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HUMAN RANDOMIZED CONTROLLED TRIAL

Effect of adjunctive systemic antibiotics on microbial populations compared with scaling and root planing alone for the treatment of periodontitis: A pilot randomized clinical trial

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Abstract

Background: To investigate the microbial shift after periodontitis being treated by scaling and root planing (SRP) with or without adjunctive antibiotics, and to assess the relationship between oral microbiota and systemic factors.

Methods: A 6-month pilot randomized controlled trial recruited 14 subjects with severe periodontitis, divided into test group and control group to receive full-mouth SRP with or without amoxicillin (500 mg) and metronidazole (200 mg) (three times a day for 7 days). Clinical examination, collection of subgingival plaque and saliva, and blood tests were performed at baseline pre-treatment, 3 months, and 6 months post-treatment. The V3V4 region of 16S DNA was sequenced; taxonomic assignment was based on the Human Oral Microbiome Database.

Results: The periodontal condition significantly improved in both groups; the test group showed a greater improvement in plaque index, probing depth, and bleeding index than the control group. The test group demonstrated significantly lower microbial richness and diversity, and less abundant *Porphyromonas* than the control group at 3 months for both subgingival microbiome and salivary microbiome. However, the microbial differences narrowed within 6 months. The subgingival and salivary microbiota shifted synergistically. Glucose was positively related to subgingival *Porphyromonas*; mean platelet volume was positively related to subgingival *Leptotrichia*.

Conclusions: Systemic administration of amoxicillin and metronidazole along with SRP had an advantage over SRP alone in clinical improvement and infection

control in both the subgingival region and saliva 3 months post-treatment. Microbial advantage nearly disappeared at 6 months; however, the clinical advantage lasted longer. The use of antibiotics also has potential benefits for systemic inflammation and glucose.

KEYWORDS
amoxicillin, dental scaling, metronidazole, microbiota, periodontitis, root planing, saliva

1 | INTRODUCTION

The oral cavity is a complex ecosystem with >700 species distributed in several habitats, including gingival sulcus and saliva.[1] The oral microbiome is a critical etiological factor of oral infectious diseases, such as periodontitis,[2] which is characterized by deep pockets, bleeding, and bone loss. With a prevalence of 52.8% to 64.6% in adults,[3] it is the dominant cause of adult tooth loss.[4,5] It has been established that periodontitis is a result of microbiological dysbiosis.[6] Therefore, biofilm-targeted treatment is an essential approach for the treatment of periodontitis.

Scaling and root planing (SRP) is the gold standard for periodontal treatment to remove calculus and biofilm.[7] However, deep pockets and furcations may limit the treatment effect of mechanical debridement for advanced periodontitis. Moreover, it cannot eliminate the bacteria that infiltrate periodontal tissue or other parts outside the periodontal pockets. Using antibiotics systemically to treat generalized severe periodontitis could help reduce those organisms that "protect" from mechanical disruption by subgingival debridement. Several studies have shown that adjunctive systemic antibiotics, combined with mechanical debridement, achieves additional clinical improvements compared with those obtained with SRP alone.[8–10] The combined use of amoxicillin (AMX) and metronidazole (MTZ) is regarded as the most promising regimen to treat periodontitis.[9–13] SRP with adjunctive AMZ + MTZ shows significant clinical benefits over SRP alone, even 1- or 2-years post-treatment.[10,14–16] Studies showed some degree of success in clinical effect with adjunctive use of antibiotics[14–16]; however, the microbial effect is not remarkable and somewhat controversial.[17,18] Some studies indicated that systemic adjunctive use of AMX + MTZ has a better microbial effect in reducing major periodontal pathogenic microbiota than SRP alone.[18,19] While another study concluded that systemic AMX + MTZ and placebos were comparable in lowering periodontal pathogens.[17]

Bacteria, colonizing, and proliferating in the subgingival region, are continuously released into saliva from the periodontal pockets. In turn, saliva, immersing all teeth, is an important source of subgingival recolonization of periodontal pathogens.[20] Close communication makes the salivary microbial community partly responsible for the rebound of pathogens.[21] Therefore, salivary infections should be controlled during periodontal treatment. Systemic administration of antibiotics has excellent advantages in inhibiting pathogens in the overall oral cavity[22] and reducing systemic inflammation induced by organism infection.[23] However, no studies have yet reported a shift in the salivary microbiome after periodontal treatment with antibiotics. Furthermore, the association between the salivary and subgingival microbiomes during periodontal treatment with antibiotics remains unclear.

Previous studies have established that severe periodontal infection is associated with systemic inflammation and metabolism.[24] Periodontal treatment can reduce or eliminate local infection,[23] which may further alleviate systemic inflammation[25,26] and alter metabolism.[27] In addition, systemic administration of antibiotics potentially benefits systemic inflammation and metabolism by regulating the microbiome overall the body.[28] In peripheral blood, glucose, white blood cells (WBC), platelets, alkaline phosphatase (ALP), cholesterol (CHO), triglycerides (TG), lipoproteins are indicators of systemic inflammation, and metabolism.[24] Investigating the relationship between microbiota and systemic parameters could help evaluate the benefits of periodontal treatment.

Therefore, this 6-month pilot randomized controlled trial aimed to investigate the microbial shift in subgingival biofilm and saliva after periodontitis treatment with or without AMX and MTZ as adjuncts to SRP, and to assess the relationship between oral microbiota and systemic factors.

2 | MATERIALS AND METHODS

This randomized controlled trial was conducted in compliance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines[29] and Helsinki Declaration of 1975, as revised in 2013.[30] This clinical study was registered at the Chinese Clinical Trial Registry (approval number: ChiCTR-TRC-1900027377) and approved by the

