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Adjunctive affects of systemic amoxicillin and metronidazole with scaling and root planning

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Abstract

Aims: The objective of this study was to investigate the effect of the systemic administration of metronidazole and amoxicillin as an adjunct to initial periodontal therapy in patients with moderate to severe chronic periodontitis.

Methods and Materials: This randomized, double blind study, involved 50 adult patients with untreated periodontitis who were randomly assigned to receive either a full-mouth scaling and root planning along with systemic metronidazole and amoxicillin (T group) or scaling and root planning with a placebo (P group). Clinical measurements including probing depth (PD), clinical attachment levels (CAL), Plaque Index (PI), and Bleeding Index (BI) were recorded at baseline and six to eight weeks after therapy. The deepest pocket was selected and samples for microbiological testing were taken. Patients received coded study medications of either 500 mg amoxicillin in combination with 250 mg metronidazole or an identical placebo every eight hours for seven days following scaling and root planning.

Results: There was a significant change in PD , CAL, PI, and BI in the T group compared to the placebo group after therapy. Parallel to the clinical changes, treatment significantly reduced the number of *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), and *P. intermedia* (Pi) compared with baseline in the T group. However, in the P group only the Pi colony count was reduced significantly (P=0.0001). After therapy, there was a significant difference between the T and P groups in the number of patients negative for Aa, Pg, and Pi.

Conclusions: The significant differences between treatment and placebo groups are in line with other studies and support the considerable adjunctive benefits of the combination of amoxicillin and metronidazole in the treatment of chronic periodontitis.

Keywords: Metronidazole, amoxicillin, periodontal disease, treatment, antibiotic therapy

Introduction

Periodontal diseases are common infections affecting a proportion of people in all populations, but severe forms of these diseases affect only approximately 10% of populations [1,2]

The principle aim of periodontal therapy is to halt further loss of periodontal attachment. This is achieved through meticulous supra and subgingival debridement resulting in the reduction of the total bacterial load.[3] Despite this therapy, some patients may experience continued

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periodontal attachment loss.[4] One possible factor responsible for post-treatment disease progression is the inability of therapy to suppress periodontal pathogens to levels compatible with periodontal health.

The presence of periodontal pathogens such as *Porphyromonas gingivalis* (Pg), *Tanerella forsythesis* (Tf), and *Actinobacillus actinomycetemcomitans* (Aa) has been linked to ongoing periodontal destruction.[5-8] Moreover, the absence of specific periodontal pathogens seems to have a negative predictive value for further attachment loss;[9] therefore, an objective of periodontal treatment might be to suppress or eliminate certain subgingival periodontal pathogens.

It has been suggested some periodontal pathogens may be inaccessible to mechanical periodontal therapy due to their ability to invade the gingiva[10] or root dentin.[11] With this in mind, it is conceivable local and/or systemic administration of effective antimicrobial agents may enhance the outcome of mechanical therapy.[12]

Reviews on periodontal treatment have suggested antimicrobial therapy may be considered in the presence of early onset disease, advanced chronic adult periodontitis, and periodontitis that failed to respond to non-surgical mechanical therapy.[13-17]

Antimicrobials may be delivered locally or systemically, but when periodontitis affects numerous teeth, the use of systemic antimicrobials has several advantages including a cost benefit.[16] The adjunctive use of systemic antibiotics such as tetracycline, metronidazole, amoxicillin/ clavulanate, or clindamycin can improve the results of the initial periodontal treatment and can more predictably suppress periodontal pathogens in juvenile and adult periodontitis patients.[18,19] Since the subgingival microbiota in advanced periodontal diseases often includes species with different antimicrobial susceptibilities, combination antibiotic therapy is recommended.[20-24]

Metronidazole is one of the most widely used antimicrobial compounds in the treatment of some types of periodontal diseases such as aggressive periodontitis and adult periodontitis. Metronidazole is thought to work specifically on the anaerobic microbiota associated with periodontal diseases.[25,26] In addition to metronidazole, *in vitro* studies indicate penicillin, especially amoxicillin, acts effectively against most periodontal pathogens.[27,28] A recent series of studies showed the combined use of amoxicillin and metronidazole can be useful in the treatment of advanced periodontitis, especially in patients harboring Aa.[3,20,21,23,29] Other studies have shown the elimination of this putative periodontal pathogen has been associated with long-term periodontal stability.[23] Recently, Berglund et al.[12] confirmed this observation. These studies showed scaling and root planning in combination with systemic metronidazole plus amoxicillin improved clinical parameters significantly better than scaling alone.

