ORIGINAL ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Microbiological Evaluation of Minocycline Microspheres Effect on the Bacteria Causing Chronic Periodontitis Using Multiplex PCR and Viable Counting: An *in-Vivo* study

Marwa M. Azab [a,*], Marina N. Naguib[b], Hany K. Shalaby [c]

[a] Microbiology and Immunology Department, Faculty of Pharmacy, Suez Canal University, Ismaillia 41522, Egypt; [b] Dentist working at ministry of health, Cairo 11865, Egypt; [c] Periodontology and Oral Diagnosis Department, Faculty of Dentistry, Suez University, Suez 43511, Egypt.

Received on: 11. 05. 2022

Revised on: 28. 05. 2022

Accepted on: 30. 05. 2022

*Correspondence Author:

Tel: +01024299630

E-mail address:

marwaazab2515@yahoo.com

Abstract

Periodontitis is an inflammatory infection of the periodontal tissue that, if left untreated, can lead to tooth loosening and, finally, tooth loss. The current study goals to identify the effectiveness of minocycline microspheres (Arestin) in the cure of chronic periodontitis, where forty chronic periodontitis patients (17 males and 23 females) between the ages of 20 and 45 were included. Patients were randomly assigned to one of two groups. 20 patients in Group I (the test group) received Arestin-based scaling and root planning (SRP) therapy (Minocycline HCl microspheres). 20 patients in Group II (Control group) who received only scaling and root planning (SRP) treatment. In order to plating on (non-selective) Anaerobic Thioglycolate Medium Base (ATMB) agar plates supplemented with horse serum (5 percent) and kanamycin-vancomycin-bacitracin, which is used for the selective recovery of obligatory Gram-negative rods (anaerobic) for colony forming unit calculations, microbiological samples were taken from both groups before treatment (baseline) and on days 30, 90, and 180 after treatment. A Multiplex PCR was used to amplify the bacterial DNA from *Tannerella forsythia (T.f.), Aggregatibacter actinomycete mcomitans (A.a.), Treponema denticola (T.d.),and Porphyromonas gingivalis (P.g.).* Groups I and II had significantly lowed microbiological analysis. For individuals with chronic periodontitis, scaling and root planning using Arestin (microbeads containing minocycline hydrochloride) may be a novel and efficient therapeutic approach.

Keywords: Arestin, Chronic Periodontitis, Multiplex PCR, Tannerella forsythia

1. Introduction:

Periodontal tissue is a type of tissue that surrounds, supports, and holds the upper and lower jaw teeth in place. It is composed of four major parts: gum, periodontal tissue, alveolar bone, and cementum **(Savage *et al.*, 2009).**

Untreated periodontitis, an inflammatory condition that affects the periodontium, can cause teeth to become loose and eventually fall off. It is consider now one of the most common oral diseases. Periodontitis can be caused by subgingival Gram-negative bacteria, such as *Tannerella forsythia (T.f.), Aggregatibacter actinomycete mcomitans (A.a.), Treponema denticola (T.d.),and Porphyromonas gingivalis (P.g.)* **(Dahan *et al.*, 2001).**

These pathogens coexist with countless other species in a highly organized plaque biofilm that colonizes, adheres to, and develops on the surface of teeth. This biofilm, known as plaque, comprises bacterial colonies that are embedded in a glycocalyx matrix. By preventing harmful substances and antibiotics from entering the biofilm, this matrix safeguards the microbes **(Dahan *et al.*, 2001).**

These bacteria's pathogenesis may involve the immediate release of proteolytic enzymes, the production of toxins like lipopolysaccharide that cause the expression of degradable enzymes, and the activation of degradative pathways by the release of cytokines from lymphocytes and macrophages as a result of the formation of an immune response **(Dahan *et al.*, 2001).**

Scaling and debridement, a non-surgical cleaning technique used to remove calculus and microbiological plaque from below the gum line, is the initial treatment of periodontitis. Then, under local anesthesia, root planning is performed with specialized curettes to improve periodontal health, but it is rarely completely successful in eliminating plaque or periodontal pathogens **(N. Jain *et al.*, 2008)**.

Periodontal surgery considered a necessary step to stop the loss of bone and to repair bone if nonsurgical treatment is unsuccessful. Advanced periodontitis is treated surgically using a variety of techniques, such as guided tissue regeneration, open flap debridement, osseous surgery, autografts, and allografts. However, these procedures have some drawbacks, including pain, swelling, and a lengthy healing period for the tissues **(Schwach-Abdellaoui *et al.*, 2000)**. Mechanical debridement has some drawbacks, such as difficulty accessing areas like root concavities and small furcation **(Ramesh *et al.*, 2016)** and other extra-dental sites that may act as bacteria reservoirs, causing early bacterial colonization of tooth surfaces after instrumentation; for this reason, the adjunctive use of chemotherapy drugs like antibiotics has been recommended **(Cortelli *et al.*, 2008).**

Antibiotics are given systemically or locally. The disadvantages of systemic administration include the emergence of resistant flora, suppression of natural flora, poor patient compliance, and more. Local drug delivery systems strips, (fibers, gels, films, and semisolid with micro or nanoparticles, vesicular systems, etc....) are controlled intra-pocket devices and systems are preferred **(N. Jain *et al.*, 2008).** Since the pathogens-specific drug can be applied directly to the periodontal pocket to achieve high concentrations, local drug administration in periodontology is thought to be more effective **(Schwach-Abdellaoui *et al.*, 2000).**

Minimally invasive dentistry is popular these days; therefore, using local drug delivery systems in periodontal diseases has increased avoiding massive surgical procedures. Tetracyclines, doxycycline, metronidazole, amoxicillin, and amoxicillin-clavulanate are just a few of the many antibiotics that can treat periodontitis **(Ramesh *et al.*, 2016).**

A tetracycline semi-derivative known as minocycline is effective against a variety of gram-positive and gram-negative anaerobes, including infections linked to adult periodontitis. As an anti-collagenase, minocycline prevents the formation of proteins in bacterial cell walls **(Cortelli *et al.*, 2008).**

Three different drug delivery methods for minocycline are available: as a microencapsulated microsphere (Arestin), a gel (Dentomycin), and an ointment form (periocline) **(Javali M. A *et al.*, 2010).**

Arestin is a bioabsorbable polymer microencapsulated with controlled, non-systemic release minocycline hydrochloride (polyglycolide-co-dl lactide). These microspheres are injected into the affected periodontal areas as a powder, microspheres range in size from 20 to 60 µ **(Williams *et al.*, 2001).**

After administration, Arestin immediately adheres to the periodontal pocket. The polymer is hydrolyzed by gingival crevicular fluid, which results in the formation of water-filled channels inside the microspheres. For continuous release of the encapsulated minocycline, these holes provide exit channels. Through the channels, the active medication can disintegrate and diffuse from the microspheres into the surrounding tissues **(Wilder, 2002).**

2. Materials and methods

2.1. The study's methodology and participant requirements:

The forty chronic periodontitis patients who took part in the current study with age ranged from 20 - 45, with a mean age of 33. There were 17 men and 23 women. The patients in two equal study groups were of comparable age range, with 33 ($\pm$8), 32.5 ($\pm$8) for the control group and test group, respectively. Subjects for this single-blind study were selected from dentist clinics. All clinical examinations were performed by the same

examiner. Inclusion criteria included: (1) systemically healthy patients; (2) without periodontal therapy before the clinical trial (6 months); (3) patients with moderate periodontitis and periodontal pockets between 5-7 mm, clinical attachment loss 3-5mm. Exclusion criteria included: (1) patients who have systemic diseases; (2) patients with the gingival recession; (3) pregnant and lactating female patients; (4) smokers; (5) patients who have taken antibiotics less than 6 months before the clinical trial; (6) history of allergy to minocycline or tetracycline derivatives. Patients were randomly split into two groups: group I (Arestin group) which contain 20 patients who received conventional periodontal treatment, including full mouth SRP plus Arestin applied on the first day and first month postoperatively; and group II (SPR group**)** which contain 20 patients, received conventional periodontal treatment (full mouth SRP) on the first day and first month postoperatively.

