Clin Oral Invest (2013) 17:1645–1660
DOI 10.1007/s00784-012-0856-4

ORIGINAL ARTICLE

Clinical and microbiological results following nonsurgical periodontal therapy with or without local administration of piperacillin/tazobactam

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Received: 15 November 2011 / Accepted: 27 September 2012 / Published online: 20 January 2013

Abstract
Objectives We assessed if adjunct administration of piperacillin/tazobactam added clinical and microbiological treatment benefits.
Materials and methods Thirty-six subjects (mean age 52.1 years (SD±10.3)) (NS by group) with chronic periodontitis were randomly enrolled receiving subgingival debridement and the local administration of piperacillin/tazobactam (test group) or debridement alone (control group). Bleeding on probing (BOP), probing pocket depth (PPD), and microbiological counts of 74 species were studied by checkerboard DNA-DNA hybridization up to month 6 after treatment.
Results >Mean PPD changes between baseline and month 6 in the test and control groups were 1.5 and 1.8 mm, respectively (NS between groups). BOP in both groups decreased from about 80 to 40 %. At 4 and 12 weeks, lower counts of the following bacteria were found in the test group (site level): *Fusobacterium* species, *Parvimonas micra*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Tannerella forsythia*, *Treponema denticola*, and a composite load of nine pathogens ($p<0.001$). At week 26, subjects receiving local antibiotics had a lower prevalence at tested sites for *Fusobacterium nucleatum* sp. *polymorphum*, *Fusobacterium periodonticum*, *P. micra*, and *T. denticola*.
Conclusions At 26 weeks, treatment with or without piperacillin/tazobactam resulted in similar BOP and PPD improvements. At week 26 and at the subject level, the prevalence of 4/74 pathogens was found at lower counts in the group receiving local antibiotics.
Clinical relevance Administration of piperacillin/tazobactam reduces the prevalence of *Fusobacterium*, *P. micra*, and *T. denticola* to a greater extent than debridement alone but with no short-term differences in PPD or BOP.

Keywords Periodontitis · Debridement · Local antibiotics · Piperacillin/tazobactam · Microbiota · Checkerboard DNA-DNA

Introduction

The routine initial treatment of both chronic and aggressive periodontitis includes supra- and subgingival debridement with the objective to remove or at least reduce the infectious burden such that clinical evidence of inflammation is reduced to a clinically acceptable level. The literature on short- and long-term results of nonsurgical mechanical periodontal therapies is extensive (i.e., [1–7]). Supportive periodontal therapy is considered as critical in maintaining initial reductions of the subgingival microbiota [8]. Data suggest that comprehensive periodontal debridement can achieve important reductions of bacterial counts that may last for up to 8 months after initial treatment [9]. The progression of site-specific periodontitis can be predicted

Electronic supplementary material The online version of this article (doi:10.1007/s00784-012-0856-4) contains supplementary material, which is available to authorized users.

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by monitoring counts of bacteria associated with periodontitis [10]. It is also known that subgingival debridement of root surfaces cannot effectively eliminate all bacteria and some bacteria may also be present within the dentin layer [11]. Although treatment often results in an immediate reduction of bacterial counts, recolonization has been reported shortly after therapy [12].

Most studies on microbiological changes following the administration of local antibiotics have focused on periodontal site-based changes. This has been consistent with a perception of a site-specific periodontal disease progression and that site-specific disease progression appears to occur in clusters of patients [13–17].

Recent studies have also demonstrated that the microbial content of the periodontal pocket is a determinant of gene expression in the gingival tissues and this controls the differential ability of periodontal species to elicit a local host response [18]. Thus, genetic factors may explain why periodontitis-susceptible subjects carry a specific pathogenic microbiota in their periodontal pockets. This concept is consistent with findings that within a periodontitis-susceptible patient, the presence of many bacterial species is similar within different sites of a patient with similar probing pocket depths (PPDs) [19].

Both systemic and local antibiotics have been advocated as adjunct antimicrobial therapies. Chronic periodontitis often presents with deep periodontal pockets at a limited number of sites. Therefore, the administration of local antibiotics with a high local concentration, and in combination with debridement, may be more effective in the management of localized periodontal infections than treatment with debridement alone. Recent studies have documented the positive effects of using systemic antibiotics to control the periodontal infection in combination with nonsurgical periodontal therapy [20–23].

Management of "refractory periodontitis" with reduction of bacteria is also possible by using a combination of a local antibiotic and subgingival debridement [24]. Others have shown that local administration of doxycycline in periodontal pockets of subjects with a smoking habit results in a significantly greater reduction in the levels of *Porphyromonas gingivalis* in comparison to debridement alone [25]. Recent studies have, however, also shown that the combined use of local administration of doxycycline and debridement of molars with furcation involvement failed to show a significant difference in vertical probing pocket depth up to 12 months after treatment and in comparison to local debridement alone [26]. A difference in horizontal furcation probing depth to the benefit of drug administration was found [26].

Data suggest that treatment with minocycline HCl microspheres as an adjunct treatment to debridement alone results in a greater reduction in the levels of *P. gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in combination than by debridement alone [27–30]. Other studies have, however, shown that slow release of local antibiotics may not provide sustainable control of the subgingival microbiota and may not be different from that of subgingival debridement alone [31, 32]. Thus, probing pocket depth reduction correlated significantly with a decrease in the numbers and proportions of red complex bacteria 30 days after administration [31]. The other study [32] showed that controlled release of doxycycline did not significantly suppress several subgingival pathogenic microorganisms and the authors concluded that this treatment did not seem to possess no distinct advantage over broad spectra, safe and inexpensive antiseptics and that the rationale for its employment in periodontal therapy remains unclear [32]. In one study using a 14 % doxycycline gel applied subgingivally at furcation sites following supportive periodontal therapy, the results showed that when applied only once at baseline, the administration failed to reduce the frequency of the need for re-instrumentation at furcation sites during supportive therapy for a period of 12 months [26]. In the most recent study on topical administration of doxycycline at periodontal sites in subjects with therapy-resistant periodontal pockets, the added use of doxycycline yielded on average 0.1 mm more reduction in probing pocket depth than supportive therapy alone at 3 months [33]. At 26 weeks, this study failed to demonstrate better odds of improved periodontal conditions if treatment included the antibiotic or not [33]. This is consistent with the findings by others using locally administered doxycycline and that the benefits did not remain beyond 3 months [34]. The long-term clinical value of adjunct local antibiotics has been questioned [35, 36].

Thus, local administration of either metronidazole, tetracycline, doxycycline, or minocycline adjunct to supportive periodontal therapy may not provide additional significant benefits in regard to probing depth reduction or gain in clinical attachment compared to results obtained by mechanical debridement alone [35]. Recent data suggest that treatment with local administration with azithromycin in a gel preparation may provide some clinical benefits over scaling and root planning alone [37]. The microbiological impact of such local antibiotic therapy remains unclear. Some of the tested local antibiotics are no longer available on the market.

Quorum sensing and plasmid transfer may inhibit efforts with both systemic and local antibiotics [38, 39]. Data suggest that piperacillin/tazobactam appears to be effective against infections by gram-negative anaerobes [40, 41]. Antibiotic resistance to piperacillin/tazobactam seems to be less than to that to amoxicillin/clavulanic acid [42]. Piperacillin/tazobactam also appears to be effective against *Pseudomonas aeruginosa* [43, 44]. *P. aeruginosa* is known to be a key pathogen in transmitting antibiotic resistance through plasmid transfer in biofilms [45, 46]. *P. aeruginosa* has been identified in subjects with periodontitis [47, 48]. Therefore,

control of *P. aeruginosa* infection in periodontal pockets may be of importance to enhance the effects of local antibiotic administration. Currently, there are no studies available assessing the effects on *P. aeruginosa* by local periodontal administration of antibiotics.