Fleming et al.[30,31] in a study on 38 adult patients with progressive periodontitis, compared scaling plus amoxicillin (375 mg tid) and metronidazole (250 mg tid) with scaling alone. After 12 months, patients harboring Aa benefited clinically and microbiologically from the antibiotic therapy. Winkel et al.[4] evaluated the adjunctive effect of systemically administered amoxicillin and metronidazole in a group of adults who also received supra and subgingival debridement. After six months, additional improvement in probing depth (PD), clinical attachment levels (CAL), and bleeding

on probing (BOP) and additional suppression of Pg, Tf, and Pi were found in an antibiotic group than in a placebo group. Rooney et al.[2] compared the adjunctive benefits to scaling and root planning of amoxicillin and metronidazole alone as well as combined and found the combination of amoxicillin and metronidazole provided the greatest adjunctive efficacy for all outcome measures.

Lopez et al.[32] showed a one-week course of systemic metronidazole and amoxicillin every four months as the only therapy that arrested the progression of adult periodontitis and significantly improved the clinical parameters of the disease.

Lopez and his colleagues in a recent study demonstrated changes in clinical and microbiological parameters were similar after receiving systemically administered metronidazole and amoxicillin as the sole therapy or after receiving scaling and root planning only.[33] Three recent systematic reviews[34-36] on the adjunct effect of antimicrobial agents with scaling and root planning showed a beneficial effect of antimicrobials in reducing pockets and further attachment loss. These studies confirmed patients with deep pockets, progressive or active disease, or specific microbiological profile can benefit more from this adjunctive therapy. But due to differences in study methodology and lack of sufficient data it was difficult to establish definitive conclusions.

There have been only a few controlled clinical and microbiological studies done on this subject. As a result, this randomized, double blind, placebo controlled, parallel study was done to investigate the effect of initial periodontal treatment in conjunction with systemically administrated amoxicillin plus metronidazole in patients with moderate to severe adult chronic periodontitis.

Methods and Materials

Based on the results of Winkel et al.[4] with a power equal to 90%, the least sample size (for the test group and control being α=0.05) was calculated to be 50 patients.

Study Population

A total of 50 volunteers (29 female and 21 male, mean age: 34.42 ± 8.23) were selected for this study from patients referred to the Department of Periodontology in the Faculty of Dentistry at Hawler Medical University and from private dental practices for treatment of periodontitis. Selection criteria are shown in Table 1.

Study Design

This was a randomized, double blind, placebo controlled. It extended from the baseline to the end-trial over a two-month period. Prior to participation the purpose and procedures of the study were fully explained to all subjects who were entered into the study only after having conveyed written consent.

Clinical and microbiological data were collected at a non surgical pre-treatment baseline appointment and at a two month non-surgical post-treatment appointment. At each clinic, one examiner was responsible for all clinical measurements and microbiological sampling at baseline as well as the re-examination. The examiners were calibrated with each other at baseline. Clinical data were recorded from all teeth except third molars and severely malpositioned teeth. Subgingival plaque samples were obtained from one tooth in the mouth with the greatest probing depth using a universal curette. After cleaning the supragingival area, the curette was inserted into the base of the pocket and drawn coronally against the

root surface. The samples were pooled in a reduced transport fluid and sent to the laboratory within one hour. The clinical data collected were as follows:

1. PD in mm was recorded from four surfaces of each tooth distobuccal, mid-buccal, mesiobuccal, and mid-lingual using a North Carolina periodontal probe (Hu Friedy, Chicago, IL, USA).
2. Loss of attachment (LOA) at the same four sites using the clinically detected cementoenamel junction as the reference point.
3. The BI was calculated as the percent of bleeding points recorded 30 seconds after probing.[37]
4. The PI was recorded using the standard method.[38]

After the baseline visit, patients returned for full mouth scaling and root planing (S & R) carried out under local anesthesia using a standardized procedure and lasting approximately one hour. All teeth were instrumented using an ultrasonic scalar (Varios 550, NSK, JP) then re-instrumented using Gracey hand curettes (Hu Friedy, Chicago, IL, USA) and polished. Oral hygiene instruction including an appropriate interdental cleaning method was performed for all patients. On the same day patients randomly received coded study medications. They were given either 500 mg amoxicillin in combination with 250 mg metronidazole or an identical placebo consisting of lactose capsules and vitamin B1 tablets to be taken every eight hours for the following seven days. Patients were advised to use the medications after meals. One week after the scaling procedure patients were recalled for a complete mouth examination at which time S & R was done as needed. In addition oral hygiene instructions were reinforced.