2.2. Method of therapeutic agent application:

At each treatment site, the dispenser tip was put sub-gingivally until the base of the pocket, until resistance is felt. Then the handle mechanism is pressed to dispense the unit dose of microspheres while gradually removing the tip coronally away from the pocket's base.

Arestin (Orapharma, USA) is a bioresorbable polymer of microspheres with non-systemic release that contains minocycline HCL and doesn't need to be removed or adhered or dressed.

2.3. Sample collection, viable counting, DNA extraction, and PCR processing:

After carefully removing all supragingival plaque, tooth samples were isolated using cotton and subgingival plaque isolates were collected with a sterile curette. The periodontal curette was inserted into the pocket with one motion and moved coronally while scraping all sub-gingival plaque from the denuded root surface before being placed in different Eppendorf tubes with 200µl of sterile saline (phosphate-buffered) **(Sambrook & Russell, 2001).**

The samples were plated on non-selective Anaerobic Thioglycolate Medium Base (ATMB) agar plates with 5% horse serum and kanamycin-vancomycin-bacitracin as supplements, which is used to recover obligatory anaerobic Gram-negative rods. Plates incubated for 7 days at 37°C in an an-aerobic chamber (80/10/10, $N_2/H_2/CO_2$). The plates were examined for colonies after 7 days, and results were collected as a count of CFUs. For accuracy, each sample triplicate plate was kept, and the mean of all three plates was recorded **(Slots, 1982).** The collected samples were used for a genomic bacterial DNA extraction using QIAamp DNA Minikit (Cat. No.51304) purchased from QIAGEN group-Germany. The bacterial DNA from A.a., P.g., and T.f., was amplified using a multiplex PCR (T.d.). Every bacteria had its own forward primer, and a conserved reverse primer was used **(Trans SD, 1999).**

PCRs were performed in a 50 µl final volume. The master mix composed of 15 µl molecular biology water, 5 µl sample, 1 µl primer *A.a*. forward 10 pMol, 1µl primer *P.g*. forward 10 pMol, 1 µl primer *T.f.* forward 10 pMol, 2 µl conserved reverse primer 10 pMol, 25 µl readymade master mix genedirex (Cat. No. DM101-0100), lengths of the anticipated products for *A.a.* were 360 bp, *T.f.* 745 bp, and *P.g.* 197 bp. According to previous research, forward and reverse primers were used to *detect T. denticola* (T.d.) obtaining a 300 bp amplicon **(Gillespie *et al.*, 1993)**. The master mix with a final volume of 50 µl, included molecular biological water (18 µl), DNA sample (5 µl), and 1 µl for every forward and reverse primer (10 pMol/each), and 25l ready-made master mix genedirex (Cat. No. DM101-0100). The cycling conditions were 35 cycles: 1 minute (94°C) 1 minute (61ºC), and 1 minute (72ºC), a 15-minute denaturation step (94ºC) preceding the amplification cycles and a 7-minute final step (72ºC). The DNA product was observed in 2% agarose gel (Sigma, USA) at the end of the amplification process and was visualized under transillumination after ethidium bromide (Sigma, USA) staining, as shown in **table 1**.

2.4. Statistical Analysis:

One of the following tests was used to determine whether a difference was significant in the statistical comparison between the several groups:

- Student's t-test (Unpaired): For comparing the mean of two sets of numerical data (The parametric data).
- analysis of variance by Repeated measures ANOVA: For comparing among more than two related groups of numerical (The Parametric) data followed by post-hoc LSD
- Inter-group comparison of categorical data (Performed by using Pearson's chi-square test; *X2*-value) or fisher exact when indicated.

A P value <0.05 was used to determine statistical significance.

Table 1: Primer sets for 16S rRNA gene amplification for A.a., P.g., and T.f.

Bacteria name	**Primers**	**Amplicon**
Aa	(5'-TACAGGGGAATAAAATGAGATACG-3')	360 bp
Pg	(5'-ATTGGGGTTTAGCCCTGGTG-3')	197 bp
Tf	(5'-TACAGGGGAATAAAATGAGAT CG-3')	745 bp
Reverse Aa, Pg, Tf,	(5'-ACG TCA TCC CCA CCT TCC TC-3')	
Td	(5'-TAA TACCGAATGTGCTACTTTACAT-3')	300 bp
Reverse Td	(5´-TCAAAGAAGCATTCCCTCTTCTTCTTA-3')	

3- Results:

In the present research, 40 chronic periodontitis patients 17 (men) and 23 (women) with a mean age of 20 to 45 years were enrolled (33). Prior to treatment (baseline), as well as days 30, 90, and 180 after treatment for the two groups, microbiological samples were taken.

Viable counting:

The mean and standard deviation from the initial value for the infection group were 4090 ± 250. The SRP group's mean and standard deviation on day 30 were (490 ± 80) for the SRP group and 320 ± 30 for the Arestin group. On day 90, the mean and standard deviation for the SRP group were 340 ± 30, whereas those for the Arestin group were 120 ± 20. At day 180, the mean and standard deviation for the SRP group were (170 ± 20), while those for the Arestin group were (40 ± 10).At the beginning, colonies x3 in the two groups did not differ statistically significantly from one another, according to **table 2 and figure 1**. While, at days 30 and 90; There was a noticeable difference (P<0.05) in colonies x3 mean of the Arestin group (0.32 ± 0.03, 0.12 ± 0.02) when compared with SRP group (0.49 ± 0.08, 0.34 ± 0.03) respectively. On day 180, the Arestin group's colonies x3 mean (0.04 ± 0.01) had a highly significant difference (P <0.001) from the SRP group's (0.17 ± 0.02) colonies.

Table 2: Comparison of (colonies x3) between the two groups at different times

		Groups		**Independent t-test**
		SRP group	**Arestin group**	
		Mean ± SD	**Mean ± SD**	***p*-value**
Colonies x3	**Baseline**	4.09±0.25	4.09±0.25	>0.999 ns
	day 30	0.49±0.08	0.32±0.03	<0.05*
	day 90	0.34±0.03	0.12±0.02	<0.05*
	day 180	0.17±0.02	0.04±0.01	<0.001**

Data are expressed as mean standard deviation (mean±SD), P: Probability, *: significant (p<0.05), **: highly significant (P <0.001), Unpaired Student's t-test was used.