The objective of the present randomized clinical study was to assess the efficacy of local treatment with piperacillin/tazobactam in conjunction with subgingival debridement of periodontal pockets in comparison to local debridement alone using 26-week results as the study endpoint. We tested the null hypothesis that there are neither microbiological nor clinical differences in the treatment outcomes between the two study arms.

Materials and methods

The Ethics Committee at the University of Bern, Switzerland (Kantonale Ethik Kommission, Bern, Switzerland) approved the study. The study was conducted between 2007 and 2010. All participating subjects signed an informed consent. The flow chart for the study is presented in Fig. 1.

Exclusion criteria

Subjects were excluded

1. If they had received periodontal therapy within the preceding 6 months
2. If they had been treated with systemic or local antibiotics within the preceding 6 months
3. If they were allergic to piperacillin/tazobactam or penicillin
4. If they were using anti-inflammatory medications or medications known to cause gingival overgrowth

Inclusion criteria

Subjects were included

1. If they were 18 years of age or older
2. If they had four periodontal non-adjacent sites with a PPD≥5 mm requiring therapy
3. If they had a diagnosis of chronic periodontitis

Periofilm T®/Asbacare Clinic® (Medirel AS, Agno, Switzerland) is an antibiotic formulation (European Union Class III drug) which contains piperacillin and tazobactam in a formulation suitable for the treatment of periodontitis. Periofilm T® contains a powder (sodium piperacillin 100 mg, sodium tazobactam 12.5 mg) and a liquid (aminoalkyl-methacrylate copolymer, ammonium methacrylate copolymer, ethanol 95 %, and purified water). The liquid and powder are mixed immediately before administration with a supplied syringe.

Clinical measurements and treatment procedures

Subjects were allocated to the treatment group through randomization using the PASW, statistics 18.0 software (IBM/SPSS, Armonk, NY, USA). Subjects were given coded numbers and then through the software program allocated to either the test group or control group. Study subjects and therapist were strictly instructed not to inform the examiner if they had received treatment with the test drug or not. The clinical examiner had no access to dental records and was not present in the clinic when initial treatment or administration of antibiotics was performed. The allocation to the intervention group was not revealed until the data set had been locked. Bacterial samples were collected at the four selected sites from which clinical data were collected. The same brand of periodontal probes with 1-2-3-5-7-8-9-10 mm markings (SE4 Pocket probe, Deppeler SA, Rolle, Switzerland) was used to measure PPD at six sites per tooth. Bleeding on probing (BOP) of the pockets was defined approximately 10 s after the measurement of PPD. Only non-adjacent sites from different teeth were studied.

Each subject provided four teeth with a PPD≥5 mm, and these teeth were included in the study and treated with debridement as deemed necessary with hand instruments and/or ultrasound. The treatment was performed without time restrains and continued until the clinician was convinced that subgingival plaque and calculus had been removed at all the selected test teeth and neighboring teeth. In subjects with more than four sites presenting with a PPD≥5 mm, the four sites with the deepest PPD were chosen with consideration to risk for saliva contamination when performing bacterial sampling. Thus, if a test tooth had more than one site with the same (deepest) PPD, a buccal and preferably a mesio-buccal site was chosen. Third molars were excluded. In addition, no site represented sites associated with furcation involvement. All teeth were treated with nonsurgical debridement according to clinical protocol. During the study period, systemic antibiotics were not prescribed.

Oral hygiene instructions and information about the etiology of periodontitis and various treatment options were given to each subject prior to the enrollment in the study and before the initial therapy was given. Following the completion of this therapy, the selected test teeth were then treated with the adjunct local antibiotics according to what study group the subject had been assigned to by computer-based randomization. The assignment to study group was not given until the debridement had been completed.

The local antibiotic drug was administered by a clinician who was unaware of the randomization schedule and otherwise also not associated with the study. The administration

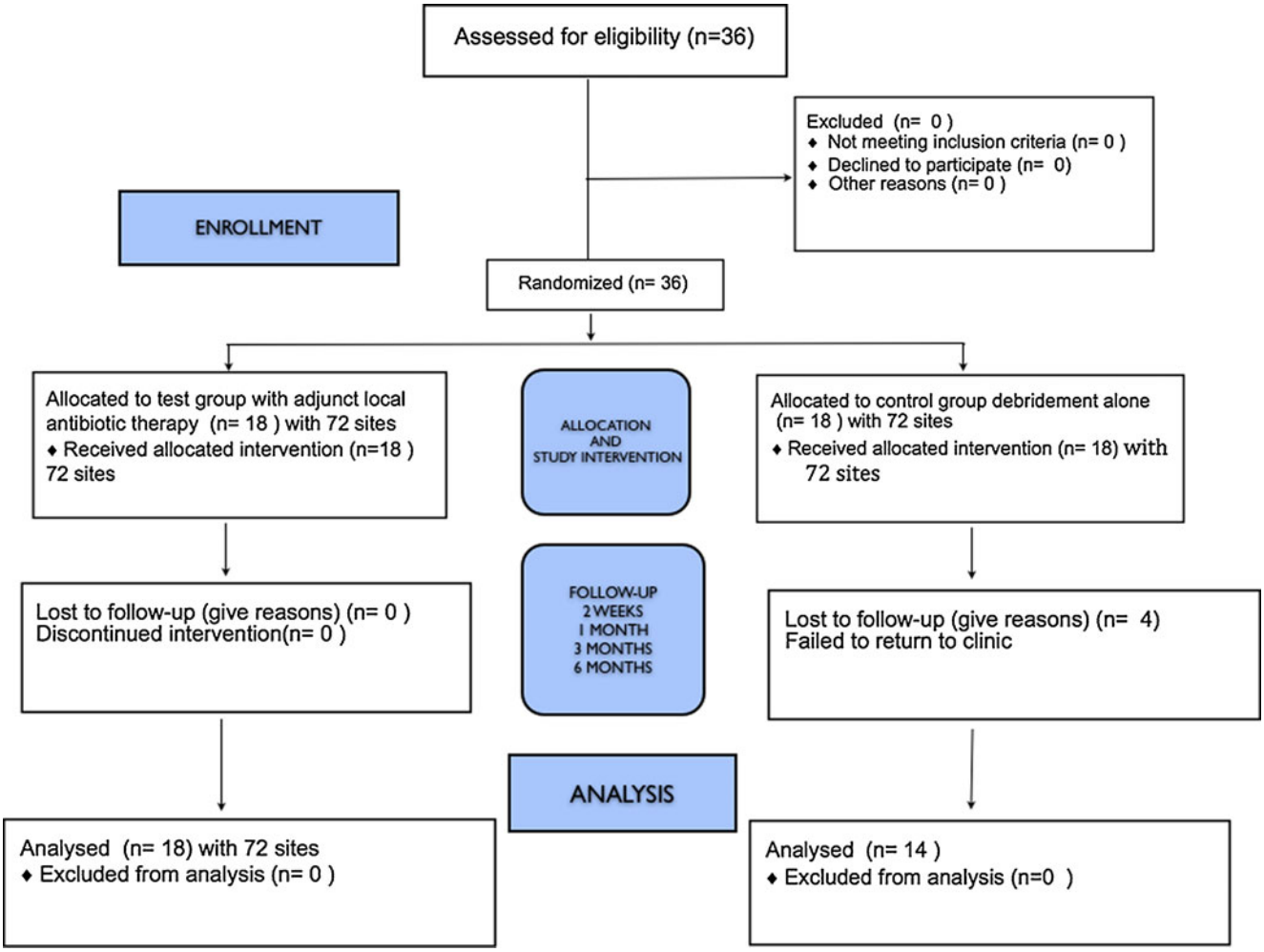

Fig. 1 Consort flow chart

of the local antibiotics was performed circumferentially in the pockets of the selected teeth. No additional treatment was performed before study endpoint at month 6. Subjects were given individualized post-treatment oral hygiene guidelines as required by routine clinical protocol at weeks 1, 2, 4, and 12.

