

# An Investigation of Antibiotic Resistance of *Aggregatibacter Actinomycetemcomitans* and *Porphyromonas Gingivalis* in Peri-implantitis Lesions

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## Abstract

**Background and Aim:** It has been shown that anaerobic and capnophilic bacteria play an important role in implant failure and loss. The present study is an in vitro research aimed to investigate the antibiotic resistance of *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) in peri-implantitis lesions of Iranian patients and to find laboratory efficiency of some antibiotic on these two bacteria.

**Materials and Methods:** In this antibiogram study, the plaque samples were obtained from peri-implantitis lesions from patients who referred to implant center of Faculty of Dentistry of Tehran University of Medical Sciences. Nine samples incubated in Aa-specific culture media and 9 samples incubated in Pg-specific culture media under anaerobic and capnophilic conditions. After 48 hours, colonies were verified by microscopic and biomedical examination, and a colony-counting device. Then the specimens were cultured in the specific culture media for antibiogram evaluations by measuring the diameter of growth inhibition zone of antibiotic standard disks of amoxicillin, co-amoxiclav, metronidazole, tetracycline, clindamycin, and ciprofloxacin.

**Results:** There was no statistically significant difference ( $P=0.74$ ) between the colony count of Aa ( $84.56 \pm 16.65$ ) and Pg ( $87.67 \pm 21.49$ ). Most of growth inhibition zone ranged between 10 and 35 mm. The Pg specimens were significantly more resistant to studied antibiotics ( $P<0.05$ ) compared to Aa. However, both groups had similar resistance to amoxicillin and tetracycline, P-values were 0.22 and 0.13 respectively.

**Conclusion:** A large number of peri-implantitis lesions contain Aa and Pg bacteria. Moreover, the majority of Aa samples were sensitive to the applied antibiotics, while almost all Pg specimens were resistant to them.

**Key Words:** Peri-implantitis, Antibacterial Drug Resistance, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*

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Received: 2 Feb 2017  
Accepted: 28 Sep 2017

➤ Cite this article as: Kadkhoda Z, Torabi S, Aliramezani A. An Investigation of Antibiotic Resistance of *Aggregatibacter Actinomycetemcomitans* and *Porphyromonas Gingivalis* in Peri-implantitis Lesions. J Islam Dent Assoc Iran. 2017; 29(4):135-140. DOI: 10.30699/jidai.29.4.135

## Introduction

Peri-implantitis is the condition of pathological inflammatory changes that occur in the soft and hard tissues around an implant. It is a progressive inflammatory destruction of the alveolar bone, which presents as deepening of the pocket probing

depth around the implant and appears by bleeding and/or pus during probing [1]. Although the etiology of peri-implantitis has not been clearly identified, environmental factors, such as infected dental implants, cigarette smoking, history of periodontal disease, the implant soft-tissue

interface, genetic factors, systemic diseases, poor oral hygiene, lack of frequent dental visits, immune system imbalance, and diabetes are considered risk factors in its occurrence [2].

It has been suggested that the tissues surrounding the implant have weaker natural barriers, and less resistant to infection, compared to those of a tooth [3]. Bacterial infection plays an important role in oral implant failure. The bacterial flora that is present in peri-implantitis is similar to the flora that is observed in periodontal diseases [4]. Previous studies have shown that the bacterial flora in failing implants is gram-negative anaerobic bacteria, such as *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Aggregatibacter actinomycetemcomitans* (Aa), which are also pathogens of periodontal disease [1]. The composition of microbial flora present in peri-implantitis is far more complicated than that which is found around healthy implants [5]. Prevalence of peri-implantitis in patients with implants and in implants themselves has been reported as 18.8% and 9.6%, respectively [6].

The main treatment goals of peri-implant diseases are control of the infection and preventing disease progression. Previous studies have reported successful treatment of peri-implantitis through antimicrobial regimens in combination with surgical or non-surgical debridement [7]. Amoxicillin and clavulanic acid were recommended for surgical treatment of peri-implantitis because of some bacterial pathogen can potentially complicate these lesions [8]. Amoxiclav (or amoxicillin and clavulanic acid) is used in the treatment of infections when we have some mixed infection and some resistant to first and general antibiotic therapy (without using any antibiogram).