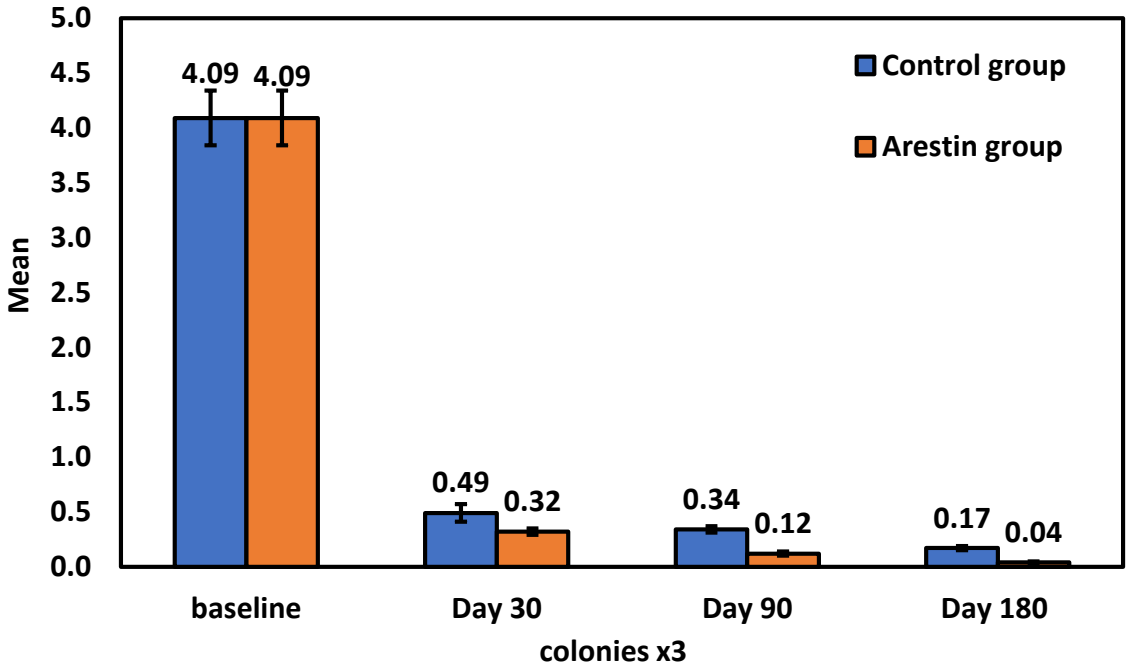


Figure 1: Bar chart representing mean values of colonies x3 in the two groups.

According to **table 3** and **figure 2**, In Arestin group; There was a high significant decrease (P<0.001) of colonies x3 mean at days 30, 90 and 180 (0.32±0.03, 0.12±0.02, 0.04±0.01) respectively, when comparing each of them with colonies x3 mean at baseline (4.09±0.25). In SRP group, there was a statistically significant decline (P <0.001) of colonies x3 mean at days 30, 90 and 180 (0.49±0.08, 0.34±0.03, 0.17±0.02) when comparing each of them with colonies x3 mean at baseline (4.09±0.25). The results are shown as means with standard deviations (mean ± SD), probability (P), significant (*): (P <0.05), highly significant (**) (P<0.001), and using a test One-way ANOVA, post-hoc Tukey analysis, and P1: significance between baseline and day 30, P2: significance between baseline and day 90, and P3: significance between baseline and day 180.

Table 3: Changes of colonies x3 mean by time within each group

Group		baseline	day 30	day 90	day 180
Arestin group	**Mean±SD**	4.09 ± 0.25	0.32 ± 0.03	0.12 ± 0.02	0.04 ± 0.01
	Post-hoc		P1 <0.001**	P2 <0.001**	P3 <0.001**
SRP group	**Mean±SD**	4.09 ± 0.25	0.49 ± 0.08	0.34 ± 0.03	0.17 ± 0.02
	Post-hoc		P1 <0.001**	P2 <0.001**	P3 <0.001**