An office secretary, who was blind to the medication, dispensed the medications. Medications for each subject were in two identically numbered packages. A sealed master code decipher was secured in a locked environment in the Department of Periodontology until the end of the study. Only in the case of adverse events was the code deciphers to be accessed. To check compliance patients were asked to return any tablets that remained after seven days. Patients returned for a follow up visit approximately two months after completion of the medication treatment phase when clinical examinations and microbiological sampling were performed again.

Microbiological Analysis

After superficially cleaning the sites with sterile gauze and drying the supra gingival area with dental unit air, samples for microbiological analysis were taken with a sterile periodontal curette inserted to maximum pocket depth. Each sample was transferred to 5 ml of reduced transport fluid. Samples were transported within one hour for microbiology analysis and immediately processed. Each sample was dispersed using a vortex mixer at the maximum setting for 30 seconds.

A ten-fold serial dilution of dispersed samples was then prepared in 25% strength Ringers solution (10^{-1}, 10^{-2}, and 10^{-5}).[39] Diluted and non-diluted samples were then inoculated on the following freshly prepared solid agar media using sterile bent glass rods:

1. Defibrinated (5%) sheep blood brucclla agar (Difco Laboratories, Detroit, MI, USA) supplemented with haemin (5 mg/l) (Merck, Darmstadt, Germany) and menadione (1 mg/l) (Merck Darmstadt, Germany) for detection of Pg and Pi.
2. Chocolate blood tripticase soy agar (Pronadisa, Madrid, Spain) supplemented with bacitracin (30

microgram/1) (Sigma, St. Louis, MO, USA) for detection of Aa.

The supplemented brucella blood agar plates were placed in an anaerobic condition created by Anaercult A (Merck HgaA, Darmstadt, Germany) and anaerobic jars. The brown pigmented colonies were then subcultured (to achieve a pure culture) and identified using the following as well as other conventional biochemical tests: [39-41]

- Gram staining.
- Aerotolerace test.
- Special potency antibiotic discs consisting of vancomycin 5 µg, colistin 10 µg, and kanamycin 1 mg (Padtan Teb, Iran).
- Indole production of tryptophan, lipase and esculin hydrolysis.

After seven days of incubation of chocolate agar plates in an atmosphere of 5% CO_2 at 37ºC, the Aa colonies were subcultured and identified by Gram staining, production of catalase, oxidase, ONPG (o-Nitrophenyl-ß-D-Galactopyranoside) test, urease, indole production of tryptophane, and fermentation of carbohydrates.[40,41]

The number of Pg, Pi, and colony forming units (cfu) were counted on the brucella blood agar plates, and the cfu of Aa were counted on the chocolate agar plates. The plates containing 30-300 colonies were used for detection of cfu and expressed in terms of 1 ml of transport medium.[42]

Statistical Analysis

For analyses of the clinical data, a patient level response variable was calculated for each parameter by computing the full mouth mean value of the scores. The mean values for PD and CAL of pockets ≥ 5mm were calculated. BOP was calculated as the mean proportion of sites of whole mouth data.

Differences between the antibiotic group (test) and placebo group (control), between baseline and re-examination were analyzed using a paired samples t-test, and the two sample unpaired t-test was applied for the analysis of differences between the test and control groups. An analysis of covariance (ANOVA) using baseline PD as a covariate was performed. The PI represented a mean mouth score.

The difference within each group between baseline and re-examination were analyzed using the Wilcoxon signed rank test. The Mann-Whitney U test was applied for analysis of differences between the two groups. Mean values for colony count for each microorganism (Pg, Pi, and Aa) before and after treatment were calculated. The number of persons who were positive for each microorganism was compared between baseline and reexamination using the McNemar's test. P values of <0.05 were considered as significant for all analyses. Statistical analysis was performed using SPSS version 10.0 software (SPSS, Chicago, IL, USA).