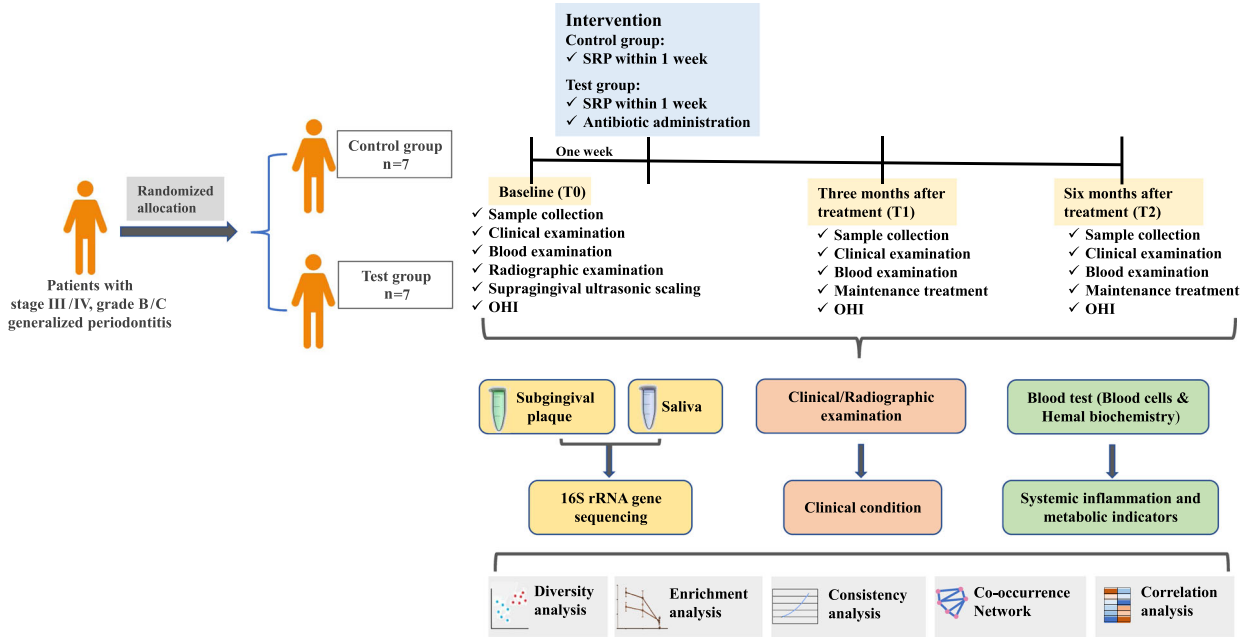

FIGURE 1 Flowchart. SRP, scaling and root planing. OHI, oral hygiene instruction

Peking University Institutional Review Board (approval no. PKUSSIRB-201627026).

2.1 | Experimental design

This 6-month, examiner-masked, pilot randomized controlled trial consisted of two parallel groups: 1) test group (SRP with systemic administration of AMX + MTZ); 2) control group (SRP alone). Clinical information, subgingival plaque samples, saliva samples, and blood samples were collected and analyzed at three time points: baseline pre-treatment (T0), 3 months post-treatment (T1), and 6 months post-treatment (T2). Figure 1 outlines the procedure of the randomized controlled trial.

2.2 | Outcome variables and sample size calculation

The primary outcome of this study was the differences in the genus *Porphyromonas* at T1 between the test group and the control group, and the secondary outcome was the differences in probing depth (PD) at T1 between the test group and the control group. The post-hoc method was used to calculate the power using PASS* with the primary outcome.

* Version 11, NCSS Statistical Software, Kaysville, UT

2.3 | Patient recruitment

Inclusion criteria:

1) At least six non-adjacent sites of six teeth with PD ≥5 mm;
2) More than 30% of sites with radiographic bone loss > 1/2 of the root;
3) Aged 40 to 65 years;
4) Residual teeth >15 (excluding hopeless teeth).

Exclusion criteria:

1) Systemic diseases or other infectious diseases (e.g., hepatitis, tuberculosis);
2) Pregnancy or lactation;
3) Allergic to amoxicillin or metronidazole;
4) Previous periodontal treatment;
5) Antibiotic therapy within 3 months;
6) Smoking, alcohol or taking any kind of drug.

Fourteen patients were recruited in this pilot study, diagnosed with generalized chronic periodontitis (1999 Classification[31]) and generalized stage III/IV, grade B/C periodontitis (2017 Classification[32]). All patients recruited signed an informed consent form before inclusion.

2.4 | Randomization and allocation concealment

The randomization sequence and allocation concealment were performed using computer-generated ratio-blocked (1:1) random numbers. Clinical examination and treatment

procedure were performed by two experienced and calibrated periodontists. The examiner was masked to the group information before completing data collection and statistical analysis.

2.5 | Treatment procedure and clinical monitoring

At the baseline, supragingival ultrasonic scaling was performed after sample collection and periodontal examination. One week later, two half-mouth SRPs were conducted within a week using a subgingival ultrasonic scaler and Gracey curets under local anesthesia. AMX 500 mg and MTZ 200 mg, three times a day, 7 days were prescribed as recommended[33] immediately after SRP in the test group. Medication compliance post-treatment was assessed by an assistant. Potential discomfort or adverse events were also recorded. Maintenance therapy by ultrasonic scaling and oral hygiene instruction was conducted at every appointment (T1, T2).

2.6 | Calibration exercise and clinical examination

Six non-study patients with periodontitis were recruited for calibration exercise. The single designated examiner recorded full-mouth PD with an interval of 24 hours between the first and second recordings. The kappa value of the self-consistency test was 0.98.

Periodontal clinical examination was performed at T0, T1, and T2 by an experienced periodontist. Clinical parameters, including full-mouth plaque index (PI, buccal, and lingual aspects),[34] PD (mesial, distal, and middle sites of buccal and lingual surfaces), bleeding index (BI, buccal and lingual aspects),[35] and attachment loss (AL, mesial, distal, and middle sites of buccal and lingual surfaces) were recorded.

2.7 | Blood examination and sample collection

At each follow-up time point, fasting venous blood was collected with anticoagulant tubes and coagulation-promoting tubes. The following indicators were detected using an automatic biochemical analyzer[†]: glucose, WBC, platelet (PLT), platelet-larger cell ratio (P-LCR), mean platelet volume (MPV), neutrophil percentage (NEUT%), monocyte percentage (MONO%), lymphocyte percentage (LYM%), ALP, total CHO, TG, high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Thereafter, unstimulated whole saliva was collected for 10 minutes by the natural outflow at 08:00 a.m.–09:00 a.m. before meals and 2 hours after brushing. Saliva samples were centrifuged for 15 minutes at 13,000 rpm (centrifugal radius: 5.5 cm), 4°C to obtain the precipitate.

Subgingival plaque samples were pooled from six non-adjacent bucco-mesial sites of Ramfjord index teeth (16, 21, 24, 36, 41, 44)[36] using sterilized curets. If the index teeth were lost or did not meet the criteria (PD ≥5 mm, bone loss >50%), other teeth in the same region were sampled.