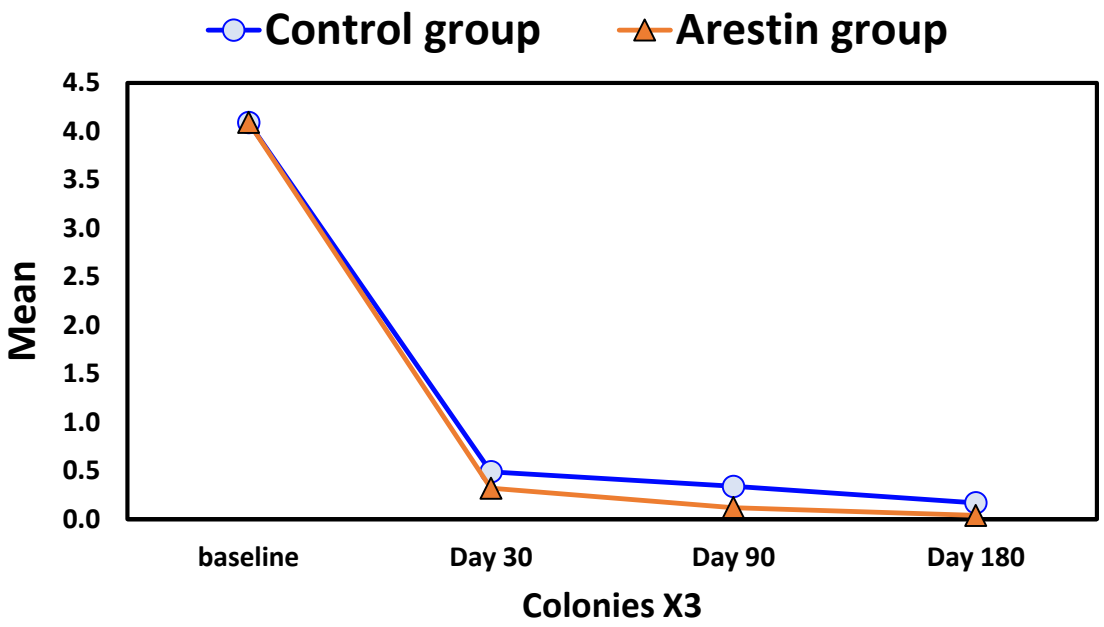


Figure 2: Line chart representing mean values of colonies x3 in the two groups

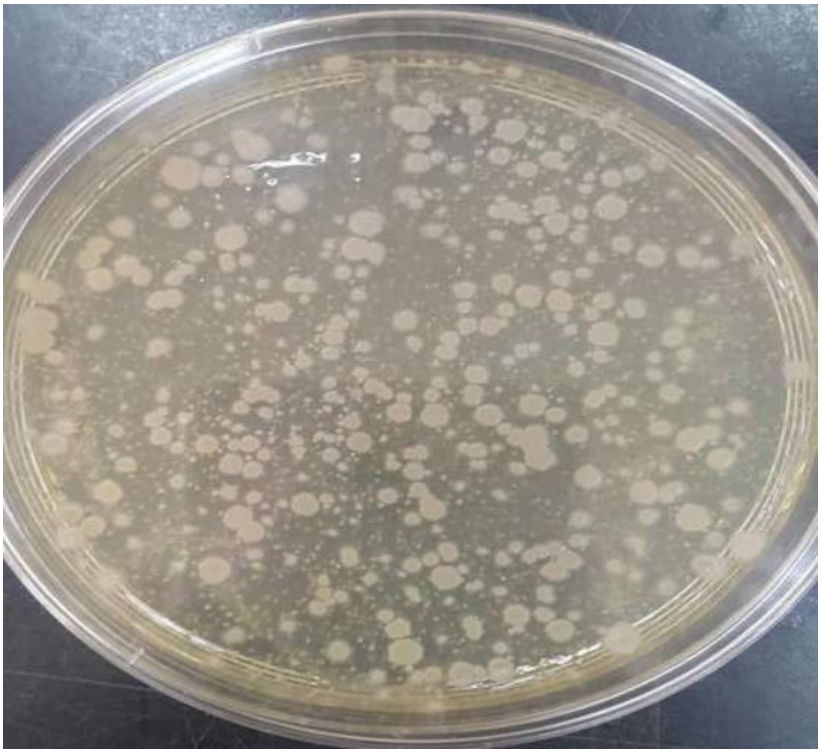


Figure 3: Bacterial growth at baseline

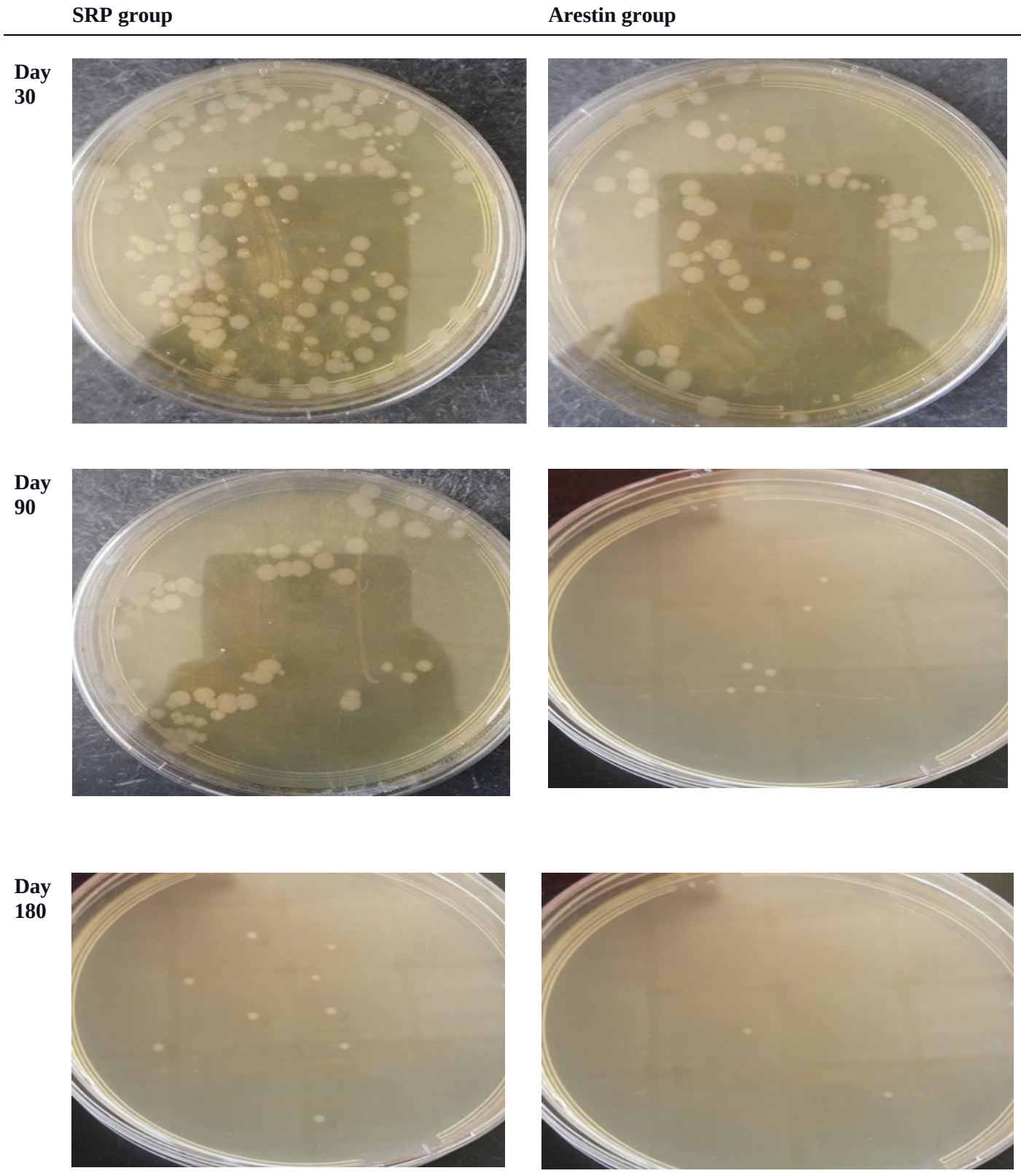


Figure 4: Change in bacterial growth by time within each group.

Polymerase Chain Reaction:

According to **table 4** and **figures (5, 6, 7, and 8)**: The presence of the four pathogens under study was not significantly different between groups according to the initial data: The detecting frequency of A.a. in both groups was less than 40%, and 100% of the subjects in both groups tested positive for T.f., P.g., and Td. At days 30, 90, both groups experienced a considerable reduction in the percentage of P.g.-positive sites, but the experimental group experienced a noticeably larger decrease. At day 180, both groups' percentages of P.g.-positive sites decreased, but the reduction in the experimental group was greater. At days 30, 90, and 180, both the experimental group and the control group experience a decrease in percentage of T.f., A.a., and T.d. positive sites, with the Arestin group experiencing a greater decrease.

Table 4: Comparison of the number and proportion of pathogen-positive sites between the two groups at various times

Pathogen	Baseline		Day 30		Day 90		Day 180	
	Arestin	**SRP**	**Arestin**	**SRP**	**Arestin**	**SRP**	**Arestin**	**SRP**
Pg	20(100%)	20(100%)	6(30%)	13(65%)	3(15%)	10(50%)	2(10%)	8(40%)
P	1.00		0.027*		0.04*		0.065	
Aa	8(40%)	7(35%)	6(30%)	5(25%)	2(10%)	3(15%)	1(5%)	2(10%)
P	1.00		1.00		1.00		1.00	
Tf	20(100%)	20(100%)	7(35%)	10 (50%)	4(20%)	7(35%)	2(10%)	5(25%)
P	1.00		0.33		0.48		0.4	
Td	20(100%)	20(100%)	10(50%)	11(55%)	7(35%)	10(50%)	9(45%)	5(25%)
P	1.00		1.00		0.33		0.32	

Data stated as frequency (Number percent), P: Probability, *: significance < 0.05, **: high significance, used test: Pearson's chi-square or fisher exact when indicated

According to the **table 5** and **figures (5, 6, 7, and 8)**: In the Arestin group; When compared to the percentage of positive sites at baseline (20=100%), a highly significant decrease (P <0.05) in the number and percentage of P.g. positive sites at days 30, 90, and 180 (6=30%, 3=15%, and 2=10%), respectively. a highly significant decrease (P <0.05) in the number and percentage of positive sites for the A.a. at days 30, 90, and 180 (6=30%, 2=10%, 1=5%) respectively when comparing them with the percentage of positive sites at baseline (8=40%). A highly significant decrease (P <0.001) in the number and percentage of positive sites for the T.f. at days 30, 90, and 180 (7=35%, 4=20%, and 2=10%) respectively when comparing them with the percentage of positive sites at baseline (20=100%). There was an extremely significant reduction (P<0.001) of positive sites for the T.d. in both the percentage and number at days 30, 90, and 180 (10=50%, 7=35%, and 5=25%) respectively when comparing them with the percentage of positive sites at baseline (20=100%).

