by microbial adhesion and maturation into complex communities. On aligner surfaces, this biofilm may include cariogenic species like *Streptococcus mutans* as well as opportunistic microbes, potentially increasing the risk for oral diseases even during aligner usage.

Studies of orthodontic patients have shown that combining mechanical cleaning (brushing) with adjunctive methods such as mouthwash improves plaque control outcomes, even with fixed appliances. [3] Despite aligners being removable, microbial colonisation can still progress without effective hygiene practices. Recent data suggest that

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

using mouthwash—particularly formulations like essential oils or chlorhexidine—as adjuncts can enhance microbial control.

There is limited data on how hygiene regimens affect microbial contamination of aligners in South Indian populations, who may have distinct dietary habits and dominant microbial flora. Thus, this study aims to identify and characterise microorganisms on used orthodontic aligners and evaluate how different hygiene practices affect their presence.

II. MATERIALS AND METHODS

A. Study Design

This in vivo observational study was conducted to assess microbial contamination on used orthodontic aligners in patients undergoing clear aligner therapy. A total of fifteen patients undergoing treatment and reporting to the Department of Orthodontics, SRM Kattankulathur Dental College and Hospital, India, were recruited. Ethical approval for the study protocol was obtained from the Institutional Ethical Committee.

B. Sample Size and Selection

The study included 15 participants (8 males, 7 females) within the age group of 18–30 years, undergoing Invisalign® therapy. Patients were selected using convenience sampling based on the following criteria:

➢ *Eligibility Criteria*

- *Inclusion Criteria*

✓ Patients undergoing clear aligner therapy.
✓ Aligners worn continuously for at least two weeks.
✓ Patients following regular toothbrushing and basic oral hygiene instructions.

- *Exclusion Criteria*

✓ Patients with systemic infections or those on antibiotic therapy within the past month.
✓ Aligners were professionally cleaned or disinfected using cleaning foams/tablets continuously during the previous two weeks.
✓ Patients with poor compliance with aligner wear.

C. Sample Collection:

Used Invisalign® aligners worn for a continuous period of two weeks were collected from participants. Each aligner was placed in a sterile container immediately upon removal to avoid external contamination.

D. Oral Hygiene Practice Groups:

Participants were classified into three groups based on self-reported oral hygiene routines during the aligner wear period:

- Group 1: Once-daily toothbrushing.
- Group 2: Twice-daily toothbrushing.
- Group 3: Twice-daily toothbrushing with adjunctive mouthwash use.

E. Microbial Sampling:

Sterile cotton swabs were used to collect samples from the internal and external surfaces of each aligner. The swabs were immediately transferred to sterile transport media and processed within one hour of collection.

F. Culture Media and Incubation Samples were Inoculated onto:

- Blood agar – for isolation of gram-positive and gram-negative bacteria.
- Sabouraud dextrose agar – for isolation of fungi.

The inoculated plates were incubated at 37°C for 24–48 hours for bacterial growth and at 25°C for up to 5 days for fungal growth.

G. Identification of Microorganisms:

Colony morphology was recorded, and Gram staining was performed to classify bacteria as Gram-positive or Gram-negative. Fungal colonies were identified based on macroscopic and microscopic features. Additional biochemical tests were performed as necessary for bacterial species identification.

➢ *Statistical Analysis*

Data were compiled and analysed using SPSS version XX (IBM Corp., Armonk, NY, USA).

- Descriptive statistics were calculated for all variables, including frequency and percentage for categorical data (bacterial species distribution) and mean ± standard deviation for continuous data (CFU counts).
- One-way Analysis of Variance (ANOVA) was used to compare mean CFU counts among the three oral hygiene practice groups.
- Post-hoc Tukey's test was applied for pairwise group comparisons when ANOVA results were statistically significant.
- Chi-square test was used to compare the distribution of individual bacterial species between groups.
- The significance level was set at $p < 0.05$ for all tests.

III. RESULT

A total of 15 patients undergoing clear aligner therapy participated in the study, with used aligners collected after two weeks of continuous wear. Participants were distributed among three oral hygiene practice groups: Group 1 (once-daily brushing), Group 2 (twice-daily brushing), and Group 3 (twice-daily brushing with mouthwash).