2.8 | DNA extraction and sequencing

Before DNA extraction, both saliva precipitates and subgingival plaque were washed three times with phosphate buffer solution (PBS, pH = 7.4, 200 mL). Bacterial genomic DNA in samples was extracted following the manufacturer's guidelines using a TIANamp Micro DNA Kit.[‡] Its quantity and quality were measured using NanoDrop 2000.[§] The V3V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR to build the library. Sequencing was performed on the Illumina MiSep PE300 platform.[**] Image analysis, base calling, and error estimation were performed using Illumina Analysis Pipeline Version 2.6.3.

2.9 | Data analysis and statistical analysis

Sequences were removed if they were <200 bp, had an inferior quality score (≤20), contained ambiguous bases, or did not exactly match the primer sequences and barcode tags. Thereafter, qualified reads were separated using sample-specific barcode sequences and trimmed using Illumina Analysis Pipeline Version 2.6. The data set was analyzed using QIIME2. Actual sequence variants (ASVs) were identified using the DADA2 pipeline.[37] All sequences were classified into different taxonomic groups based on the Human Oral Microbiome Database.[1]

Clinical parameters and microbiota between test group and control group were compared using Student *t*-test or Mann-Whitney *U* test. The comparison before and after periodontal treatment was conducted using paired *t*-test or Wilcoxon signed-rank test. Principal coordinates analysis (PCoA) was performed to examine the similarity of microbial composition between different

[†] Olympus AU 5400, Brentwood, NH

[‡] TIANGEN BIOTECH, Beijing, China
[§] Thermo Scientific, Waltham, MA
[**] Realbio Technology, Shanghai, China

samples. The correlation of microbiota was analyzed by Spearman correlation coefficients; the co-occurrence network was visualized with Cytoscape 3.8. The similarity of the microbial shift in the subgingival region and saliva was presented with ratios as described previously.[38] The correlation of microbiota, periodontal parameters, and systemic indicators was tested using Spearman correlation coefficients and presented as heatmaps. Statistical analyses and visualization were performed using R 3.3.2.

3 | RESULTS

Demographic characteristics and clinical parameters are presented in Table 1. A total of 84 samples (42 subgingival plaque and 42 saliva samples) from 14 patients were analyzed (Fig. 1). All patients complied with the treatment processes; none were lost to follow-up. No adverse events occurred. The sample size power, at 0.96, was appropriate for this study. A mean value of 28,985 clean tags (21,942 to 34,412) was generated. Finally, we observed an average of 1,496 ASVs (average length 419 bp), consisting of 11 phyla, 24 classes, 38 orders, 60 families, 111 genera, and 334 species.

3.1 | SRP with systemic antibiotics had a superior clinical effect than SRP alone

The demographic characteristics and clinical parameters of the patients recruited in the test and control group were comparable at baseline. Full-mouth PD, PD ≥5 mm (%), PI ≥2 (%), BI ≥2 (%), and AL significantly decreased after treatment in both the test and control group (P <0.05); some parameters further decreased from month 3 to month 6. PD significantly decreased from 4.33 mm (baseline) to 3.43 mm (month 3) and 3.26 mm (month 6) in the control group, whereas it significantly decreased from 4.47 to 3.13 mm (month 3) and 3.14 mm (month 6) in the test group. PD at month 3 was significantly lower in the test group than in the control group (P <0.05). The percentage of PD ≥5 mm was also lower in the test group than in the control group although without significant difference. BI ≥2 (%) at month 3 and 6 was significantly lower in the test group than that in the control group (P <0.05). The reduction of BI ≥2 (%) in the test group was also greater than that in the control group (P <0.05). AL also experienced a slightly greater reduction in the test group than in the control group. For the sampled sites, PI, PD, BI, and AL also significantly decreased after treatment (P <0.05). The test group showed a greater reduction in PI than the control at month 3 and 6 (Table 1).

3.2 | SRP with systemic antibiotics had a superior microbial effect than SRP alone

3.2.1 | Shift of subgingival microbiome after SRP with or without antibiotics

Microbial richness presented by Chao1 in the test group significantly decreased from baseline to month 3 (P <0.05), then slightly increased until month 6. In the control group, microbial richness significantly decreased from 3 months to 6 months post-treatment. Microbial diversity presented by Shannon in the test group slightly decreased from baseline to month 3, then significantly increased from month 3 to month 6 (P <0.05). However, in the control group, microbial diversity increased from baseline to month 3, then decreased at month 6. Both microbial richness and microbial diversity in the test group were significantly lower than those in the control group at 3 months (P <0.05, Fig. 2A). PCoA showed multivariate microbiome dissimilarities among the three time points in the two groups (P <0.05). The samples before treatment (baseline) clustered from samples post-treatment, whereas samples at month 3 and 6 had some overlap (Fig. 2B). The composition also changed significantly during the observation period. Core genera (relative abundance > 1%) with significant differences are presented in Figure 2C. SRP with or without antibiotics showed significant reductions in some genera, including *Porphyromonas*, *Treponema*, *Filifactor*, *TM7 G-5*, *Peptostreptococcaceae XI G-6*, *Fretibacterium*, *Dialister*, and *Peptococcus*. The genus *Tannerella* only decreased in the test group. Nevertheless, the relative abundances of some genera, such as *Actinomyces*, *Rothia*, *Neisseria*, *Capnocytophaga*, *Lautropia*, and *Cardiobacterium*, were elevated after treatment in both groups (P <0.05).