Microbiological sampling

All microbiological samples were taken before probing pocket depth assessments had been performed. The selected sites representing the deepest non-adjacent sites within the subject had been defined at a previous visit. GCF was collected with sterile endodontic paper points (absorbent paper points size 50, Dentsply/Maillefer, Ballaigues, Switzerland). The paper points remained in situ for 15 s and were then placed in dry Eppendorf tubes (1.5 ml natural flat cap microcentrifuge tubes, Starlab, Ahrensburg, Germany). Bacterial samples were taken before treatment and at 2, 4, 12, and 26 weeks after intervention.

Analysis of subgingival bacterial samples

The vials were stored at −20 °C and processed after a standard storage period of 3 months. To each sample, 0.15 ml TE (10 mM Tris–HCl, 1 mM EDTA, pH 7.6) and 0.5 ml NaOH were added. All samples were analyzed by checkerboard DNA-DNA hybridization technique. The 74 species assessed are presented in Table 1. The checkerboard DNA-DNA hybridization was performed as described elsewhere [48, 49]. Briefly, bacterial DNA was extracted, concentrated on nylon membranes (Roche Diagnostics GmbH, Mannheim, Germany), and fixed by cross-linking using ultraviolet light (Stratalinker 1800, Stratagene, La Jolla, CA, USA). The membranes with fixed DNA were placed in a Miniblotter 45 (Immunetics, Cambridge, MA, USA). Signals were detected by using the Storm Fluor-Imager (Storm 840, Amersham

Table 1 Reference bacteria strains included in the DNA-DNA checkerboard analysis

Species	Collection	Species	Collection
Actinomyces israelii	ATCC 12102	*Lactobacillus jensenii*	GUH 160339
Actinomyces naeslundii (type I + II)	ATCC 43146	*Lactobacillus vaginalis*	GUH 078092
Actinomyces neuii	GUH 550898	*Leptotrichia buccalis*	ATCC14201
Actinomyces odontolyticus	ATCC 17929	*Mobiluncis curtisii*	GUH 070927
Aggregatibacter actinomycetemcomitans (a)	ATCC29523	*Mobiluncus mulieris*	GUH 070926
Aggregatibacter actinomycetemcomitans (Y4)	ATCC 43718	*Neisseria mucosa*	ATCC 33270
Aerococcus christensenii	GUH 070938	*Parvimonas micra*	ATCC 19696
Aanaerococcus vaginalis	GUH 290486	*Peptoniphilus* sp.	GUH 55097
Atopbium parvulum	GUH 160323	*Porphyromonas endodontalis*	ATCC 35406
Atopobium vaginae	GUH 010535	*Porphyromonas gingivalis*	ATCC 33277
Bacteroides ureolyticus	GUH 080189	*Prevotella bivia*	GUH 450429
Bifidobacterium biavatii	GUH 071026	*Prevotella disiens*	GUH 190184
Bifidobacterium bifidum	GUH 070962	*Prevotella intermedia*	ATCC 25611
Bifidobacterium breve	GUH 080484	*Prevotella melaninogenica*	ATCC 25845
Bifidobacterioum longum	GUH 180689	*Propionibacterium acnes*	ATCC 11727/2
Campyobacter gracilis	ATCC 33236	*Proteus mirabilis*	GUH 07092
Campylobacter rectus	ATCC 33286	*Pseudomonas aeruginosa*	DSMZ 50071
Campylovacter showae	ATCC 51146	*Selenomonas noxia*	ATCC 43541
Capnocytophaga gingivalis	ATCC 33612	*Staphylococcus anaerobius*	DSMZ 20714
Capnocytophaga ochraceae	ATCC 335945	*Staphylococcus aureus*	ATCC 25923
Capnocytophaga sputigena	ASTCC 33612	*Staphylococcus aureus* (yellow)	GUH 070921
Corynebacterium nigricans	GUH450453	*Staphylococcus aureus* (white)	GUH 070922
Cornerbacterium aurimucosum	GUH 071035	*Staphylococcus epidermis*	GUH 130381
Dialister sp.	GUH 071045	*Staphylococcus haemolyticus*	DSMZ 20263
Escherichia coli	GUH 070903	*Streptococcus agalactiae*	GUH 230282
Eikenella corrodens	ATCC 23834	*Streptococcus anginosus*	ATCC 33397
Enterococcus faecalis	GUH 170812	*Streptococcus constellatus*	ATCC 27823
Enterococcus faecalis	ATCC 29212	*Streptococcus gordonii*	ATCC 10558
Fusobacterium nucleatum nucleatum	ATCC 25586	*Streptococcus intermedius*	ATCC 27335
Fusobacterium nucleatum polymorphum	ATCC 10953	*Streptococcus mitis*	ATCC 49456
Fusobacterium nucleatum naviforme	ATCC 49256	*Streptococcus oralis*	ATCC 35037
Fusobacterium periodonticum	ATCC 33693	*Streptococcus pneumoniae*	DSMZ 11866
Gardnerella vaginalis	GUH 080585	*Streptococcus sanguinis*	ATCC 10556
Haemophilus influenzae	ATCC 49247	*Streptococcus mutans*	ATCC 25175
Helicobacter pylori	ATCC 43504	*Tannerella forsythia*	ATCC 43037
Lactobacillus acidophilus	ATCC 11975	*Treponema denticola*	ATCC 35405
Lactobacillus crispatus	GUH 160342	*Treponema socranskii*	D40DR2
Lactobacillus gasseri	GUH 17085	*Varibaculum cambriense*	GUH 070917
Lactobacillus iners	GUH 160334	*Veillonella parvula*	ATCC 10790

ATCC American Type Culture Collection; *D* sample from Forsyth Institute, Boston, MA, USA; *GUH* Ghent University Hospital Collection, Ghent, Belgium

Biosciences, Piscataway, NJ, USA) with a setup of 200 μm and 600 V. The digitized information was analyzed by a software program (ImageQuant, Amersham Pharmacia), allowing comparisons of the density of the 19 sample lanes against the two standard lanes (10^5 or 10^6 cells). Signals were converted to absolute counts by comparisons with these standards [34].

Statistical methods

We assumed at 25 % difference in the proportion of positive test results at study sites for *P. gingivalis* (15 %/40 %) at 26 weeks after intervention. Using the four sites in 18 subjects from each group and anticipating that two subjects in each group would

not complete the study (n=64 per group), the power would be 88 %. The Kolmogorov–Smirnov test was used to assess whether the data set had a normal distribution pattern or not. Independent t tests (equal variance not assumed) and Mann–Whitney U tests to screen for bacteria with significant differences in bacterial counts were performed. Subject-based bacterial counts were calculated based on the number of sites with bacteria present at week 26. General linear model multivariate ANOVA using Sidak's correction for multiple observations and with smoking, age, and gender as covariates was used to assess differences by bacterial presence at the subject level. The PASW, statistics 18.0 software for MAC OS X version 10.6.7 was used for the analysis of the data (IBM SPSS).