In the SRP group: A highly significant decline in significance was noticed (P<0.001) in positive sites for the P.g. in the number and percentage at days 30, 90, and 180, Pg (13=65%, 10=50%, and 8=40%) respectively when comparing them with the percentage of positive sites at baseline (20=100%). No significant difference in the percentage and number of positive sites for A.a. at days 30, 90, and 180 (5=25%, 3=15%, and 2=10%) respectively when comparing them with the percentage of positive sites at baseline (7=35%). A highly significant decrease (P<0.001) in the percentage and number of positive sites for the T.f. at days 30, 90, and 180 (10=50%, 7=35%, and 5=25%) respectively when comparing them with the percentage of positive sites at baseline (20=100%). There was a significant decrease (P<0.001) in the number and percentage of positive sites for the T.d. at days 30, 90, and 180 (11=55%, 10=50%, and 9=45%) respectively when comparing them with the percentage of positive sites at baseline (20=100%).

Table 5: Changes over time in each group's total number and percentage of pathogen-positive sites

	Baseline	Day 30	Day 90	Day 180	P
Pg					
Arestin group	**20 (100%)**	**6 (30%)** **P1 <0.001****	**3 (15%)** **P1 <0.001**** **P2=0.45**	**2(10%)** **P1<0.001**** **P2 = 0.11** **P3 = 1.00**	**<0.001****
SRP group	**20 (100%)**	**13 (65%)** **P1<0.05***	**10 (50%)** **P1 <0.001**** **P2=0.33**	**8 (40%)** **P1 <0.001**** **P2=0.11** **P3=0.52**	**<0.001****
Aa					
Arestin group	**8 (40%)**	**6 (30%)** **P1 = 0.74**	**2 (10%)** **P1 = 0.065** **P2 = 0.2**	**1 (5%)** **P1<0.05*** **P2 = 0.09** **P3 = 1.00**	**0.01***
SRP group	**7 (35%)**	**5 (25%)** **P1=0.7**	**3 (15%)** **P1=0.27** **P2=0.69**	**2 (10%)** **P1=0.12** **P2=0.4** **P =1.00**	**0.22**
Tf					
Arestin group	**20 (100%)**	**7 (35%)** **P1<0.001****	**4 (20%)** **P1<0.001**** **P2=0.33**	**2 (10%)** **P1<0.001**** **P2 = 0.12** **P3 = 0.66**	**<0.001****
SRP group	**20 (100%)**	**10 (50%)** **P1 <0.001****	**7 (35%)** **P1 <0.001**** **P =0.33**	**5 (25%)** **P1 <0.001**** **P2=0.19** **P3=0.73**	**<0.001****
Td					
Arestin group	**20 (100%)**	**11 (55%)** **P1 <0.05***	**10 (50%)** **P1 <0.001**** **P2=0.75**	**9 (45%)** **P1<0.001**** **P2=0.52** **P3=0.75**	**0.001***
Control group	**20 (100%)**	**11 (55%)** **P1 <0.001***	**10 (50%)** **P1 <0.001**** **P2=0.75**	**9 (45%)** **P1<0.001**** **P 2=0.52** **P3=0.75**	**0.001***

Data are expressed as a frequency (percentage), P: Probability, *: significance <0.05, **: high significance. When showed, Fisher exact or Pearson's chi-square were used for tests. P1 represents significance vs. baseline, P2 represents significance vs. Day 30, and P3 represents significance vs. Day 90.

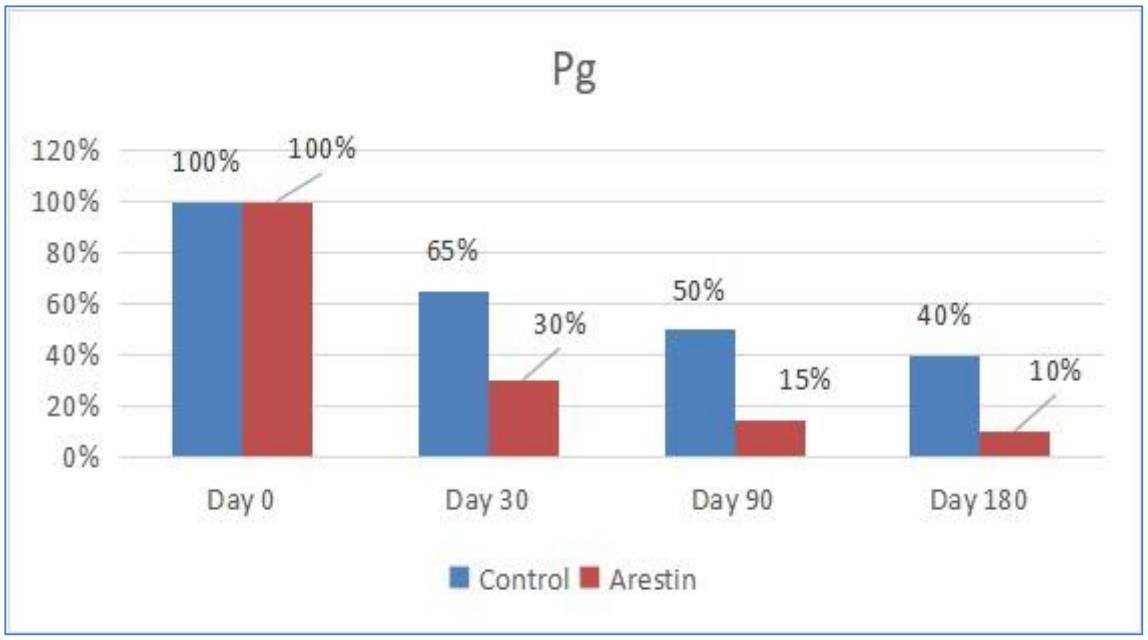


Figure 5: The percentage of positive Pg sites in the groups (Bar Chart).

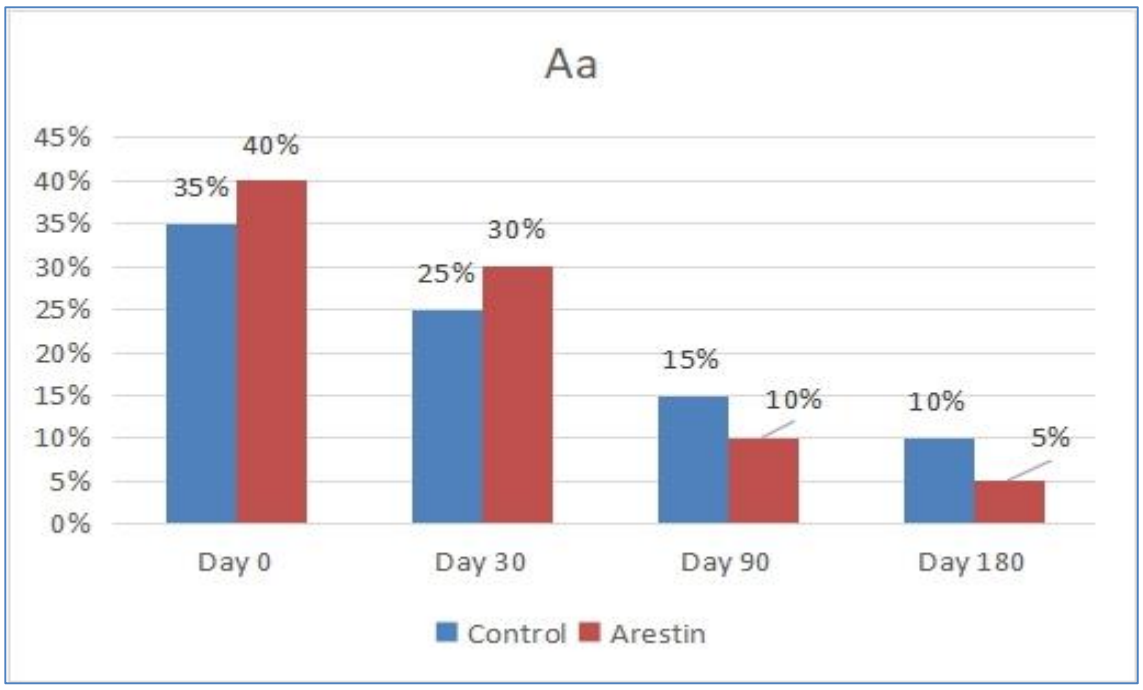


Figure 6: The percentage of Aa positive sites in the two groups (Bar Chart).