Rams et al. [2] studied some bacterial resistance in the peri-implantitis lesions and found that at in vitro conditions, *Prevotella intermedia/nigrescens* or *Streptococcus constellatus* were resistant to clindamycin, amoxicillin, doxycycline, and metronidazole in 46.7%, 39.2%, 25%, and 21.7% of the peri-implantitis subjects, respectively. In the in vitro conditions, only 6.7% subjects revealed resistant to both amoxicillin and metronidazole [2]. There are not enough laboratory documents about microbial resistance to antibiotic therapy in

peri-implantitis lesions, especially in the case of Aa and Pg probably due to difficulties of anaerobic microbial culture techniques. Many clinicians prefer to use variations of antibiotic regimens for treatment of peri-implantitis lesions without any antibiotic sensitivity tests such as antibiogram test. In the present study, we have investigated the sensitivity of Aa and Pg to some of the antibiotics by antibiogram test. Results of such studies could help clinicians to consider some microbial resistant and choose a suitable antibiotic regimen as an adjunctive treatment to the mechanical therapy of peri-implantitis treatment.

### Materials and Methods

This research was an in-vitro antibiogram study of specimens collected from peri-implantitis lesions of patients who treated in the implant center of the School of Dentistry, Tehran University of Medical Sciences (Ethics Committee approval number: IR-TUMS.REC.1395-2819).

Peri-implantitis lesions in the patients were previously diagnosed by a trained periodontist and prosthodontist and referred to the implant center for sampling and laboratory study. The inclusion criteria were: to have at least one peri-implantitis lesion in either upper or lower jaw; a minimum of 6 months and maximum of two years since the implant have been loaded; no antibiotics consumptions for at least a month prior to the study; and absence of any severe systemic diseases. After obtaining consent from the patients and before treatment, lesions with a probing depth of  $\geq 5$  mm were examined via x-ray in order to identify the bone defects and lesions with proximal bone loss of two implant threads were selected for the study. In the present study, there was no need for matching the oral hygiene of patients and only the presence and detection of Aa and Pg was required.

Samples were collected from each patient after isolating and cleaning the peri-implantitis lesion by inserting a sterile paper point in the pockets for 10 seconds. The paper points were immediately placed in thioglycolate medium and transferred to the laboratory. In the laboratory, samples were incubated in Brucella agar medium enriched with sheep blood, vitamin K, hemin, and horse and calf serum for less than half an hour. At this point patients and cultures were examined, however, not

all specimens grew on the anaerobic culture medium. Unfortunately, only a few of the specimens responded to the anaerobic culture medium. Successful specimens were frozen and 18 samples were prepared for further experiments, 9 on Aa and 9 on Pg specific culture medium. Therefore, the sample size was less than it was expected because of samples that failed to culture due to the sensitivity of anaerobic bacteria to atmosphere's  $O_2$  during sampling and/or transferring, or delicacy of their culture conditions. The Aa-specific culture medium contained bacitracin and vancomycin, and the Pg-specific culture medium contained bacitracin, colistin, and polymyxin B antibiotics. The specimens were incubated under anaerobic conditions for 48 hours at  $37^\circ C$ . The Pg inoculated plates were cultured using GasPak Anaerobic System A (Anaerocult, Merck, Darmstadt, Germany) while the Aa inoculated plates in addition to GasPak A, had 5%  $CO_2$  flow. The Aa and Pg colonies were monitored and two bacterial cultures were compared based on the presence or absence of their colonies. In positive cultures, Aa colonies were star-shaped and Pg colonies contained black pigments. To ensure the presence of colonies, a direct smear of each bacterium was prepared, and both bacteria were identified through Gram stain by a microbiologist. In addition, biochemical tests were performed to identify and confirm the presence of these bacteria including spot indole test, oxidase and catalase tests, and lecithinase and lipase test. The results of spot indole test and oxidase and catalase tests, Aa responded positively while they were negative for Pg. however, the results of lecithinase and lipase tests were positive for Pg and negative for Aa. In order to carry out the sugar test, arabinose, maltose, mannitol, mannose, and galactose sugars were added to the Aa liquid medium culture and placed in a capnophilic environment for 48 hours, after which the test results were evaluated. Since Pg is a saccharolytic bacterium, the sugar test was not applicable. In order to record the quantity of the bacteria, the number of colonies in each sample were counted with a colony-counting device. An antibiogram test was performed using the disc diffusion method. Test plates were inoculated and kept for 48h in an anaerobic and microaerophilic with the  $CO_2$  flow for Aa and Pg respectively.