Results

A total of four subjects were lost to follow-up before the first visit after the intervention. Due to the fact that they never returned for the bacterial samplings or clinical follow-up, no data after enrollment could be collected from these four subjects in the control group. The data from these subjects were excluded from the analysis. Data were studied in 18 subjects (4/18, 22.2 % smokers) from the test group including 72 test sites and in 14 (2/14, 14.3 % smokers) subjects from the control group including 56 test sites. The mean age of the study population was 52.1 years (SD±10.3) in both groups and thus with no statistically significant difference by study groups. During the study period, no adverse events were identified.

Clinical data: probing pocket depth and bleeding on probing at sites from which bacterial samples were taken

The study included 41.0 % incisors and cuspids, 20.1 % premolars, and 38.8 % molars. Baseline overall PPD values at sites from which bacterial samples were collected varied between 5 and 11 mm (overall mean 6.8 mm, SD±1.3 mm). At baseline, the mean PPD in the control and test groups were 7.0 mm (SD±1.2) and 6.8 mm (SD±1.3), respectively (NS). At week 26, the corresponding PPD values were 5.2 mm (SD±1.5) and 5.1 (SD±1.4), respectively (NS). At baseline, all PPD values were >5 mm. At week 26, 42.9 % of the sites in the control group had a PPD value >5 mm, whereas 35.5 % of the sites in the test group had a PPD value >5 mm. The mean difference (decrease) in PPD between baseline and week 26 in the control group was 1.8 mm (SE±0.3; 95 % CI 1.2, 2.3; p<0.001). The mean difference (decrease) in PPD between baseline and week 26 in the test group was 1.5 mm (SE±0.2; 95 % CI 1.1, 2.0; p<0.001). Both at baseline and at week 26, general linear model univariate analysis with smoking status and sample site as covariates, statistical analysis failed to demonstrate study group differences in PPD values both at baseline and at week 26.

At baseline and at sites from which bacterial samples were taken, BOP was present at 85.1 % in the control group and at 82.1 % in the test group (NS). At week 26, BOP was found at 44.9 % in the control group and at 38.2 % sites in the test group. Statistical analysis failed to demonstrate group differences in BOP scores at either time point.

Considering smoking status, statistical analysis failed to demonstrate differences in PPD values. At baseline, the mean difference in PPD by smoking status was 0.3 mm (SE±0.3; 95 % CI −0.2, 0.8; p=0.24). The mean PPDs in smokers and nonsmokers at baseline were 6.5 mm (SD±1.3) and 6.8 mm (SD±1.3) (p=0.24). The mean PPDs in smokers and nonsmokers at week 26 were 4.9 mm (SD±1.2) and 5.2 mm (SD±1.5), respectively (p=0.38).

Microbiology

Analysis by Kolmogorov–Smirnov test failed to identify a normal distribution pattern by bacterial counts. Based on the screening of the 74 bacterial species included in the checkerboard DNA-DNA hybridization method, the 15 species with the highest prevalence rates at baseline and 2, 4, 12, and 26 weeks after treatment in the test and control groups were identified. The mean values of each of these species by study group are presented in descending order (Figs. 2a, b; 3a, b; 4a, b; 5a, b; and 6a, b). It is noteworthy that at baseline, the mean values of these bacteria were similar by study group. With few exceptions, the same bacteria were identified including *Aggregatibacter actinomycetemcomitans* (Y4), *Fusobacterium nucleatum* spp. *naviforme*, *Fusobacterium nucleatum* spp. *nucleatum*, *Parvimonas micra*, *P. gingivalis*, *P. aeruginosa*, and *T. forsythia.* The total count of a composite pathogen group including these species and also including *T. denticola* and *Staphylococcus aureus* was computed. The load of this composite bacterial group was further assessed (see below).

At 2, 4, and 12 weeks, it is obvious that shifts in the prevalence ranking order of species had occurred in both study groups. Consistently, *A. actinomycetemcomitans* (Y4) was present at much higher levels in the control group. Other species were also present at higher counts in the control group. At 26 weeks, most differences between the two groups had disappeared. The prevalence rates (percent) at the bacterial level $\geq 1.0\times10^5$ cells for each of the bacteria included in the composite pathogen group are shown in Table 2. In the control group and at 4 weeks, a relevant decrease in the prevalence rate based on the $\geq 1.0\times10^5$ cells detection level was not found for any of the species. At week 4 in the test group, *P. gingivalis*, *P. aeruginosa*, and *T. forsythia* decreased ≥20 % in detection prevalence rates. At 26 weeks, the decrease remained in the test group for *P. aeruginosa* and *T. forsythia.*

At baseline, smokers had a higher bacterial count of *T. forsythia* than nonsmokers (p=0.008). This difference did

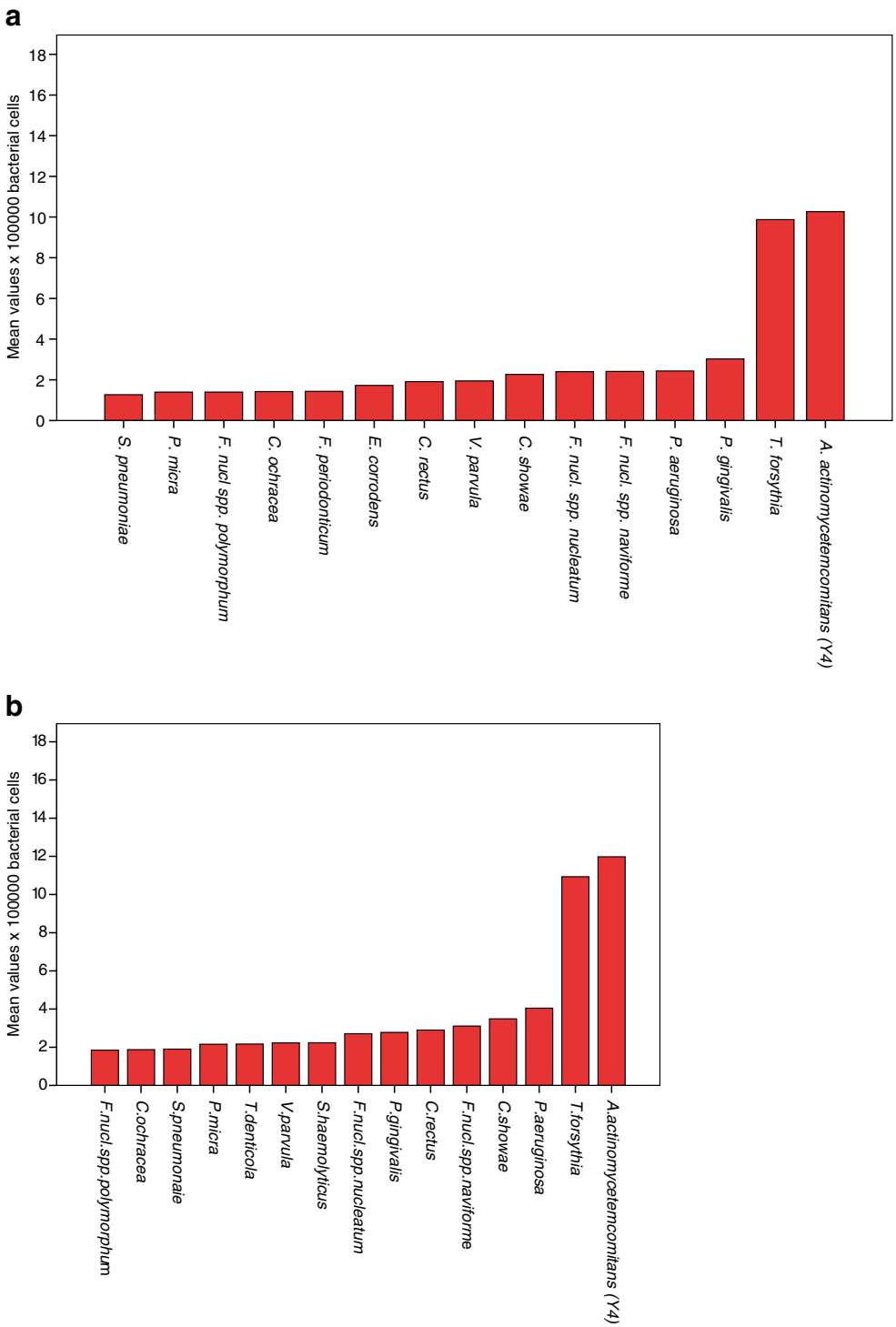

Fig. 2 **a** Baseline mean bacterial counts in descending order for the 15 most prevalent species in the control group. **b** Baseline mean bacterial counts in descending order for the 15 most prevalent species in the test group