3.2.2 | Shift of salivary microbiome after SRP with or without antibiotics

In saliva, microbial richness presented by Chao1 significantly decreased from baseline to month 3 in the test group and was significantly lower than that in the control group (P <0.05). Microbial diversity at month 3 presented by Shannon in the test group was also significantly lower than the control group (P <0.05, Fig. 3A). The PCoA plot also showed an overlap of samples among the three time points in the control group. However, the test group showed a significant transition before and after treatment (P <0.05, Fig. 3B). Core genera (relative abundance > 1%) with significant differences are presented in Figure 3C. In the control group, the microbial composition showed lit-

TABLE 1 Demographic characteristics and clinical parameters before and after treatment

	Control group (n = 7)			Test group (n = 7)		
	Baseline (T0)	**Month 3 (T1)**	**Month 6 (T2)**	**Baseline (T0)**	**Month 3 (T1)**	**Month 6 (T2)**
Age (years)	43.57 ± 6.63			42.57 ± 3.29		
Sex (Male%)	42.86%			57.14%		
BMI (kg/m^2)	23.57 ± 1.50			22.74 ± 0.55		
Tooth loss	2.29 ± 1.14			2.49 ± 1.12		
Full mouth						
PI ≥2 (%)	95.90 ± 8.63	63.64 ± 32.46*	48.85 ± 33.03*	100 ± 0	49.94 ± 34.90*	29.29 ± 31.70*
Reduction		32.26 ± 28.74	47.05 ± 30.36		50.06 ± 34.90	70.71 ± 31.70
PD (mm)	4.33 ± 0.40	3.43 ± 0.29*#	3.26 ± 0.35*	4.47 ± 0.50	3.13 ± 0.16*#	3.14 ± 0.20*
Reduction		0.88 ± 0.36	1.07 ± 0.34		1.34 ± 0.37	1.33 ± 0.38
PD ≥5 mm (%)	40.28 ± 12.85	13.73 ± 7.45*	10.48 ± 10.83*	41.93 ± 11.38	5.96 ± 3.23*	6.45 ± 4.31*
Reduction		26.55 ± 11.72	29.60 ± 9.07		35.97 ± 10.28	35.49 ± 10.16
BI ≥2 (%)	95.11 ± 6.76	73.36 ± 20.17*#	51.60 ± 11.60*#	98.63 ± 3.36	52.37 ± 8.40*#	37.57 ± 7.69*#
Reduction		21.75 ± 15.50#	43.51 ± 10.12#		46.25 ± 9.43#	61.05 ± 9.38#
AL (mm)	3.13 ± 0.97	2.56 ± 0.93*	2.42 ± 0.80*	3.01 ± 0.66	2.46 ± 0.75*	2.48 ± 0.78*
Reduction		0.44 ± 0.20	0.58 ± 0.13		0.49 ± 0.40	0.46 ± 0.44
Sampled teeth						
PI ≥2 (%)	95.24 ± 11.66	60.00 ± 42.13*	45.24 ± 35.60*	100 ± 0	50.00 ± 38.05*	32.86 ± 32.24*
Reduction		35.24 ± 36.60#	50.00 ± 31.18#		50.00 ± 38.05#	67.14 ± 32.24#
PD (mm)	4.21 ± 0.62	3.37 ± 0.44*	3.22 ± 0.38*	4.32 ± 0.52	3.11 ± 0.21*	3.14 ± 0.26*
Reduction		0.84 ± 0.39	0.98 ± 0.29		1.20 ± 0.39	1.18 ± 0.41
BI ≥2 (%)	92.86 ± 9.37	66.90 ± 20.81*	53.33 ± 17.34*	98.81 ± 2.92	56.90 ± 27.26*	55.71 ± 26.99*
Reduction		25.95 ± 17.52	39.52 ± 13.21		41.90 ± 27.84	43.10 ± 27.62
AL (mm)	3.20 ± 1.19	2.50 ± 1.01*	2.35 ± 0.80*	3.38 ± 0.71	2.81 ± 1.02*	2.86 ± 0.92*
Reduction		0.70 ± 0.56	0.86 ± 0.50		0.57 ± 0.44	0.51 ± 0.36

BMI, body mass index; PI, plaque index; PD, probing depth; BI, bleeding index; BOP, bleeding on probing; AL, attachment loss.
Reduction, the drop-out value from baseline to month 3 or month 6.
*Significant difference with baseline.
#Significant difference between the test group and the control group; P <0.05.

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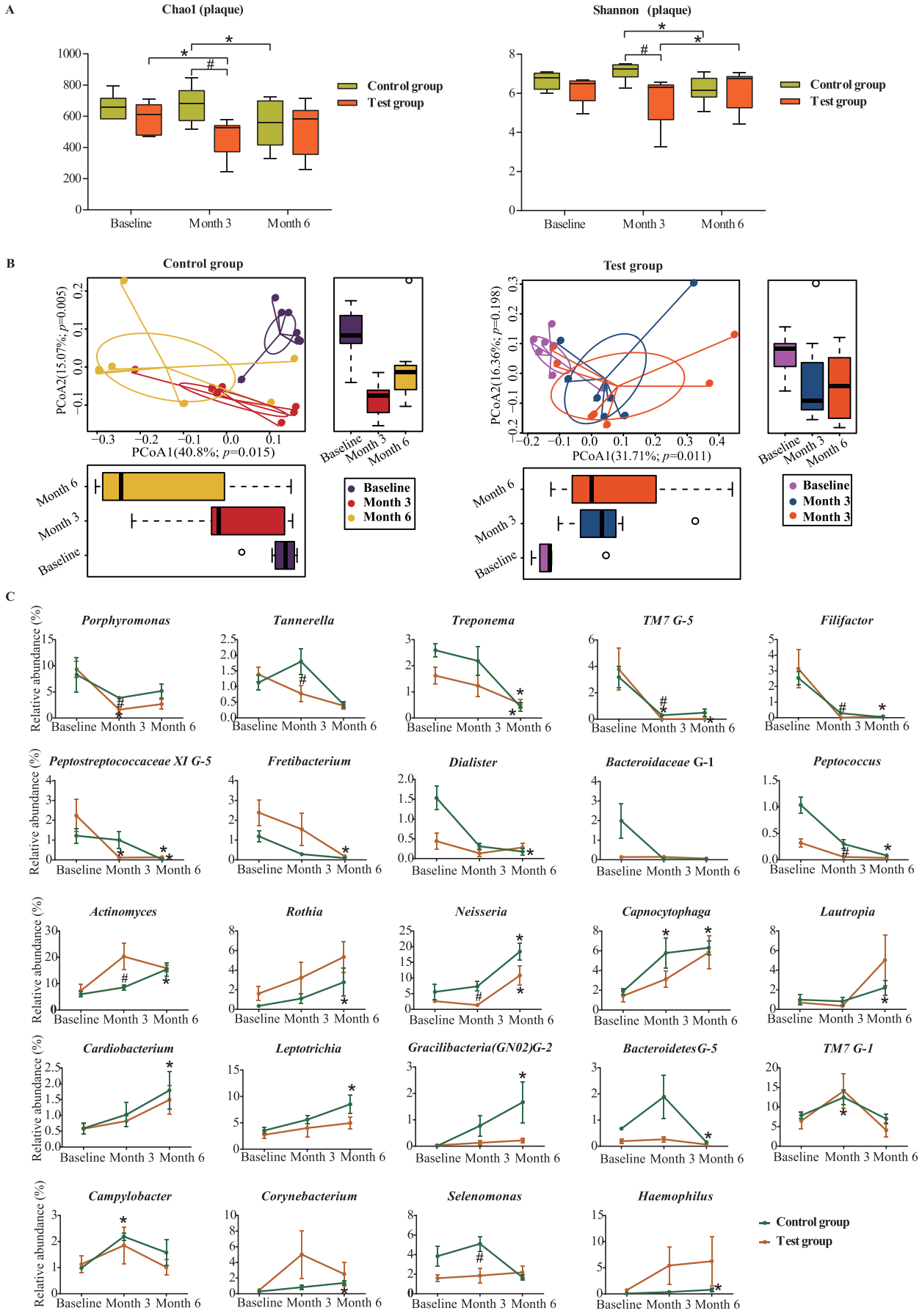

