Table 5 Bacterial counts for each species (fifth root-transformed) at each time point and group with between-group testing with Mann-Whitney test (*) or *t* test for independent samples (+), according to normality of data

Bacteria		Aligner		Bracket	Test	*P*
S. mutans (transformed)	*n*	Mean (SD)	*n*	Mean (SD)		
Count at T0	15	9.44 (6.06)	15	12.20 (7.24)	+	0.27
Count at T1	15	8.60 (6.05)	15	11.02 (7.73)	+	0.35
Count at T2	15	8.87 (6.14)	15	11.09 (6.71)	+	0.35
L. acidophilus (transformed)	*n*	Median (IQR)	*n*	Median (IQR)		
Count at T0	15	0 (0–0)	15	0 (0–0)	*	1.00
Count at T1	15	0 (0–0)	15	0 (0–0)	*	0.76
Count at T2	15	0 (0–0)	15	0 (0–0)	*	0.76
S. sanguinis (transformed)	*n*	Mean (SD)	*n*	Mean (SD)		
Count at T0	15	23.93 (11.67)	15	32.05 (5.24)	+	0.02
Count at T1	15	21.32 (10.55)	15	41.08 (10.02)	+	< 0.001
Count at T2	15	22.43 (9.49)	15	34.75 (7.63)	+	0.001

IQR interquartile range, *NT* not tested, *SD* standard deviation

patients (*P* for interaction = 0.11). This was further explored by stratified analyses (Appendix) and indicated a variation pattern of *S. sanguinis* that was similar to that of s-PlI: the *S. sanguinis* counts showed a tendency to reduce through time among the aligner patients, while *S. sanguinis* counts tended to increase through time among the bracket patients.

Discussion

The aim of the present prospective cohort study was to compare the salivary levels of cariogenic bacteria among adolescent patients treated with either thermoplastic aligners or fixed self-ligating appliances. The results indicated no difference in the salivary levels of *S. mutans* or *L. acidophilus*, although patients treated with thermoplastic aligners had lower salivary *S. sanguinis* levels than those treated with self-ligating appliances (Table 6). Oral microbiota attachment in orthodontic patients has been mainly associated with increased risk of *S. mutans* and lactobacilli colonization, among other species, thus initiating a series of events, which may lead to the development of demineralizations or caries [3, 8].

As far as the periodontal parameters are concerned, a statistically significant difference in both plaque scores (s-PlI) and gingivitis scores (s-GI) was found between fixed appliances and thermoplastic aligners, which favored the latter (Tables 2 and 3). This agrees with previous data indicating that teenagers treated with aligners display better compliance with oral hygiene, less plaque, and subtler gingival inflammatory reactions than those treated with fixed appliances [24]. The ease of oral hygiene maintenance with the clear aligners most likely allows patients to maintain, or possibly even improve, their oral hygiene. A recent systematic review pointed out that periodontal health indexes are significantly improved during clear aligner treatment, in particular when these appliances were compared to fixed appliances. However, the level of evidence was moderate for all the included studies [17]. Additionally, oral hygiene was significantly associated with patient sex, with male patients having significantly higher plaque scores than female patients (Table 3). Furthermore, pre-treatment oral hygiene levels were significantly associated with plaque scores and gingivitis during treatment (Table 3). Finally, no clear variation pattern of oral hygiene was seen through time, which agrees with Clements et al. [25], who demonstrated that the mean average papillary bleeding scores did not change in a statistically significant manner during aligner treatment.

In the present study, instructions were given to brush the thermoplastic appliances once daily. However, a recent study demonstrated that the use of a vibrating bath with cleaning solution protocol reduced biofilm adherence more than regular brushing or immersion of the aligner in chlorhexidine mouthwash [26]. The use of a chlorhexidine mouthwash as an adjunct to oral hygiene at home does not seem to be necessary for patients undergoing aligner treatment, at least for the first 8 months of treatment [27].

Additionally, several appliance-related factors might influence the intraoral performance of thermoplastic aligners. The material used for the fabrication of the thermoplastic aligners in this study was PET-G, which is the most widely used material for the fabrication of both aligners and retainers [28]. The material used for the Invisalign (Align Technology, Santa Clara, Calif) aligners is polyurethane-based and seems to have