not exist at week 26. Analysis by general linear model multivariate analysis with smoking status, and sample site as covariates, with the above listed species, and the composite bacterial counts as dependent variables and adjustment for multiple comparisons by Sidak correction for multiple observation found significantly higher bacterial counts in the control group at baseline for the following species: *P. micra*, *S. aureus* ($p<0.01$), and with the largest difference for *T. denticola* (mean difference -1.3×10^5 cells; 95 % CI -2.1, -0.4×10^5; $p=0.005$). The corresponding results from the analysis at weeks 2, 4, 12, and 24 are presented in Table 3. Thus, specifically at weeks 4 and 12, significantly lower counts of the following bacteria were found in the test group: *A. actinomycetemcomitans* (Y4), *F. nucleatum* species, *P. micra*, *P.*

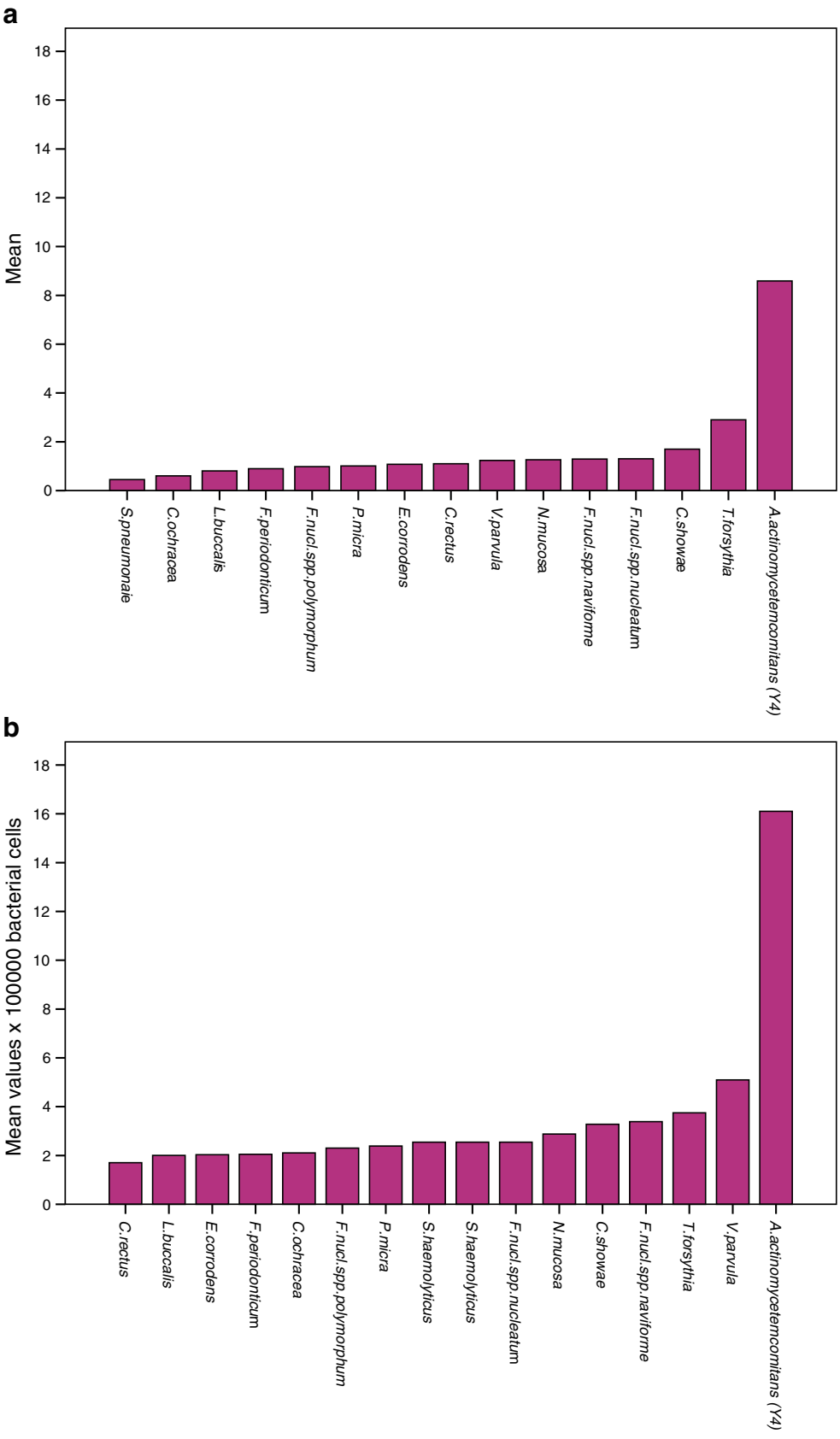

Fig. 3 **a** Week 2 mean bacterial counts in descending order for the 15 most prevalent species in the control group. **b** Week 2 mean bacterial counts in descending order for the 15 most prevalent species in the test group