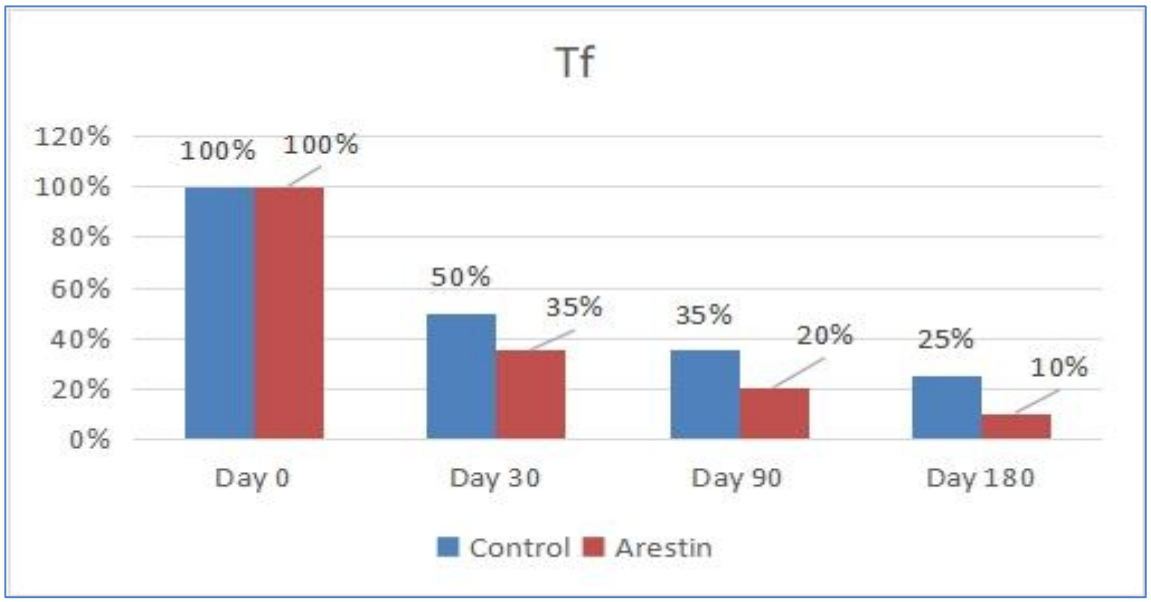


Figure 7: The percentage of Tf positive sites in the two groups (Bar Chart).

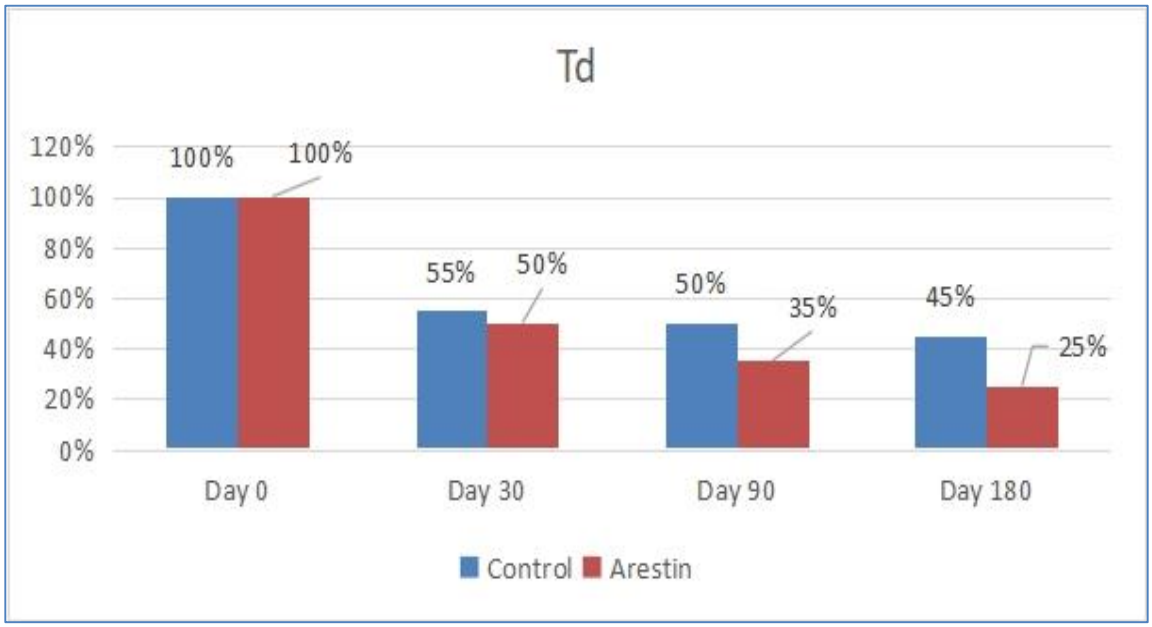
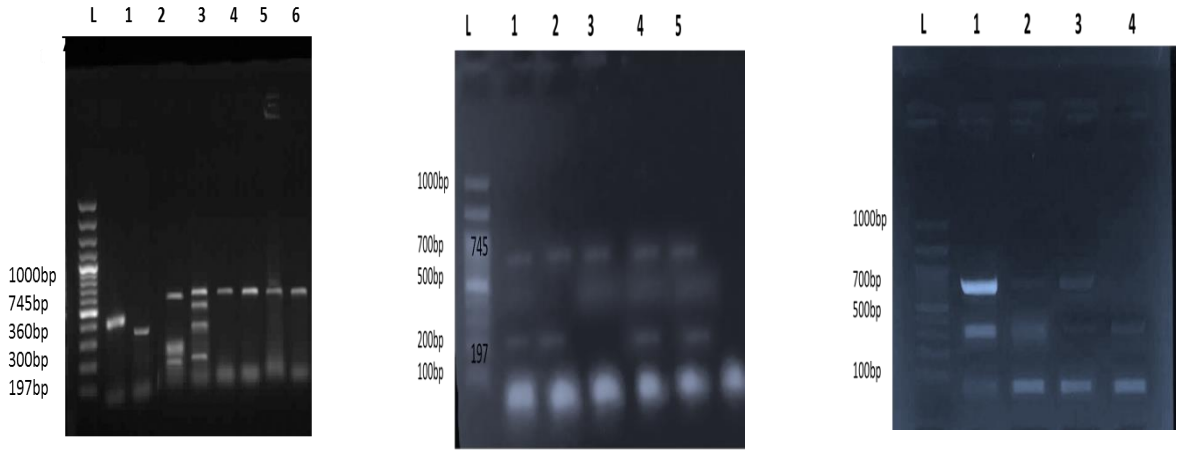
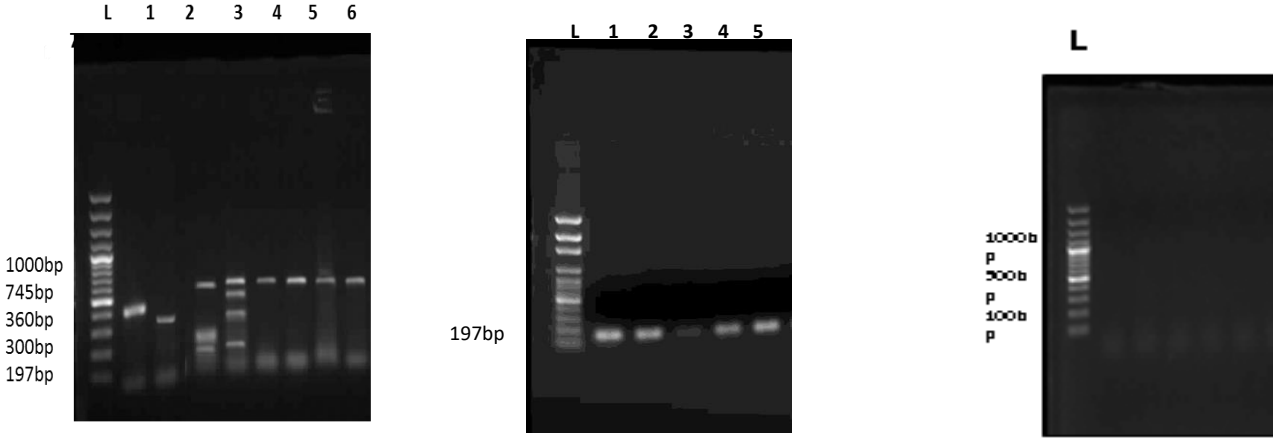