Antibiotic standard disks (mast disc, Mast group Ltd, UK) of amoxicillin ( $10 \mu g$ ), co-amoxiclav ( $20 \mu g$ ), metronidazole ( $5 \mu g$ ), tetracycline ( $10 \mu g$ ), clindamycin ( $10 \mu g$ ), and ciprofloxacin ( $5 \mu g$ ) (8,9) were placed on the agar surface. The plates were incubated for further 48 hours followed by measuring the diameter of the inhibition zone in millimeters.

The collected data from colony counting and antibiogram test were analyzed using Statistical Package for the Social Sciences software, version 19.0 (SPSS Inc., Chicago, IL, USA). A t-test was used to compare the prevalence of each variable in the Aa and Pg groups. The level of statistical significance was considered to be  $P < 0.05$ .

## Results

Eighteen positive specimens collected from patients with peri-implantitis lesions were divided into two groups. Each group of nine samples was cultured in an anaerobic and microaerophilic with the  $CO_2$  flow for Aa and Pg respectively.

The age of the patients with the Aa positive culture ranged between 27 and 61 years with the mean of  $42.4 \pm 11.7$ , while patients with Pg positive culture were between 30 and 64 years with the mean of  $45.4 \pm 13.1$ . Patients in the Aa group were five men and four women and Pg group consisted of two men and seven women. The probing depth was between 5 to 9 mm with the mean of  $6.8 \pm 1.3$  in Aa group. Although the Pg group had the same probing range (5-9 mm), the mean was  $6.4 \pm 1.3$ . In both groups, five lesions were in the upper jaw and four were in the mandible.

The colony count of Aa and Pg cultures were positive in peri-implantitis lesions. Comparison of colony count of Aa ( $84.56 \pm 16.65$ ) and Pg ( $87.67 \pm 21.49$ ) did not show a statistically significant difference ( $P$ .value=0.74).

The comparison of antibiotic resistance and the mean diameter of the growth inhibition zone of Aa and Pg bacteria are summarized in Tables 1 and 2, respectively. The results of the antibiogram of two bacterial groups were significantly different except for amoxicillin and tetracycline, the  $P$ -values were 0.15 and 0.15, respectively. This means, overall, the specimens in Aa group were more susceptible to antibiotic than Pg group. However, samples in Aa and Pg groups had similar resistance to

**Table 1.** Comparison of Aa and Pg resistance to different antibiotics; values are presented as number (percentage)

antibiotic	Aa N(%)	Pg N(%)	P-Value
Amoxicillin	3(33.33)	7(77.78)	0.15
Co-amoxiclav	1(11.11)	7(77.78)	0.02
Metronidazole	1(11.11)	9(100)	<0.001
Tetracycline	3(33.33)	7(77.78)	0.15
Clindamycin	0(0)	6(66.67)	0.009
Ciprofloxacin	0(0)	7(77.78)	0.002

**Table 2.** The diameter of growth inhibition zone (in millimeter) of different antibiotic disks on the Aa and Pg culture plate. Values in parentheses are the range of inhibition zone for each antibiotic

Antibiotics	Aa	Pg	P-Value (t-test)
Amoxicillin	10±9.21(0-28mm)	4.44±9.17(0-25mm)	0.22
Co-amoxiclav	19.11±9.33(0-30mm)	5.56±11.3(0-30mm)	0.01
Metronidazole	21±9.57(0-30mm)	0±0(0-0mm)	<0.001
Tetracycline	18.33±14.79(0-35mm)	7.22±14.39(0-35mm)	0.13
Clindamycin	29.22±7.08(15-35mm)	7.67±13(0-34mm)	<0.001
Ciprofloxacin	30.44±6.27(20-35)	2.78±5.65(0-15)	<0.001

amoxicillin and tetracycline the P-values were 0.22 and 0.13, respectively.