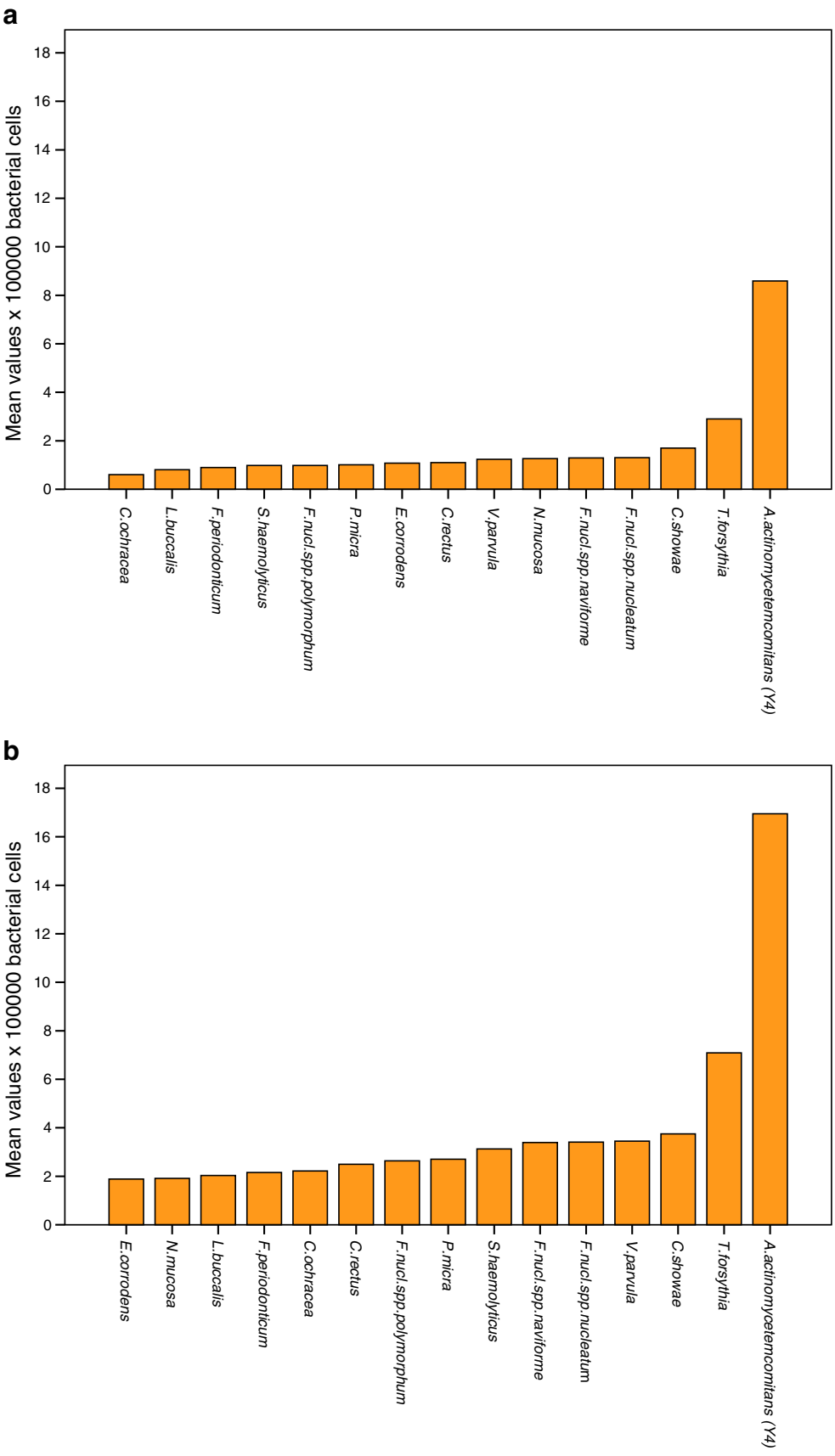

Fig. 4 **a** Week 4 mean bacterial counts in descending order for the 15 most prevalent species in the control group. **b** Week 4 mean bacterial counts in descending order for the 15 most prevalent species in the test group

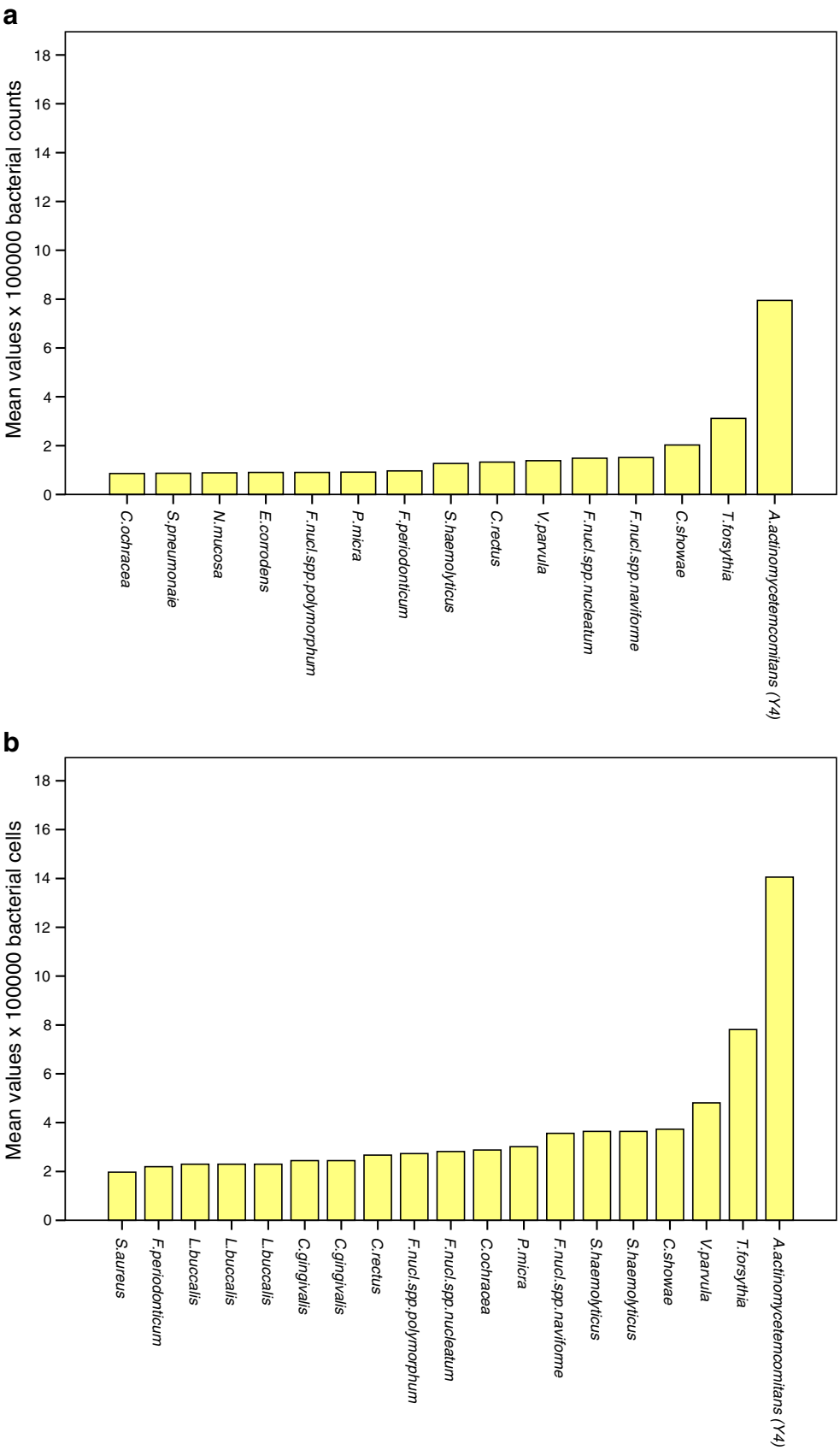

Fig. 5 **a** Week 12 mean bacterial counts in descending order for the 15 most prevalent species in the control group. **b** Week 12 mean bacterial counts in descending order for the 15 most prevalent species in the test group