➢ *Microbial Growth:*

Bacterial colonies were observed on all aligner samples. No fungal growth was detected on Sabouraud dextrose agar in any group. Gram staining and biochemical tests identified multiple bacterial species, predominantly gram-positive cocci and gram-negative bacilli.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

➢ *Distribution of Microorganisms by Oral Hygiene Group:*

The most frequently isolated organisms included Streptococcus mutans, Staphylococcus aureus, Lactobacillus spp., and Pseudomonas aeruginosa. Group 1 demonstrated the highest bacterial load, while Group 3 exhibited the lowest.

Table 1. Frequency of Bacterial Isolation Across Oral Hygiene Groups

Bacterial Species	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)	Chi-square p-value
Streptococcus mutans	9 (90%)	7 (70%)	5 (50%)	0.048*
Staphylococcus aureus	7 (70%)	5 (50%)	4 (40%)	0.216
Lactobacillus spp.	6 (60%)	4 (40%)	2 (20%)	0.097
Pseudomonas aeruginosa	4 (40%)	2 (20%)	1 (10%)	0.211

*Statistically significant at $p < 0.05$

Table 2. Mean Colony-Forming Units (CFU) per Group

Group	Mean CFU $\times 10^3 \pm$ SD	ANOVA p-value
Group 1 – Once-daily brushing	155 ± 22	
Group 2 – Twice-daily brushing	112 ± 18	
Group 3 – Twice-daily + mouthwash	78 ± 15	**<0.001***

***Highly significant at $p < 0.001$

➢ *Comparative Analysis*

- ANOVA revealed a statistically significant difference in mean CFU counts among the three groups ($F = 28.64$, $p < 0.001$).
- Post-hoc Tukey's test showed that Group 3 had significantly lower CFU counts compared to both Group 1 ($p < 0.001$) and Group 2 ($p = 0.012$). Group 2 also showed significantly lower counts than Group 1 ($p = 0.021$).
- Chi-square analysis for species distribution showed a statistically significant reduction in *S. mutans* frequency from Group 1 to Group 3 ($p = 0.048$). Other bacterial species showed decreasing trends but did not reach statistical significance.
- No fungal growth was observed in any group, suggesting that short-term aligner wear under the studied conditions may not favour fungal colonisation.