FIGURE 2 The shift of microbial profiling in subgingival dental plaque. **A**) Alpha diversity (microbial richness by Chao1, microbial diversity by Shannon). **B**) Principle Coordination Analysis (PCoA) by unweighted Unifrac distance. **C**) The microbial changes of core genera (relative abundance >1%). *Significant difference with baseline. #Significant difference between the test group and the control group. $P < 0.05$

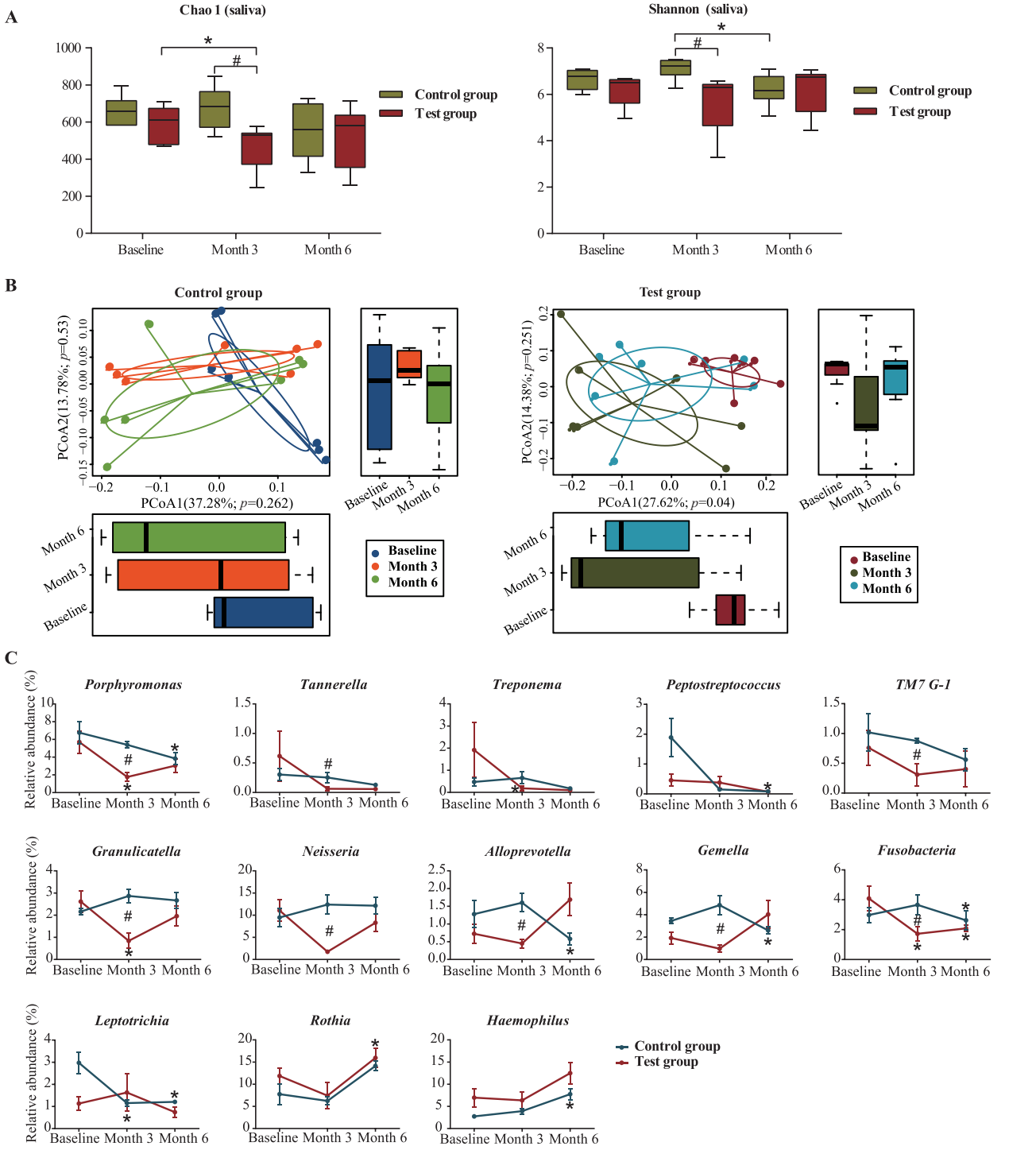

FIGURE 3 The shift of microbial profiling in saliva. **A**) Alpha diversity (microbial richness by Chao1, microbial diversity by Shannon). **B**) Principle Coordination Analysis (PCoA) by unweighted Unifrac distance. **C**) The microbial changes of core genera (relative abundance >1%). *Significant difference with baseline. #Significant difference between the test group and the control group. *P <0.05*

tle change before and after treatment. However, in the test group, it dramatically changed after treatment with significant decreases in the genera *Porphyromonas*, *Treponema*, *Granulicatella*, and *Fusobacteria* ($P < 0.05$).