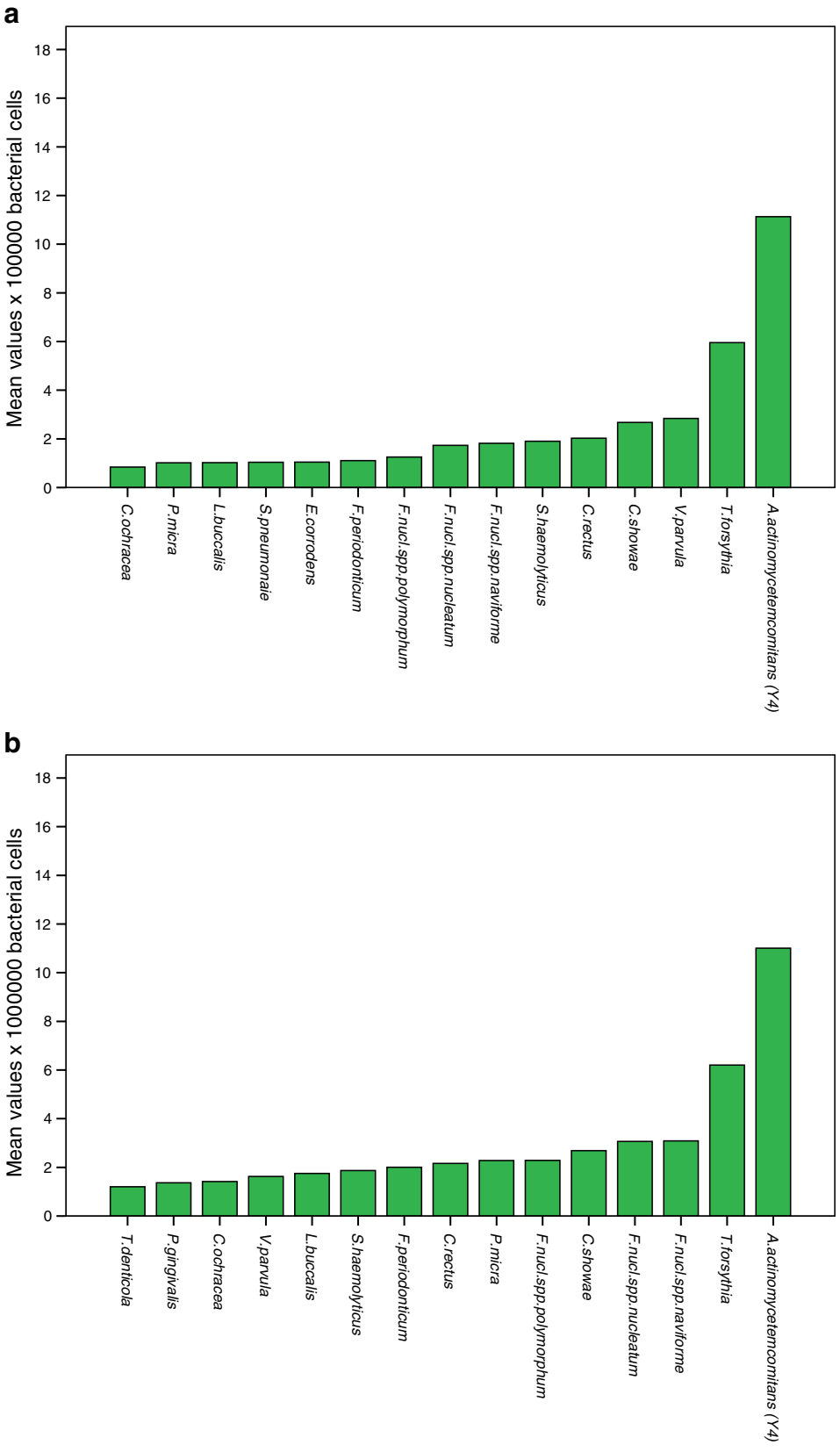

Fig. 6 **a** Week 26 mean bacterial counts in descending order for the 15 most prevalent species in the control group. **b** Week 26 mean bacterial counts in descending order for the 15 most prevalent species in the test group

gingivalis, *P. aeruginosa*, *S. aureus*, and *T. denticola* were found in the test group. At week 26, only *F. nucleatum* spp. *polymorphum*, *F. periodonticum*, *P. micra*, *P. aeruginosa*, and *T. denticola* were found at lower counts in the test group.

Table 2 Distributions of selected bacteria at different sample times in the test and control groups by the detection level for a positive test result ($\geq \times 1.0^5$ bacterial cells) that is the standard detection level for the checkerboard DNA-DNA hybridization method

Composite species	Baseline		2 weeks		4 weeks		12 weeks		24 weeks	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
A. actinomycetemcomitans	88.0	80.4	65.2	78.3	71.4	83.0	71.1	76.0	71.6	73.0
F. nucleatum naviforme	54.3	70.4	44.9	60.9	38.1	55.3	47.0	68.0	49.4	59.5
P. micra	51.1	66.7	28.1	63.0	34.5	68.1	61.0	64.5	39.5	63.2
P. gingivalis	38.0	58.5	13.5	21.7	16.7	29.8	16.9	30.0	24.7	29.7
P. aeruginosa	43.7	61.5	24.5	61.8	13.4	74.3	26.2	61.5	17.8	50.0
S. aureus	28.3	51.0	14.6	52.2	15.1	53.2	27.7	52.0	23.5	35.1
T. forsythia	68.5	76.5	42.7	67.4	41.7	61.7	41.0	60.0	48.1	57.8
T. denticola	47.8	68.6	30.3	58.7	32.1	66.0	37.3	50.0	37.0	73.0

A. actinomycetemcomitans is represented by the Y4 reference strain

Subject-based microbiological analysis

The five microbiological species that were found to differ by the site-specific analysis were further investigated. At baseline and at week 26, the number of sites that were defined as being positive for these five species identified as being present at a bacterial level $\geq 1.0 \times 10^5$ cells (primary detection level for the checkerboard method) were provided a subject-based number. Analysis by general linear model multivariate ANOVA including smoking habit, subject age, and gender as covariates, and using Sidak's correction for multiple observations, the analysis failed to identify baseline differences in a number of positive sites for the five species studied. At week 26, however, the same analysis identified that significantly fewer positive sites were found in subjects who had been treated with the antibiotics for the following species: *T. denticola* ($p<0.001$), *F. nucleatum* spp. *polymorphum* ($p=0.001$), *P. micra* ($p=0.006$), and *F. periodonticum* ($p=0.013$).

Discussion

There are currently no other clinical data on the efficacy to reduce bacterial counts in periodontal pockets by a single administration of piperacillin/tazobactam in subjects with moderate to advanced periodontitis. *P. gingivalis* was chosen as the target pathogen and as the primary outcome measure because it has been studied extensively in association with periodontitis [8, 23–25, 30, 35, 37]. Several other bacterial species demonstrated a greater susceptibility to the intervention in the test group and this effect remained also at week 26 for some species but not for *P. gingivalis*, *T. forsythia*, *or A. actinomycetemcomitans*.

The limitation of the present study is that the control subjects were not treated with a placebo drug administration. Nevertheless, the clinical examiner and the laboratory staff members were blinded to the protocol assignment to control for bias. Another limitation is that the evidence of bacterial changes following periodontal interventions from other studies does not easily provide information that can be utilized for statistical power analysis. Thus, we assumed based on our laboratory experiences that a 20–25 % difference could be anticipated. A decrease amounting to approximately 20–25 % was obtained in the test group at weeks 2 and 4 for *P. gingivalis* and *P. aeruginosa*. At week 26, this remained the case for *P. aeruginosa*, suggesting that the administration of piperacillin/tazobactam has a relevant effect but limited to the control of *P. aeruginosa* subgingival colonization. One of the reasons why the reduction in bacterial counts was limited may be the result of less than optimal control of gingival inflammation as noticed by the rather high proportion of BOP at study endpoint.

The decreases in PPD and BOP obtained in the present study are consistent with the other studies on subgingival debridement not using antibiotics [1–7]. The extent of PPD reduction and decrease in BOP in the present study suggested a clinical effective outcome of therapy provided in both groups. Furthermore, the extent of PPD reduction in both study groups in the present study was comparable, or greater to PPD reductions after combined local debridement and local antibiotics in other studies [30, 50]. In the present study, we treated subjects who were diagnosed with moderate to severe chronic periodontitis, and we only assessed interproximal conditions. In other similar studies, periodontal sites with more shallow PPDs have been studied [18, 21, 38]. It is well known that the reduction of PPD in the range of 1.5 to 2.0 mm can be obtained by debridement alone in deep periodontal pockets [1–3]. The extent of possible probing depth reduction may also be limited by anatomical factors such as the extent and topography of alveolar bone loss and attachment loss.