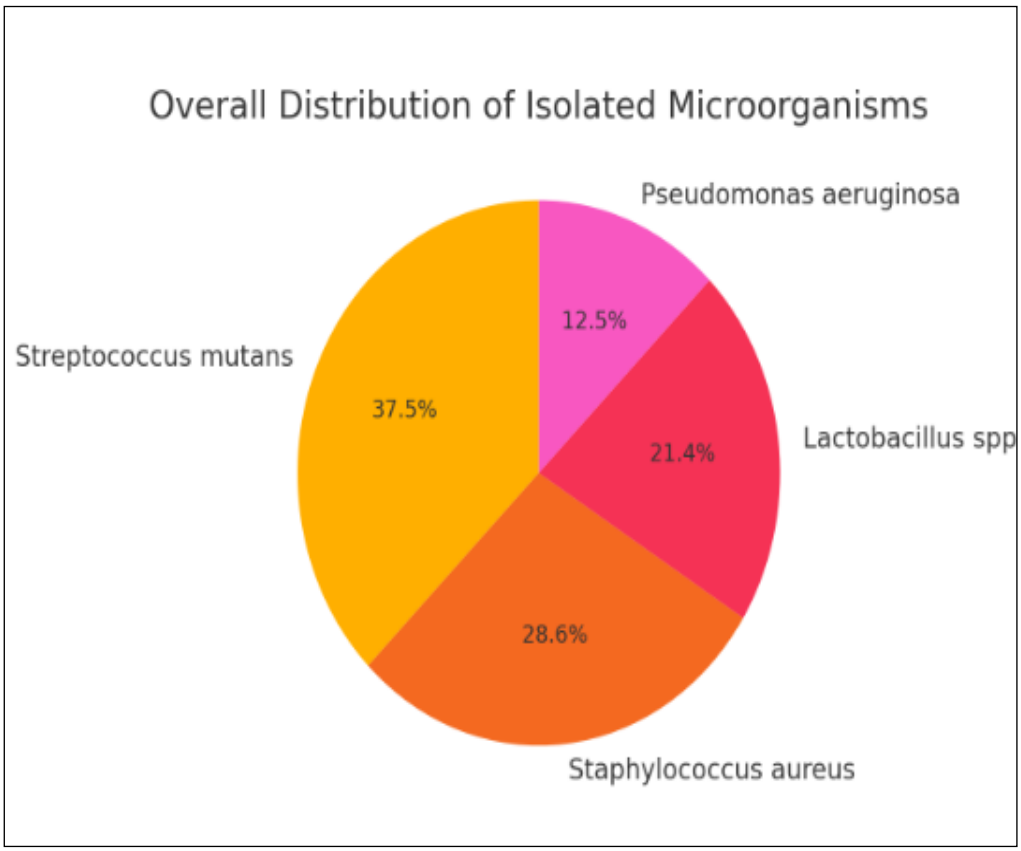

Fig 1: Overall Distribution of Isolated Microorganisms

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

Figure 1: demonstrates that *Streptococcus mutans* was the most frequently isolated microorganism, accounting for the largest proportion of colonies across all samples, followed by *Staphylococcus aureus*, *Lactobacillus spp.*, and *Pseudomonas aeruginosa*. This predominance of *S. mutans* aligns with its established role as a primary coloniser of dental biofilms and its strong adherence capability to polymeric surfaces such as aligners.

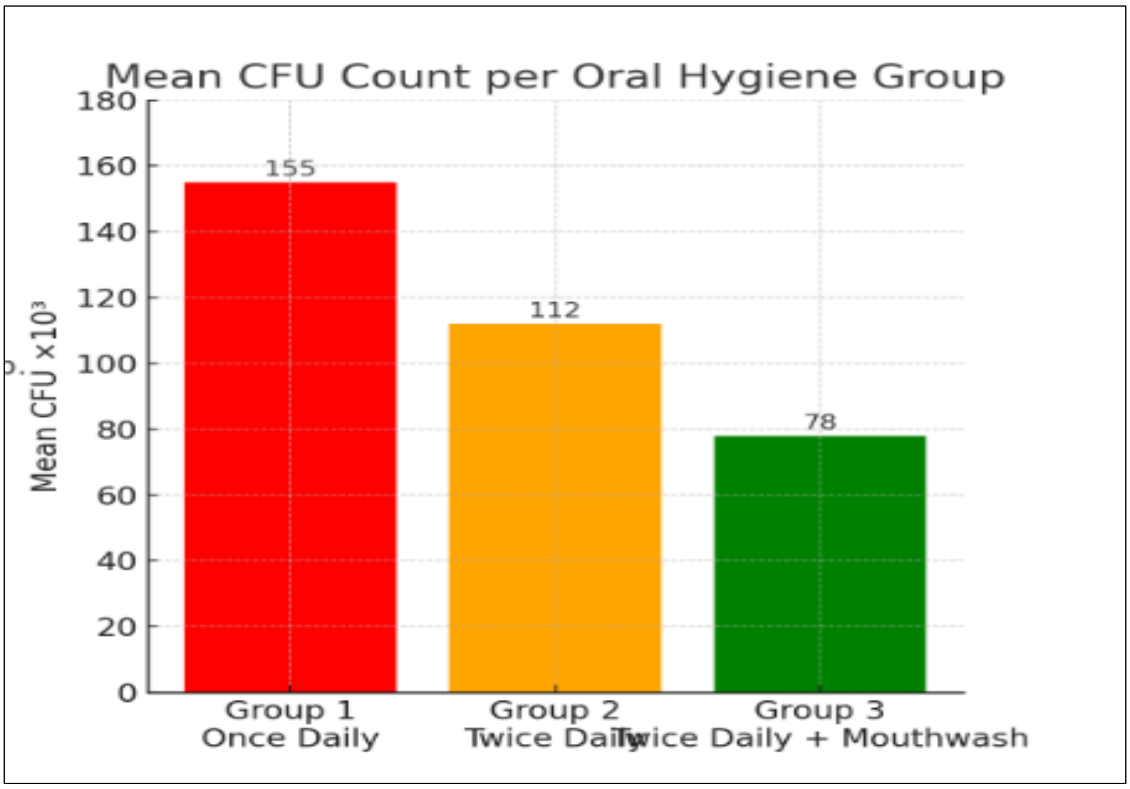

Fig 2: Mean CFU per Oral Hygiene Group

Figure 2 indicates a clear downward trend in microbial load with improved oral hygiene practices. Group 1 (once-daily brushing) exhibited the highest mean bacterial counts, while Group 3 (twice-daily brushing with mouthwash) showed the lowest. This suggests that increased brushing frequency and adjunctive antimicrobial mouthwash use are effective in reducing bacterial colonisation on aligners.