3.2.3 | Microbial difference between test group and control group

The test group showed a greater reduction of microbiota than the control group in both the subgingival biofilm and

saliva. For subgingival microbiota, genera *Porphyromonas*, *Tannerella*, *TM7 G-5*, *Filifactor*, *Peptococcus*, *Neisseria*, and *Selenomonas* were significantly less abundant in the test group than in the control group, and the genus *Actinomyces* was significantly more abundant in the test group than in the control group at month 3 (P <0.05, Fig. 2C). At month 6, there was no significant difference between two groups. Salivary microbiota also showed significant differences at month 3; however, the difference disappeared at month 6. At month 3, genera *Porphyromonas*, *Tannerella*, *TM7 G-1*, *Granulicatella*, *Neisseria*, *Alloprevotella*, *Gemella*, and *Fusobacteria* were significantly less abundant in the test group than in the control group (P <0.05, Fig. 3C).

The co-occurrence networks showed differences in the symbiotic relationships between the test and control groups. At month 3, the subgingival microbiota showed more symbiotic correlations in the control group than in the test group. *Capnocytophaga*, *Leptotrichia*, and *Treponema* showed multiple relationships in both groups; *Tannerella, Bacteroidetes G-5*, *Cardiobacterium*, and *Peptostreptococcaceae XIG-5* were only involved in the network of the control group. The co-occurrence relationship of salivary microbiota in the test and control groups was analogous at month 3. At month 6, the number of correlations rebounded in the test group. *Prevotella*, *Lautropia*, *Veillonella*, *Neisseria*, and *Selenomonas* harbored multiple symbiotic relationships in both groups. However, *Porphyromonas* and *Leptotrichia* only occurred in the network of the control group, whereas *Streptococcus* and *Campylobacter* only occurred in the network of the test group. The co-occurrence network of salivary microbiota at month 6 in the test group was sparser and weaker than in the control group (Fig. 4A).

3.3 | Similar shifts of the microbiome in subgingival biofilm and saliva

The correlation between microbial changes in the subgingival plaque and saliva was computed and plotted (Fig. 4B). ASV ratios [ASV (month 3 or 6)/ASV (baseline)] are presented in the plot to characterize the microbial changes. The ASV ratio in the subgingival biofilm was highly correlated with that in the saliva (P <0.001). The association of the subgingival microbiome and salivary microbiome in the control group was slightly stronger than that in the test group (Spearman correlation coefficient r = 0.5488, P <0.001 for control group at month 3; r = 0.4656, P <0.001 for control group at month 6; r = 0.3327, P = 0.01 for test group at month 3; r = 0.3826, P = 0.003 for test group at month 6).

3.4 | Microbiota signatures associated with the periodontal condition and systemic indicators

Figure 5A shows the relationship between the periodontal parameters and systemic indicators. As presented in the heatmap, full-mouth periodontal parameters, consisting of PD, BI, and AL, were negatively related to the implementation of SRP and the use of antibiotics (P <0.05). Body mass index (BMI) was positively associated with P-LCR and MPV and negatively associated with MONO% and PLT (P <0.05). NEUT% and LYM% showed the opposite relationship (P <0.05). The glycolipid metabolic indexes, including glucose, CHO, ALP, LDL-C, and TG, were positively associated (P <0.05).

The relationship between periodontal parameters, inflammatory indexes, metabolic indexes, and core genera (relative abundance >1%) was tested (Fig. 5B). In subgingival dental plaque, *Porphyromonas*, *Treponema*, *Prevotella*, *Fusobacterium*, *Filifactor*, *Saccharibacteria TM7 G-5*, and *Peptostreptococcaceae XIG-6*, were positively correlated with periodontal parameters (PD, BI, AL, and PI), and negatively correlated with the implementation of SRP and the use of antibiotics. In contrast, *Actinomyces* and *Capnocytophaga* in subgingival dental plaque were negatively related to periodontal parameters and positively related to the implementation of SRP and the use of antibiotics. The genus *Porphyromonas* in both subgingival dental plaque and saliva was significantly negatively correlated with the implementation of SRP and the use of antibiotics. Salivary *Porphyromonas* was positively related to PD; salivary *Fusobacterium* was positively correlated with BI. *Fusobacterium*, *Granulicatella*, and *Neisseria* were negatively related to antibiotic use (P < 0.05). *Gemella* and *Alloprevotella* in saliva were positively associated with BMI (P < 0.05). Glucose was positively related to *Porphyromonas* in subgingival plaque and negatively related to *Haemophilus* in saliva (P < 0.05). Subgingival *Leptotrichia* were negatively correlated with PLT, although positively related to MPV (P < 0.05).

4 | DISCUSSION

In this study, SRP with adjunctive systemic antibiotics showed better clinical and microbiological effects than SRP alone. Periodontal treatment with systemic antibiotics had clear advantages in reducing probing depth, suppressing periodontal bleeding, and reducing microbiological dysbiosis in subgingival plaque and saliva compared with mechanical debridement alone. The microbial advantage nearly disappeared 6 months post-treatment; however, the