Some data have shown that local administration of doxycycline or minocycline in addition to debridement in subjects who smoke results in greater reduction in the frequency of *P.*

Table 3 Microbiological differences by study groups after intervention at weeks 2, 4, 12, and 26

Time point	Species	Mean differences × 10^5 cells	95 % CI × 10^5 cells	*p* values
Week 2	*A. actinomycetemcomitans* Y4	8.8	1.6, 16.0	0.001
	F. nucleatum naviforme	1.3	0.5, 2.1	0.002
	F. nucleatum polymorphum	1.3	0.5, 2.1	0.002
	F. periodonticum	0.9	0.1, 1.7	0.02
	S. aureus	1.0	0.6, 1.4	0.001
	T. denticola	0.7	0.4, 1.1	0.001
	Composite bacteria	12.3	0.4, 24.1	0.042
Week 4	*A. actinomycetemcomitans* Y4	7.9	2.5, 13.3	0.005
	F. nucleatum naviforme	2.2	1.0, 3.4	0.000
	F. nucleatum polymorphum	1.7	1.0, 2.5	0.000
	F. periodonticum	1.3	0.7, 1.9	0.000
	P. micra	1.8	1.0, 2.5	0.000
	P. gingivalis	1.1	0.3, 1.9	0.006
	P. aeruginosa	1.5	1.0, 2.1	0.000
	S. aureus	1.0	0.7, 1.2	0.000
	T. forsythia	4.5	0.8, 8.2	0.017
	T. denticola	1.3	0.7, 1.9	0.000
	Composite bacteria	21.3	12.1, 30.5	0.000
Week 12	*F. nucleatum naviforme*	2.2	1.0, 3.4	0.001
	F. nucleatum nucleatum	1.4	0.6, 2.3	0.001
	F. nucleatum polymorphum	1.9	1.3, 2.5	0.000
	F. periodonticum	1.3	0.8, 1.8	0.000
	P. micra	2.1	1.2, 3.0	0.000
	P. gingivalis	0.4	0.1, 1.8	0.032
	P. aeruginosa	1.1	0.5, 1.6	0.000
	S. aureus	1.4	0.8, 1.9	0.000
	T. forsythia	5.6	1.5, 9.8	0.009
	T. denticola	1.2	0.6, 1.8	0.000
	Composite bacteria	18.3	9.8, 26.8	0.000
Week 26	*F. nucleatum polymorphum*	1.1	0.1, 1.9	0.029
	F. periodonticum	0.8	0.1, 1.5	0.019
	P. micra	1.2	0.3, 2.0	0.008
	P. aeruginosa	0.5	0.1, 1.0	0.046
	T. denticola	0.6	0.3, 1.0	0.002

gingivalis [22, 25]. In the present study, smoking did not seem to have an impact on the study outcomes neither on PPD nor BOP changes or microbiological changes. This may be explained by the low prevalence of smokers in the study. Smoking, subject age, and gender were included as covariates in the subject-based analysis but did not significantly influence the results.

Although subject-based factors must be considered, it is generally perceived that chronic periodontitis is tooth/site-specific [13–17]. In the present study, each subject contributed four individual test sites representing the sites with the most advanced periodontitis. Thus, no subject was overrepresented providing more data than any other subject. Several studies have used site-based analysis and performed microbiological sampling only from mesio-buccal surfaces [8, 22, 24, 50–56]. There appears to be a defined order in bacterial species succession in early supragingival and subgingival biofilm redevelopment after professional cleaning. The site-specific development of periodontitis may be the result of the symbiotic effects due to co-aggregation in subgingival biofilms including *P. gingivalis*, *T. denticola*, and *T. forsythia* [53].

Thus, the presence and counts of *P. gingivalis*, *T. denticola*, and *T. forsythia* may suggest the stability of periodontal conditions at individual sites at teeth. The observations that local treatment with antibiotics can reduce the counts of these

species are important [31, 50]. In the present study, the adjunct administration of piperacillin/tazobactam resulted in more reduction of not only *P. gingivalis*, *T. denticola*, and *T. forsythia* but also other species associated with co-aggregation in biofilms (i.e., *F. nucleatum*) and other bacteria that are associated with several diseases (*P. aeruginosa* and *S. aureus*) and identified not only in periodontitis but also in subjects with peri-implantitis [48, 57–60].

It should also be noticed that the changes in bacterial counts over time were not consistently the same by different species. This may reflect the fluctuating state of bacterial growth and changes in the development of biofilms at different sites from which samples were taken. To some extent, it may also reflect measurement errors in sampling which might be the greatest error and by the laboratory procedures. The fact that the bacterial counts of *P. aeruginosa* and *S. aureus* at study endpoint did not differ by study group could be viewed as a positive finding in that these two species did not show evidence in counts that might suggest antibiotic resistance or other advantages by the medication.

The present study identified that without the use of the antibiotic, limited changes were found after debridement among the target bacteria. Recolonization of bacteria also occurred in the test group, and this is consistent with other studies [35]. Recolonization of bacteria following periodontal surgery in newly established shallow periodontal pockets also occurs soon after surgery [60]. This is consistent with the general concept that mechanical elimination of bacteria in a biofilm is not possible. Oral bacteria in biofilm comprise a complex community depending on the interface between the host and the microbial community as a whole [61]. Elimination of bacteria associated with periodontitis may therefore not be possible using local administration of antibiotics [32]. In addition to plasmid transfer and antibiotic resistance, there is a mechanical protective glycolax layer that protects the biofilm and prevents penetration of antibiotics, and debridement may not effectively eliminate this glycolax in deep periodontal pockets.

In the present study, high counts of *P. aeruginosa* were found in the post-treatment findings in subjects in the control group. While piperacillin/tazobactam appears to be effective against *P. aeruginosa* [43, 44], this may explain why lower counts of *P. aeruginosa* were found in the test groups. In the present study, high counts of *A. actinomycetemcomitans* were found both at baseline and especially throughout the study in the control group, suggesting that subgingival debridement alone cannot significantly reduce or eliminate this microorganism. This observation is consistent with other studies suggesting that *A. actinomycetemcomitans* is difficult to manage through mechanical debridement alone [32, 62, 63]. The reduction of *A. actinomycetemcomitans* was, however, also limited in the test group. Although bacteria commonly viewed as putative pathogens in periodontitis, i.e., *T. forsythia* and *P. gingivalis*, were similarly affected by study procedures, the pathogenic capacities of *P. micra*, *Fusobacterium* species, and *T. denticola* should not be minimized. The lower prevalence of these species in the test group should be considered as having a beneficial impact on periodontal status. There are many studies to suggest that *P. micra*, *Fusobacterium* species, and *T. denticola* are present at high counts in cases with periodontitis (i.e., [8–10]).

In conclusion, the present study identified similar improvements in clinical periodontal outcomes at week 26 in subjects treated with nonsurgical debridement with or without a one-time administration of a local antibiotic (piperacillin/tazobactam). At the subject level, the local antibiotic therapy controlled the colonization of *T. denticola*, *F. nucleatum polymorphum*, *F. periodonticum*, and *P. micra*.

Acknowledgments We appreciate and thank Medirel AS Agno Switzerland who funded the study. We would also like to thank Ms. Marianne Weibel and Ms. Regula Hirschi-Imfeld for the laboratory work and support of this study. We appreciate the work by the dental staff at the University of Bern, Switzerland.

Conflict of interest None of the authors have a conflict of interest. All authors met the authorship requirements listed by the ICJME guidelines. Medirel AS Agno, Switzerland sponsored the study. The sponsor also provided antibiotics in prepared commercial packages with instructions.

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