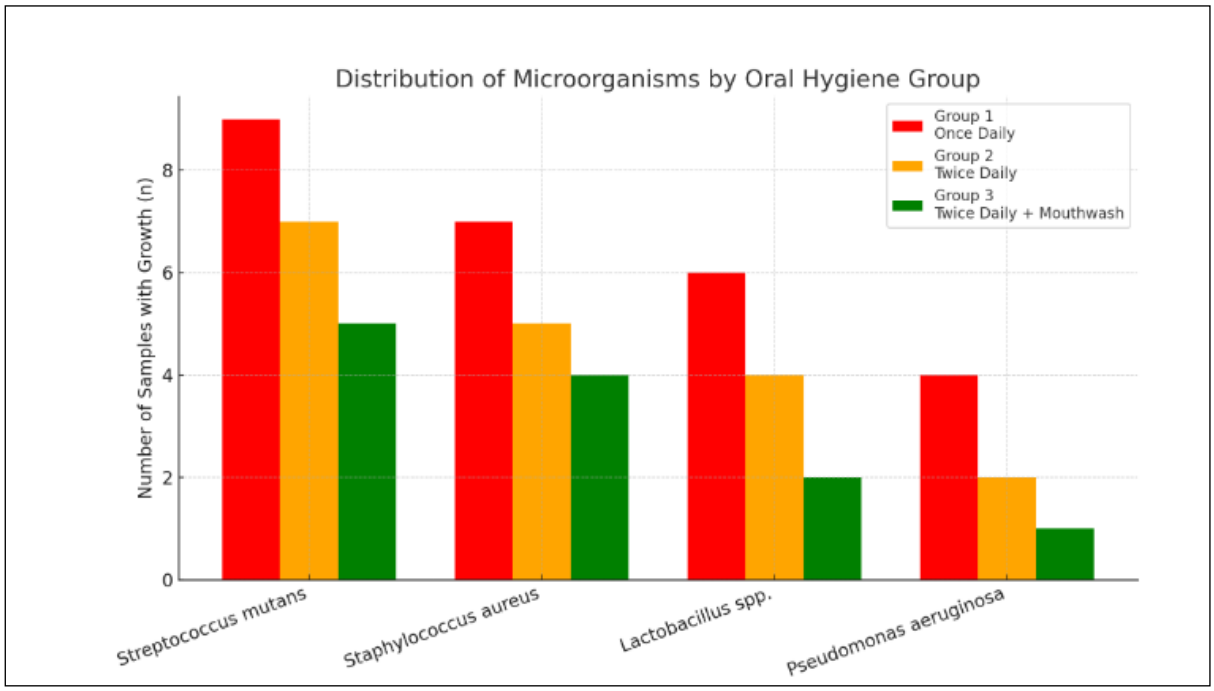

Fig 3: Distribution of Microorganisms by Oral Hygiene Group

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

Figure 3 shows the distribution of species by hygiene group, further supporting this trend. All bacterial species showed a reduction in isolation frequency with improved hygiene, with *S. mutans* showing the most pronounced decline from Group 1 to Group 3. The reduction in *Pseudomonas aeruginosa* was also notable, particularly between Groups 2 and 3, suggesting that adjunctive mouthwash use may help suppress less common but potentially pathogenic gram-negative species.

The absence of fungal growth across all samples, despite the presence of a moist intraoral environment, may be attributed to the relatively short aligner wear duration (two weeks) and regular patient adherence to basic hygiene instructions, which may have prevented conditions favourable for fungal colonisation.

IV. DISCUSSION

The present study investigated microbial contamination in used orthodontic aligners worn by patients from a South Indian population. All aligners exhibited bacterial colonisation, with Streptococcus mutans being the predominant isolate, while no fungal growth was detected.

Bacterial Profile and Biofilm Formation
The predominance of S. mutans aligns with its established role in plaque biofilm formation and the pathogenesis of dental caries. Its ability to adhere to smooth surfaces and produce extracellular polysaccharides enhances colonisation on thermoplastic materials like polyurethane used in clear aligners (Kwon et al., 2021).[4] Similar microbial patterns have been reported in studies on both fixed and removable orthodontic appliances (Levrini et al., 2016; Lucchese et al., 2018).[5,7] The detection of S. aureus, Lactobacillus spp., and Pseudomonas aeruginosa suggests that aligners can harbour both oral commensals and opportunistic pathogens, potentially increasing the risk for localised or systemic infections in susceptible individuals.