Results

Five patients left the study for different reasons leaving 50 patients (29 females and 21 males, mean age 34.5 years) enrolled and managed using the study protocol. They completed all examinations throughout the study period. Random assignment resulted in 22 patients (9 males and 13 females) being assigned to the placebo (P) group and 28 (12 males and 16 females) to the test group (T). The mean age of the P group was 35 years ranging from 19 to 51 years, and the T group was 34 years ranging from 17 to 49 years. Table 2 shows the summary of subjects and descriptive statistics in each group.

Clinical Findings

At baseline, no statistically significant differences existed between

groups for any clinical parameter (Table 3). Following therapy the periodontal condition in both groups improved. The paired t-test showed the changes in probing pocket depth were significant in both groups (P=0.00001). Reduction of the PD in the P and T groups were 1.5±0.45 and 2.1±0.67, respectively.

The two sample t-test showed the difference between the two groups was significant (P=0.00001). Covariance analysis using baseline PD as a covariate showed the final significant difference between the two groups was independent of the initial pocket depth. Clinical attachment gain was significant in both P and T groups (P=0.00001). It was 1.15 mm in the P group versus 1.92 mm in the T group. The PI was reduced significantly compared to the baseline in both groups (P=0.00001) and between the two groups (P=0.05). Similar to PI, the differences in BI within groups between baseline and re-examination were significant (P=0.00001). The BI decreased in the T group more significantly than in the P group (P<0.05). Table 3 shows the clinical findings in both groups.

Microbiological Findings: All patients in the P group and 26 of the 28 patients in the T group had complete bacteriologic samples. At baseline, three patients (11.5%) in the T group were negative for all three microorganisms (Aa, Pg, and Pi). By the end of the study the number reached fifteen (57.7%) in the T group. However, in the P group only one patient (4.5%) was initially negative for Aa, Pg, and Pi; in the end six patients (27.3%) were negative for these microorganisms. The difference between the two groups was significant. Parallel with the clinical changes, the treatment of the T group reduced the number of Aa, Pg, and Pi significantly compared to baseline (P=0.003, 0.021, and 0.0001, respectively). In the P group only the Pi colony count was reduced significantly (Pv=0.0001) and showed a significant difference between the two groups at the end of the study (P= 0.026) (Table 4). The number of patients positive for Pg, Aa, and Pi in the T group decreased significantly after therapy, but in the P group only patients positive for Aa decreased significantly. Some patients who were culture negative for a bacterium at baseline became positive after treatment. This occurred in one patient for Pg and one patient for Aa. *Prevotella melaninogenica* and *Prevotella denticola* were found in two patients.

Discussion

The rationale for the use of a systemic antibiotic in the treatment of periodontal infections is to rapidly suppress target microbial species and expedite the establishment of a host compatible microbiota. Amoxicillin and metronidazole were of particular interest due to their very different mechanisms of action and spectrum of activity. There is a considerable body of literature based on clinical and microbiological data that amoxicillin and metronidazole provide adjunctive benefits to non-surgical therapy for periodontal diseases, including refractory disease.[3,12,20,21,23,30,31]

The objective of this study was to investigate the effect of initial periodontal treatment in conjunction with systemically administrated amoxicillin plus metronidazole in a double blind, placebo controlled, randomized study in adult periodontitis patients. The choice of this systemic antibiotic regimen was based on an earlier report showing predictable and long-term suppression of subgingival Aa and Pg.[23] Considerable effort was

made to blind both the subjects and the examiner. Ethically the subjects had to know they may receive either antibiotics or two placebos. The patient information sheet explained the medication would be in the form of capsules and tablets. Medications were dispensed in identical packets identified by subject number only. The dispenser of the medication did not know the identity of the prescribed agents. The decipher code to the medication was only broken at the time of the statistical analysis and after all data had been collected.

The selection criteria represented the absolute minimum requirements, albeit a little conservative for the definition of advanced disease, and were considerably exceeded in the final subject selection.