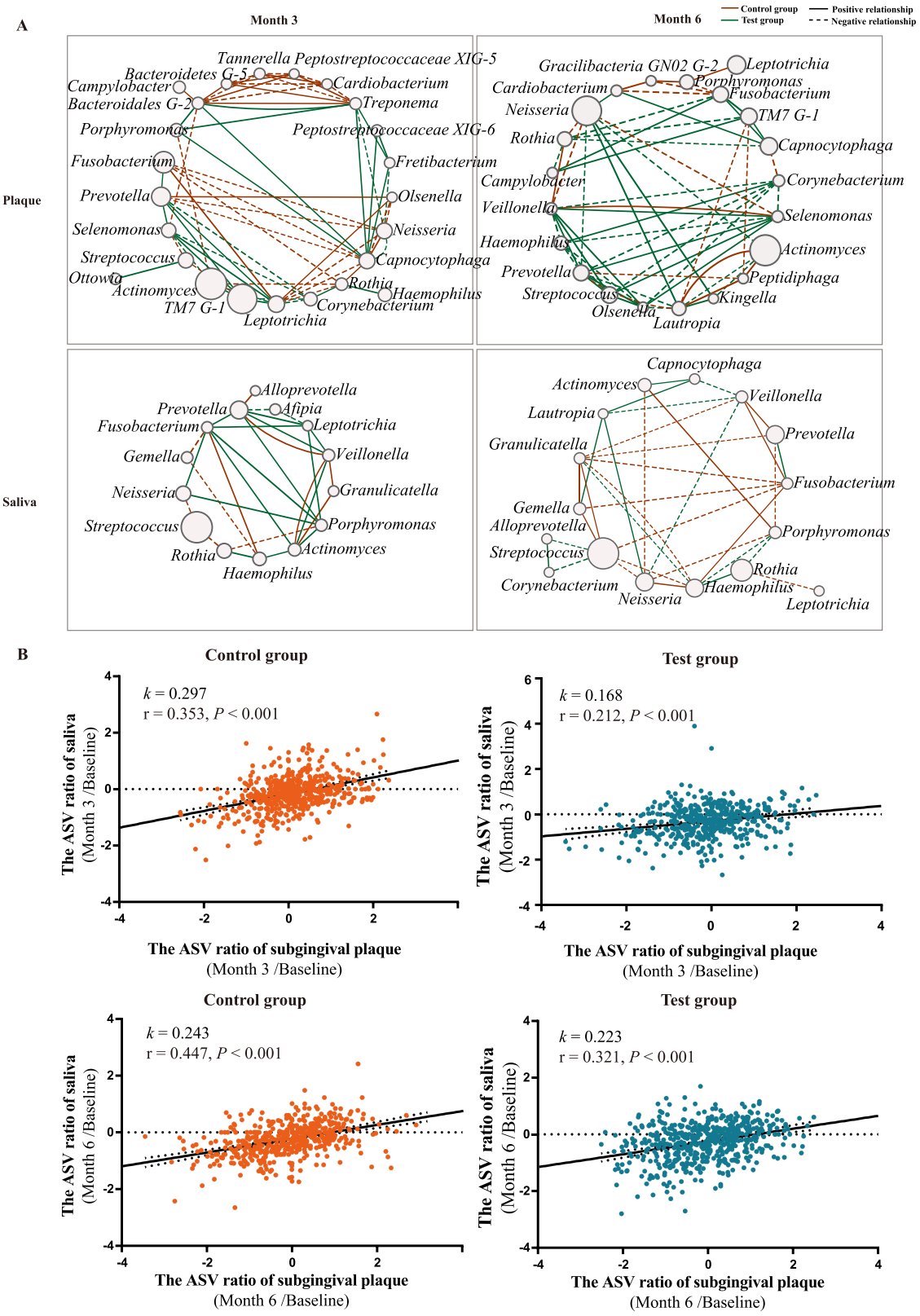

FIGURE 4 The differences and similarities of the microbial shift in subgingival region and saliva. **A**) Microbial co-occurrence networks. The symbiotic networks of core genera (relative abundance >1%) were tested by Spearman correlation with $P < 0.05$. The size of nodes was determined by the relative abundance of genera. Red connecting lines represent relationships of the control group, and green connecting lines represent relationships of the test group. The thickness of connecting lines was determined by the correlation coefficient. Dashed lines represent negative relationships; solid lines represent positive relationships. **B**) The similar shift of microbiota in subgingival biofilm and saliva. Linear regression and Spearman correlation were used to test the relationship between salivary microbiota and subgingival microbiota