## Discussion

Nowadays, the use of implant treatments has been increased, therefore, the probability of peri-implantitis lesions has also been increased. In the 2013 report of the American Academy of Periodontology, history of periodontal disease and poor hygiene were identified as the two of the leading risk factors of peri-implantitis [10]. In the present study, an antibiogram study was performed

on the Aa and Pg, two of main pathogens in peri-implantitis.

In the Leonhardt et al. [11] study, specimens from healthy and infected implants were obtained. The population contained both edentulous and individuals with teeth. Although they did not provide any data on colony count and antibiogram, they established the presence of anaerobic and capnophilic pathogens including Aa and Pg in most peri-implantitis lesions [11]. Their study suggested that the presence of periodontal pathogens in the periodontal pockets could be a

source of implants' contamination in patients with teeth [11].

Koukos et al. [12] studied genes encoding resistance of bacteria and discovered that members of the "red complex" (Pg, *Treponema denticola*, *Tannerella forsythia*) along with *Prevotella* and *Fusobacterium* spp had a significant role in peri-implantitis lesions and some bacterial pathogen like *Staphylococcus aureus* are involved in the initiation of peri-implantitis. They have also found that with the exception of one case, none of these pathogens have existed around healthy implants. Therefore, it has been concluded that elimination of critical bacteria from the oral cavity by antibiotic therapy, especially amoxicillin and co-amoxiclav is one the best way for peri-implantitis treatment [12]. The present study was an in vitro study which evaluated the antibiotic resistance of Aa and Pg by measuring the inhibition zone. Samples were obtained from pockets around the peri-implantitis lesions and only Aa and Pg positive specimens were included in the study. Probably for that reason, contrary to Koukos et al. [12] study, the results of the present study showed resistance to amoxicillin and amoxiclav.

van Winkelhoff and Wolf [13] investigated the colonization of anaerobic bacteria, such as Aa and Pg, in the dental pockets and saliva of periodontal patients immediately after implant loading, and after 6 and 12 months. A significant amount of all studied microorganisms, with the exception of Aa, was detected in the pockets and saliva of periodontal patients in all steps of the study. They have concluded that periodontal pockets are the main source of pathogens like Pg, to initiate peri-implantitis lesions [13]. However, dissimilar to the present study, no Aa was detected in their study.

Hultin et al. [14] have found a large quantity of Aa in peri-implantitis lesions. They have also observed that 54% of the semi-toothless patients of the control group were contaminated with Aa [14]. These findings are comparable to the results of present study, although conflict with van Winkelhoff and Wolf [13] findings.

Boever and Boever [15] reported a decrease in Aa and Pg after 10 days, one, three, and 6 months of treatment based on the samples collected from

pockets around implants. unlike the present study, they did not perform antibiogram analysis [15], while in our study, patient treatment and their follow up was not the purpose of the study.

In the present study, a number of Aa samples were responsive to amoxicillin, co-amoxiclav, metronidazole, tetracycline, clindamycin, and ciprofloxacin, however, antibiogram analysis revealed that the majority of Pg samples were resistant to these antibiotics. This is of great relevance in the antibiotic treatment of resistant peri-implantitis lesions in which Pg is identified.

## Conclusion

Peri-implantitis lesions contain a relatively large quantity of Aa and Pg bacteria, nevertheless, we have lost a number of specimens due to the technique sensitivity of the culture and some problems during transferring the samples. In this study, the anaerobic culture technique was used for antibiogram study. Results of in-vitro antibiogram showed that most of the Aa samples were sensitive to antibiotics used in this study and almost all Pg

specimens were resistant to these antibiotics. Due to the multi-etiological nature of peri-implantitis lesions, it is desirable to combine surgical techniques with nonsurgical procedures like selective antibiotic therapy for successful treatment. The present study could be helpful in reducing treatment failure of peri-implantitis by selecting effective antibiotic.

## Acknowledgement

This study was supported by Tehran University of Medical Sciences and Health Services grant No. 89-02-69-10052

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