The follow-up time might also be considered short, however, the aim of the study focused on the adjunctive benefits of antimicrobials. In other cited studies with metronidazole and amoxicillin or any adjunctive antimicrobial study, the actual maximum benefits would be seen within a three-month post-treatment period. Follow-up after six months would likely be irrelevant to the adjunctive action and changes would be independent of any antimicrobial action.[2]

Re-evaluation with microbiological testing within one to three months after antimicrobial therapy is desirable to verify the elimination or marked suppression of the putative pathogens and to screen for possible super-infecting organisms.[24] Re-population of most pathogens to pre-treatment levels usually requires four to eight weeks following periodontal instrumentation.[24]

All clinical parameters improved significantly after the initial periodontal therapy, but in this study the periodontal therapy was followed by an additional systemic antimicrobial therapy with amoxicillin plus metronidazole. The results showed the use of these systemic antibiotics as an adjunct to supra and subgingival debridement in adult periodontitis patients and provided better clinical outcomes than scaling and root planing alone. In particular, significant decreases in BI, CAL, and PD were seen in the antibiotic treated subjects compared with those receiving a placebo. However, these differences in PD and CAL were small and the clinical significance dubious because the measurement error inherent in the probing assessment is ±1 mm for measurements at different time points by the same assessor.[43]

The positive clinical findings for metronidazole combined with amoxicillin are in general agreement with the overall results of review papers reporting the beneficial effect of adjunct therapy.[30-32]

These findings are consistent with the other studies cited [2,3,4,12,20,21,23] except for Flemmig et al.[30] Direct comparisons with other studies are confounded to some degree for several reasons. The dosage used in the present study was different from other studies. The dosage of amoxicillin in the present study was 500 mg while other studies used 375 mg. The present study design differed from previous studies that only compared combined chemotherapy with no chemotherapy.[12,30,31] Specifically, placebo tablets and capsules were not used or only combined chemotherapy was used on patients who had failed to respond to mechanical non-surgical or surgical debridement procedures.[3,20,21,23] Therefore, the latter studies are uncontrolled studies and the stated differences must be noted in the results interpretation.

In the present study probing depth, considered by many as one of the most

important measures of disease severity, was significantly influenced by adjunctive antimicrobial therapy. This was in agreement with Berglund et al.,[12] Winkel et al.,[3,4] and Rooney et al.[2] The effect of each treatment on attachment levels were proportionately less than on pocket depth although the differences were significant. Less effect on attachment gain reflects the treatment impact at a tissue level. Pocket depths could have possibly been reduced by three mechanisms: inflammation reduction, establishment of a long junction epithelium, and the organization of collagen. Each mechanism can influence probe penetration. Loss of attachment is not dependent on the degree of inflammation and, therefore, will not change directly in proportion to a change in pocket depth.

It has been shown the absence of BOP is a good predictor of stability.[2] The adjunctive antimicrobial administration group compared to the placebo group markedly reduced the BI.

Plaque scores in both groups also improved throughout the study. Previous studies have suggested a microbiological goal of periodontal treatment might be the elimination of certain periodontal pathogens, such as Aa and Pg from periodontal pockets as well as suppression of other periodontal pathogens below certain threshold levels.[9] The results of the present study showed this treatment goal was more predictably achieved in patients treated with adjunctive metronidazole and amoxicillin than in patients receiving the placebo.

In addition to the short-term outcome of periodontal treatment the absence of key pathogens such as Aa, Pg, and Pi seems to affect long-term periodontal stability and is probably a prerequisite for long-term clinical improvement of the tissues.[9,12,23] The present findings confirm previous observations that the use of systemic amoxicillin and metronidazole is effective in suppressing Aa and Pi below cultivable levels and in reducing the number of patients culturing positive for Pg.[3,12,20,21,23]

Scaling alone had a limited effect on the intraoral detection frequency of Aa and Pg. This finding is consistent with Danser et al.[44] which indicates the effect of mechanical periodontal therapy is limited to a quantitative reduction of these pathogens in subgingival plaque. However, non-dental habitats in the oral cavity remain unaffected, thus, leaving a reservoir for subgingival reoccurrence. Subgingival levels of Aa and Pg above 3×10^4 and 6×10^5, respectively, have been shown to significantly increase the risk for new periodontal attachment loss.[45]

Thus, the assessed antimicrobial therapy resulting in a suppression of subgingival Aa and Pg (1.62×10^4 and 5.23×10^4, respectively) appears to reduce the risk for periodontal disease progression. Pi was significantly reduced in both groups. This finding supports the concept of mechanical therapy alone being enough to suppress Pi. In the test group some patients remained positive for Pg and Aa post-treatment. One explanation could be inadequate compliance with the test medication.[4] Another possible reason could be insufficient sub and supragingival debridement. Other reasons for failure to eliminate Pg and Aa might be their location within the periodontal pocket and/or the protection provided within a biofilm.[4]

Conclusion

This study has shown systemic use of metronidazole and amoxicillin in conjunction with initial periodontal treatment in chronic periodontitis patients achieved significantly better

clinical and microbiological results than initial periodontal treatment alone. Further, long-term randomized controlled trials employing larger study populations are needed to determine the efficacy of the assessed adjunctive antimicrobial therapy on the prevention of disease progression in patients harboring Aa, Pg, and Pi.