Figure 8: The percentage of Td positive sites in the two groups (Bar Chart).



A)Day 0 B) 1 and 3 months. c) 6 months

Figure 9: Showing positive sites for the pathogens on the Agarose gel in the SRP group



A)Day 0 B) 1 and 3 months. c) 6 months

Figure 10: Showing positive sites for the pathogens on the Agarose gel in Arestin group.

4. Discussion:

It is well known that pathogenic microorganisms present in the subgingival area cause most destructive types of periodontal infection, and inhibiting or eradicating these microorganisms improves periodontal health **(R. Jain *et al.*, 2012).**

Local antibiotic delivery into the pocket results in greater retention of highly concentrated drug within target tissue; it can achieve a 100 fold higher concentration of an anti-microbial agent in sub-gingival sites, providing bactericidal effect for most peri-pathogens while having no effect on microflora living in other parts of the body **(Haffajee *et al.*, 1995; Kafle *et al.*, 2018).**

Minocycline is a semi-synthetic tetracycline derivative with a larger spectrum of action **(Atilla G., Balcan *et al.*, 1996).** Minocycline's method of action is like that of other tetracyclines: it binds to bacterial ribosomal subunits and stops proteins from synthesizing (**Asadi *et al.*, 2020).** Minocycline concentrations in human cleavage fluid were also observed to be higher than the minimum inhibitory concentrations (MICs) for periodontal bacteria following 14 days of local injection using (Arestin) **(Williams RC *et al.*, 2001).**

It was proposed that healing of diseased periodontal pockets is induced by the possibility of minocycline absorbing to calcified dental structures, where it may operate as a transitory reservoir of the antimicrobial agent during the substantial phase **(Pandit *et al.*, 2013)**.

The purpose of this study was to determine whether Arestin might be employed as a local drug delivery strategy in addition to scaling and root planing in the treatment of chronic periodontitis.

In contrast to previous studies that did not measure CFUs, CFUs were measured in this study at baseline, after SRP, as well after Arestin medication usage. The test site had a statistically significant drop in CFUs when compared to the control site.

Regarding microbiological analysis in the present study after 1,3 as well as 6 months, Arestin group showed higher statistically significant mean % reduction of positive sites compared with SRP group, this can be attributed to being minocycline HCL (a semi-synthetic tetracycline), which is effective against *A.a., T.f., P.g., and T.d.*. In addition to being antibacterial, it inhibits collagenase, which is produced by *P. g.*, neutrophils, and fibroblasts from periodontal tissues **(Miyake *et al*., 1995)**. Minocycline, like all tetracyclines, exhibits substantivity, adsorption, and subsequent desorption from dentine while retaining antimicrobial activity **(Ramesh *et al.*, 2016).**

The reduction in bacteria in the Arestin group could be because of minocycline, which has many advantages over other tetracyclines, including anti-inflammatory and antibiotic properties, improved absorption, increased antimicrobial activity, and negligible toxicity. Minocycline is fat soluble, so it can easily enter various bodies. Periodontal sulcus fluid and saliva, for example, can act locally at the site of infection **(Bacha, 2014)**. The reduction in bacterial positive sites in the SRP group could be attributed to the removal of bacterial deposits; patient plaque control could have resulted in favorable subgingival microbial changes **(M. Jain *et al.*, 2013).**

This finding was consistent with Deng S et al. **(Deng S et al., 2015)**, who conducted a study to investigate the independent or combined efficacy of locally applied 2 percent minocycline and SRP in periodontitis (chronic periodontitis) treatment by assessing both clinical parameters and loads of four major periodontal bacteria. Although SRP was previously thought to be the gold standard treatment for CP, only Pg among the four selected periodontal pathogens showed a statistically significant reduction in response to SRP alone, and Pg's sensitivity to SRP was significantly reduced when compared to minocycline administered locally. Pg was more sensitive to minocycline than Fn, but Aa and Pi had no reaction. Aa had the lowest prevalence of the four bacteria studied, which was consistent with previous findings **(Bacha, 2014; Verónica B. Chiappe *et al*., 2015).** This could be because Aa is more closely linked to aggressive periodontitis than CP. Goodson JM et al. discovered similar results **(J. Max Goodson *et al*., 2007; Rylev M, 2008).**

This was in line with the findings of SersosoY et al. **(Y Soeroso et al., 2017)**, who carried out a study to assess the clinical and microbiological impact of a 2 percent minocycline HCl gel applied locally as an adjunct to SRP to treat chronic periodontitis. Both groups showed a decrease in germ quantity two months after baseline. T.d. and T.f. count changes in the test group (2-6 months) were substantially lower in the experimental group than in the control group. There was significant regrowth of P.g., T.f., and T.d. in the control group from 2 to 6 months, and of P.g. and T.f. from 3 to 6 months. The number of the three bacteria in the test group increased significantly over the course of six monthsAlthough SRP was helpful in temporarily decreasing bacteria, it may not be effective in suppressing the re-growth of three peri-pathogenic bacteria without treatment in 6 months.

Chiappe.V et al. (Verónica B. Chiappe et al., 2015) discovered a similar result when they evaluated the microbiological effects of su-bgingival minocycline micro-granules used as an adjunct to SRP compared to SRP alone in chronic periodontitis patients and concluded that at days 30 and 90 Pg, Tf, Td, and Aa were higher with SRP. There was a decrease in both groups at day 90, Pg decrease was significantly greater in the SRP with minocycline micro-granules group, but the number of sites with Td was lower. ($p < 0.05$).