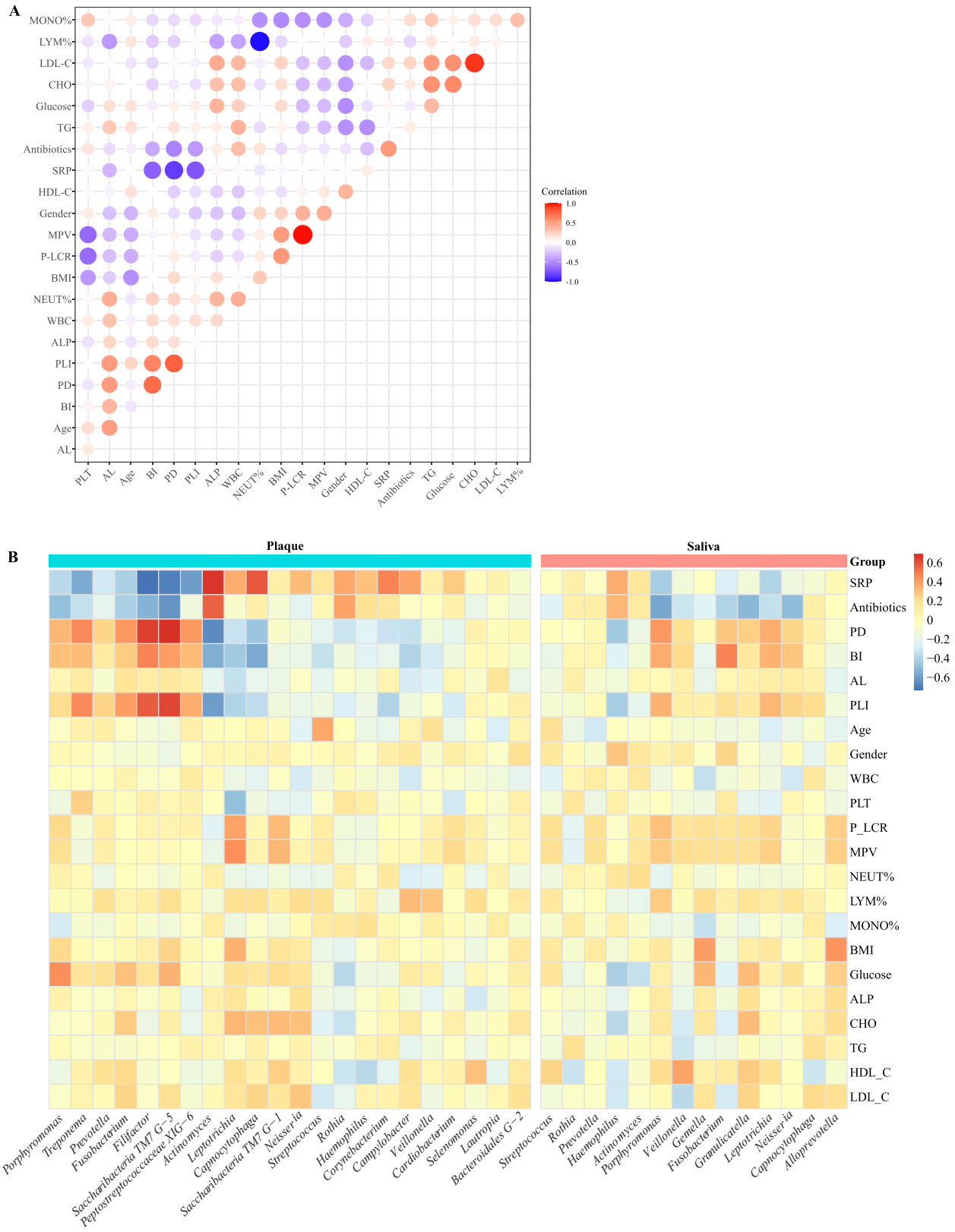

FIGURE 5 The correlation of microbiota, periodontal parameters, and systemic indicators. **A**) The correlation among periodontal parameters, treatment measures, and systemic indexes. **B**) The association of microbiota with periodontal parameters and systemic indexes. SRP, scaling and root planing; PD, probing depth; BI, bleeding index; AL, attachment loss; PI, plaque index; BMI, body mass index; WBC, white blood cells; PLT, platelet; P-LCR, platelet-larger cell ratio; MPV, mean platelet volume; NEUT%, neutrophil percentage; MONO%, monocyte percentage; LYM%, lymphocyte percentage; ALP, alkaline phosphatase; CHO, cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. The correlation was tested by Spearman rank correlation. $P < 0.05$

clinical advantage lasted longer. Some microbiota in the subgingival region and saliva were associated with inflammatory and metabolic indicators in peripheral blood, suggesting that the systemic condition of patients suffering from severe periodontitis might benefit from oral infection control.

A vital finding to emerge from the analysis is the superior clinical effect of SRP with adjunctive antibiotics over SRP alone. PD is the most applicable parameter for assessing the response to periodontal treatment.[39] The present study demonstrated a 1.34 mm full-mouth PD reduction with SRP + AMX + MTZ and 0.88 mm full-mouth PD reduction with SRP alone at month 3. Previous studies have reported approximately 1 mm (0.46 to 2.91 mm) PD reduction after being treated with SRP + AMX + MTZ.[13,17,40–44] The results also demonstrated a significant gain of clinical attachment in this study, which was consistent with previous studies.[8,13,17,40–44] In addition, monitoring periodontal bleeding, as an important indicator of inflammatory lesions, could help to objectively evaluate the clinical effect of periodontal treatment.[45] The analysis based on this study showed significant improvement in bleeding after SRP with AMX + MTZ, which corroborates the findings of a great deal of the previous work.[9,41]

Furthermore, the microbiological effect of adjunctive antibiotics along with SRP was also remarkable. Lower microbial diversity and sparse co-occurrence network in the test group than in the control group, indicated a better effect in reducing dysbiosis of the microbiome when treated with adjunctive antibiotics. Remarkable reductions were observed in *Porphyromonas*, *Tannerella*, and *Treponema* levels in both the subgingival region and saliva. These genera were significantly less abundant in the test group than the control group at 3 months post-treatment. Despite only limited studies providing a global overview of the microbiome in this field, our results were consistent with previous observations on specific genera.[14,19] It is worth noting that the differences between the test group and the control group were narrowed at month 6. There were only slight microbial differences between two groups 6 months post-treatment, as previously reported by Bizzarro et al.[18] The short-duration medication regimen may partly account for the rebound of the microbiota. Borges et al.[11] found greater clinical improvement with 400 or 250 mg of MTZ plus 500 mg of AMX/TID/14 days than a 7-day regimen, suggesting that a longer medication regimen might bring a better microbial benefit. This bears further investigation.

It is interesting to note that the salivary and subgingival microbiome changed synergistically during the 6-month observation period. Saliva assembles the bacteria from different niches overall the cavity including subgingival region,[46] making it a reservoir. After periodontal treatment, bacteria can be transmitted from the saliva to colonize in the subgingival region in turn.[21] Therefore, infection control of saliva is important to reduce the rebound of subgingival pathogens. The additional benefit of antibiotics for infection control in both the subgingival region and in saliva may be reinforced by their synergistic relationship.

The use of antibiotics and the implementation of SRP failed to show a direct association with systemic indicators in this study. However, they were significantly negatively related to the abundance of *Porphyromonas* in both subgingival dental plaque and saliva. Glucose was strongly correlated with *Porphyromonas* in the subgingival plaque. In addition, the level of *Haemophilus* in saliva, which dramatically increased after treatment, was negatively related to PI, PD, and glucose. This indicated that oral microbiota might be a connector between periodontal condition and glucose, even in non-diabetic patients. In this study, *Leptotrichia* in subgingival plaque increased post-treatment, and was positively related to MPV. MPV in peripheral blood is considered as an important indicator of inflammation[47] and has been confirmed to be negatively related to the severity of periodontitis.[48] The result indicated that the periodontal condition could be reflected by systemic MPV. We could preliminarily infer that periodontal treatment might benefit systemic metabolism and inflammation by regulating the subgingival microbiome. This requires validation in further studies.

This is the first study to evaluate the microbiological shift in both saliva and subgingival biofilm after SRP with systemic administration of antibiotics. It provided an overview of dynamic shifts in the two microbial communities during the 6-month observation period. In addition, this study is the first to evaluate the association between systemic factors and microbiota in this field, although with limited sample size. The shortage of this study is lacking placebo, potentially causing bias due to patient knowledge. Besides, pooled samples were sequenced and analyzed to explore the overall feature of the subgingival microbiome at the patient level, facilitating the evaluation of its relationship with the salivary microbiome and systemic parameters. However, pooled samples may miss the information on specific sites and different niches. Furthermore, the microbial effect of adjunctive antibiotics with a higher dose of metronidazole, longer medication regimens, and longer observation with clinical endpoints could be evaluated in further studies.

5 | CONCLUSIONS

This pilot randomized controlled trial demonstrated an overwhelming advantage of SRP with adjunctive AMX +

MTZ compared with SRP alone in improving the periodontal condition, reducing dysbiosis of both subgingival and salivary microbiome. The microbial advantage was prominent at 3 months post-treatment but disappeared by 6 months post-treatment, however, the clinical advantage remained significant at 6 months post-treatment. Besides, periodontal treatment has potential benefits for systemic glucose and inflammation. In summary, these findings highlight the significance of the systemic administration of antibiotics for patients with generalized stage III/IV, grade B/C periodontitis.

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AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the conception and design of this study. Hongye Lu conducted data collection and analysis. Lu He undertook the study design and periodontal treatment. Dongsiqi Jin performed sample collection. Yunxuan Zhu performed the clinical examinations. Lu He, Huanxin Meng, and Hongye Lu were involved in data interpretation, drafting the manuscript, and revising it critically.

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