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Table 1. Selection criteria for subjects.

Inclusion Criteria	Exclusion Criteria
• Moderate to severe chronic periodontitis according to the criteria of the American Academy of Periodontology. • 3 natural teeth in each quadrant. • ≥1 site in at least 3 of the 4 quadrants with a probing pocket depth ≥ 5 mm and clinical attachment loss of > 3 mm, showing bleeding on probing and radiographic evidence of alveolar bone loss.	• Professional scaling and root planning or surgical periodontal therapy. • Systemic or topical periodontal antibiotic therapy six months prior to initiation of the study. • Pregnancy, lactating, or planning a pregnancy. • Systemic diseases such as diabetes. • Known HIV infection. • Acute necrotizing gingivitis. • Use of non-steroid anti inflammatory drugs. • Use of mouth rinses. • Smoking. • Known hypersensitivity to amoxicillin (penicillin) and metronidazole.

Table 2. Summary of subjects, descriptive statistics in each group.

Characteristics	Test Group (N=28)	Placebo Group (N=22)
Age	33.96±7.75	35±8.96
Male	12 (42.86%)	9 (40.91%)
Female	16 (57.14%)	13 (59.09%)
Teeth	28.32±3.89	27.14±2.96
Site	19.61±11.77	13.95±5.75

Table 3. Mean (SD) PD and CAL at sites ≥ 5mm and the full mouth mean (SD) PI and BI for the placebo group and the test group before and after treatment.

	Pocket Depth (SD) mm		Clinical Attachment Level (SD) mm		Plaque Index (SD)		Bleeding Index (SD) %	
Group	Test	Placebo	Test	Placebo	Test	Placebo	Test	Placebo
Pre-treatment	5.79(0.61)	5.62(0.55)	5.8(1.11)	5.34(0.72)	1.87(0.54)	1.7(0.50)	96(10.98)	93.1(12.48)
Post-treatment	3.59(0.72)	4.11(0.54)	3.87(0.99)	4.17(0.85)	0.85(0.33)	1.5(0.37)	32.23(17.82)	38.5(13.07)
P value	0.00001*	0.00001*	0.00001*	0.00001*	0.0001†	0.0001†	0.00001*	0.00001*
Change	2.19(0.67)	1.51(0.45)	1.92(0.67)	1.15(0.46)	1.17(0.6)	0.65(0.46)	63.8(19.5)	50.6(22.3)
P (Test vs. Placebo Group)	0.0001‡		0.0001‡		0.002§		0.034‡	

*****: Paired t test; †: Wilcoxon sign rank test; ‡: Two sample t test; **§**: Man Whitney U test

Table 4. Mean (±SD) ($\times 10^4$ CFU/ml) number of *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* in the placebo and the test groups before and after treatment.

Group	A. actinomycetemcomitans			P. gingivalis			P. intermedia		
	Pre-treatment	Post-treatment	P value*	Pre-treatment	Post-treatment	P value*	Pre-treatment	Post-treatment	P value*
	Mean (SD) ($\times 10^4$CFU/ml)	Mean (SD) ($\times 10^4$CFU/ml)		Mean (SD) ($\times 10^4$ CFU/ml)	Mean (SD) ($\times 10^4$ CFU/ml)		Mean (SD) ($\times 10^4$ CFU/ml)	Mean (SD) ($\times 10^4$ CFU/ml)	
Placebo	6.68 (13.36)	5.23 (21.33)	0.161	157.95 (636.20)	61.03 (219.16)	0.086	274.23 (502.28)	15.14 (23.73)	0.0001
Test	19.42 (44.45)	1.62 (7.84)	0.003	89.08 (335.76)	5.81 (25.44)	0.021	739.73 (1372.74)	155.17 (784.22) ‡	0.0001