5. Conclusion

Minocycline microspheres (Arestin), when used with SRP to treat chronic periodontitis, showed statistically significant results that were better than SRP alone.

6. References:

Asadi, A., Abdi, M., Kouhsari, E., Panahi, P., Sholeh, M., Sadeghifard, N., Amiriani, T., Ahmadi, A., Maleki, A., & Gholami, M. (2020). Minocycline, Focused on mechanisms of resistance, antibacterial activity, and clinical effectiveness; Back to Future. *Journal of Global Antimicrobial Resistance*, 73(2), 270-277.

Atilla G., Balcan M., Bicakei N., K. A. (1996). The effect of nonsurgical periodontal and adjunctive minocycline hydrochloride treatments on the activity of salivery proteases. *J Periodontol*, *61*, 1–6.

Bacha, P. M. K. and S. (2014). Systemic antimicrobial therapy (minocycline) as an adjunct to non-surgical approach to recurrent chronic generalized gingival hyperplasia. *J Indian Soc Periodontol*, *18*(2), 249–253.

Cortelli, J. R., Aquino, D. R., Cortelli, S. C., Carvalho-Filho, J., Roman-Torres, C. V. G., & Costa, F. O. (2008). A double-blind randomized clinical trial of subgingival minocycline for chronic

periodontitis. *Journal of Oral Science*, *50*(3), 259–265.

Dahan, M., Nawrocki, B., Elkaïm, R., Soell, M., Bolcato-Bellemin, A., Birembaut, P., & Tenenbaum, H. (2001). Expression of matrix metalloproteinases in healthy and diseased human gingiva. *Journal of Clinical Periodontology*, *28*(2), 128–136.

Deng S, Wang Y, Sun W, Chen H, W. G. (2015). Scaling and root planning and locally delivered minocycline could reduce the load of Prevotella intermedia in an interdependent pattern, which correlated with symptomatic improvements of chronic periodontitis: a short-term randomized clinical trial. *Therapeutics and Clinical Risk Management*, *11*, 1795–1803.

Gillespie, M. J., Smutko, J., Haraszthy, G. G., & Zambon, J. J. (1993). Isolation and partial characterization of the Campylobacter rectus cytotoxin. *Microbial Pathogenesis*, *14*(3), 203–215.

Haffajee, A. D., Dibart, S., Kent Jr, R. L., & Socransky, S. S. (1995). Clinical and microbiological changes associated with the use of 4 adjunctive systemically administered agents in the treatment of periodontal infections. *Journal of Clinical Periodontology*, *22*(8), 618–627.

J. Max Goodson, John C. Gunsolley, Sara G. Grossi, Paul S. Bland, Joan Otomo-Corgel, I., & Frances Doherty, and J. C. (2007). Minocycline HCl Microspheres Reduce Red-Complex Bacteria in Periodontal Disease Therapy. *J Periodontol*, *78*(1568–1579).

Jain, M., Dave, D., Jain, P., Manohar, B., Yadav, B., & Shetty, N. (2013). Efficacy of xanthan based chlorhexidine gel as an adjunct to scaling and root planing in treatment of the chronic periodontitis. *Journal of Indian Society of Periodontology*, *17*(4), 439.

Jain, N., Jain, G. K., Javed, S., Iqbal, Z., Talegaonkar, S., Ahmad, F. J., & Khar, R. K. (2008). Recent approaches for the treatment of periodontitis. *Drug Discovery Today*, *13*(21–22), 932–943.

Jain, R., Mohamed, F., & Hemalatha, M. (2012). Minocycline containing local drug delivery system in the management of chronic periodontitis: A randomized controlled trial. *Journal of Indian Society of Periodontology*, *16*(2), 179.

Javali M. A, S. Bhudevamma, A. Fathima, H. A. (2010). Localy Delivery of Antimicrobials in Periodontics : A Comprensive Review. *Heal Talk*, *3*(2), 35–38.

Kafle, S., Pradhan, S., & Gupta, S. (2018). Locally Delivered Tetracycline Fibres in the Treatment of Chronic Periodontitis. *Journal of Nepalese Society of Periodontology and Oral Implantology*, *2*(2), 45–48.

Miyake Y, Tsuruda K, Okuda K, Widowatti, I. Y., & H, S. (1995). In vitro activity of tetracyclines, macrolides, quinolones, clindamycin, and metronidazole against periodontopatghic bacteria. *J Periodont Res*, *30*, 290–293.

Pandit, N., Dahiya, R., Gupta, R., Bali, D., & Kathuria, A. (2013). Comparative evaluation of locally delivered minocycline and metronidazole in the treatment of periodontitis. *Contemporary Clinical Dentistry*, *4*(1), 48.

Ramesh, A., Prakash, A. P., & Thomas, B. (2016). Local Drug Delivery in periodontal diseases…… A Review. *Journal of Health and Allied Sciences NU*, *6*(01), 74–79.

Rylev M, K. M. (2008). Prevalence and distribution of principal periodontal pathogens worldwide. *J Clin Periodontol*, *35*, 346–361.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: a laboratory manual, 854 Cold Spring Harbor Laboratory Press. *New York*, *855*.

Savage, A., Eaton, K. A., Moles, D. R., & Needleman, I. (2009). A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *Journal of Clinical Periodontology*, *36*(6), 458–467.

Schwach-Abdellaoui, K., Vivien-Castioni, N., & Gurny, R. (2000). Local delivery of antimicrobial agents for the treatment of periodontal diseases. *European Journal of Pharmaceutics and Biopharmaceutics*, *50*(1), 83–99.

Slots, J. (1982). Selective medium for isolation of Actinobacillus actinomycetemcomitans. *Journal of Clinical Microbiology*, *15*(4), 606–609.

Trans SD, R. J. (1999). Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection for actinobacillus actinomycetemcomitans, Bacteriodes Forsythus and porphyromonas gingivalis. *J Clin Microbiol*, *37*, 3504–3508.

Verónica B. Chiappe, Mariel V. Gómez, C. R., & Marilina Fresolone, Adalí Pecci, H. J. R. (2015). Subgingivaly applied minocycline microgranules in sugjects with chronic periodontitis. A randomized

clinical and microbiological trial. *Acta Odontol. Latinoam*, *28*(2), 122–131.

Wilder, R. S. (2002). Treating chronic periodontitis with the local delivery of minocycline microspheres. *Contemporary Oral Hygiene Jan/Feb*.

Williams, R. C., Paquette, D. W., Offenbacher, S., Adams, D. F., Armitage, G. C., Bray, K., Caton, J., Cochran, D. L., Drisko, C. H., & Fiorellini, J. P. (2001). Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. *Journal of Periodontology*, *72*(11), 1535–1544.

Williams RC, Paquette DW, Offenbacher S, Adams DF, Armitage GC, Bray K, Caton J, Cochran DL, D. C. and F. J. (2001). Treatment of periodontits by local administration of minocycline microspheres:a controlled trial. *J Periodontol*, *72*, 1535–1544.

Y Soeroso, T Akase, H Sunarto,, Y Kemal, 1 R Salim, M Octavia, A Viandita, J Setiawan, and B. B. (2017). The risk reduction of recurrent periodontal pathogens of local application minocycline HCl 2% gel, used as an adjunct to scaling and root planing for chronic periodontitis treatment. *Ther Clin Risk Manag*, *13*, 307–314.