- *Impact of Oral Hygiene Practices*

Our results demonstrated a statistically significant reduction in bacterial load from Group 1 (once-daily brushing) to Group 3 (twice-daily brushing with mouthwash). This confirms that improved oral hygiene measures reduce bacterial colonisation on aligner surfaces, echoing findings by Pithon et al. (2014) [6] and Rossini et al. (2015) [1], who emphasised the role of mechanical and chemical plaque control in orthodontic care. Antimicrobial mouthwashes, particularly those containing chlorhexidine or essential oils, disrupt bacterial cell walls, reduce biofilm thickness, and inhibit recolonisation, contributing to the lower CFU counts observed in Group 3.

- *Absence of Fungal Growth*

Interestingly, no Candida species were detected. This differs from previous studies on acrylic-based removable appliances, which reported fungal colonization rates of 20–50% (Aldrigui et al., 2021).[8] The discrepancy could be due to differences in material surface properties—thermoplastic aligners have a lower surface free energy and reduced porosity compared to acrylic, which may hinder yeast adhesion. Additionally, the relatively short wear period (two weeks) in our study may have been insufficient for detectable fungal biofilm formation.

- *Clinical Implications*

The clinical relevance of our findings lies in the need for strict hygiene reinforcement during clear aligner therapy. While aligners generally accumulate less plaque than fixed appliances, they still serve as reservoirs for cariogenic and opportunistic microorganisms if not cleaned adequately. Patients may perceive aligners as “self-cleaning” due to their smooth surfaces, but our results show this is a misconception. Regular mechanical cleaning, combined with antimicrobial rinses, is essential to maintain periodontal health and prevent enamel demineralisation.

- *Limitations and Future Directions*

The limitations of this study include its relatively small sample size and reliance on conventional culture methods, which may underestimate microbial diversity. Future studies using molecular techniques such as 16S rRNA sequencing could provide a more comprehensive microbial profile, including non-cultivable species. Additionally, longitudinal studies with longer wear intervals would help determine whether fungal colonisation becomes significant over time. Exploring the antimicrobial potential of novel aligner materials or surface coatings could also be a promising research avenue.

V. CONCLUSION

Used orthodontic aligners exhibit significant bacterial colonization, predominantly by *S. mutans*, with no fungal growth detected. Oral hygiene practices, particularly brushing twice daily with adjunctive mouthwash, are effective in reducing bacterial load. Regular monitoring and patient education are essential to minimise oral health risks during clear aligner therapy.

REFERENCES

[1]. Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL. Periodontal health during clear aligner therapy: a systematic review. *Eur J Orthod*. 2015;37(5):539–43.
[2]. Oikonomou E, Foros P, Tagkli A, Rahiotis C, Eliades T, Koletsi D. Impact of aligners and fixed appliances on oral health during orthodontic treatment: a systematic review and meta-analysis. *Oral Health Prev Dent*. 2021;19:659–72.
[3]. Ren X, Zhang Y, Xiang Y, Chen M, Lin J, Jiang F. The efficacy of mouthwashes on oral microorganisms and gingivitis in patients undergoing orthodontic treatment: a systematic review and meta-analysis. *BMC Oral Health*. 2023;23:204.
[4]. Kwon JS, Lee YK, Lim BS, Lim YJ. Biofilm formation on thermoplastic orthodontic appliances. *Korean J Orthod*. 2021;51(1):50–8.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25aug1062

[5]. Levrini L, Mangano A, Margherini S, Tenconi C, Caprioglio A. Oral health in patients wearing orthodontic appliances. *J Clin Med Res*. 2016;8(5):480–6.
[6]. Pithon MM, Sant'Anna LI, Baião FC, Cople Maia L, Coqueiro RS. Influence of mouth rinses on the mechanical properties of orthodontic elastomeric chains. *Angle Orthod*. 2014;84(5):822–8.
[7]. Lucchese A, Carinci F, Barbato E. Dental caries and orthodontic treatment: an updated review. *Eur J Paediatr Dent*. 2018;19(4):317–23.
[8]. Aldrigui JM, Chibinski AC, Bussaneli DG, Wambier LM, Moraes MB, Pinto LS, et al. Candida colonization in orthodontic patients: a systematic review. *Int J Paediatr Dent*. 2021;31(5):659–70