

## Subgingival Pathogen Microflora in Romanian Patients with Periodontitis

**Language:** English

**Authors:**

Assist. Prof. Dr. Dr. Stefan-Ioan Stratul, Victor Babes University of Medicine, Timisoara, Romania  
 Dr. Alin Dinca, Dr. Mirona Mesaros, Dr. Anca Benta, Dental Clinic Dr. Stratul, Timisoara, Romania

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**Introduction**

Large amounts of information on the periodontitis-related germs are available in the literature, from data originating in various parts of the world. Studies demonstrate a lower prevalence of Aa in European and American than Asian population, and subjects with periodontitis harbor Aa more than twice as frequently in Thailand as in Romania or Holland (Basak et al., 2003). To date, except for one prevalence study (Ali et al., 1996), no recent information exists on the characteristics of periodontal microflora in Romanian subjects.

**Objectives**

To determine, by PCR technique the occurrence of the most common periodontopathogenic species in Romanian subjects with various periodontal conditions, the interspecies relationship and the relationship with the basic clinical parameters.

**Material and Methods**

The study population consisted of 54 patients referred with the clinical diagnosis of localized aggressive periodontitis (LagP = 16), generalized aggressive periodontitis (GagP = 24) and chronic periodontitis (CP = 14) during May through September 2004. Periodontists (SS, AD, MM, AB) assessed the Pocket depth (PD) and the Clinical attachment level (CAL) with a PCP 15 probe (Hu-Friedy Co., Chicago, USA), the Plaque Index (PI - Silness and Loe), the bleeding on probing (BOP) and performed radiographic periodontal examinations. The criteria for inclusion in the study were: 1) patients were Romanian and long-term inhabitants of the city of Timisoara and surrounding regions; 2) they were free from systemic diseases; 3) they had never received periodontal surgery; 4) they have not received SRP or antibiotics within the preceding 6 months; 5) they had at least 20 natural teeth; 6) patients had at least one molar with PD > 4mm and CAL > 5 mm in 2 quadrants. Subgingival microbiological samples were collected from all patients with sterile paper points (Hain Lifescience GmbH, Nehren, Germany). In each patient, a total of 5 pockets with PD > 5 mm and CAL > 6 mm were chosen for sampling. At least half of the pockets were from different quadrants. The samples were pooled in a single vial for each patient. The samples were subject to PCR analysis by the Hain Lifescience GmbH laboratories (Nehren, Germany). The samples were processed within 4 days after sampling. All samples were tested for the presence of Aa, Pg, Tf, Td and Pi. The chi-square and Kruskal-Wallis non-parametric tests were used to assess the differences between the 3 diagnostic groups.

**Results**

In all germs, excepted Aa, the detection frequency did not significantly differ between the CP, LagP and GagP groups. The detection frequency of Aa significantly differs, continuously increasing between the CP (14.3%), LagP (43.8%) and GagP (45.8%) groups (p=0.016). Additionally, the detection frequency of Pi was significantly higher in patients with LagP (81.3%) than in patients with CP (14.3%). The mean proportions of Aa was higher in GagP than in LagP and CP respectively (p=0.005). The mean proportion of Td was higher in LagP than in CP and GagP respectively (p=0.028). Significant positive correlations were found between the levels of Aa and Pg, and between the levels of Pg and Tf in all periodontal conditions. Same positive correlations between the germs were found in patients with GagP, with additional positive correlations between the mean overall PD, CAL and Td. In LagP patients, significant positive correlations were found between PI and the level of Pg, between the BOP and the level of Td, and a strong positive correlation between the mean overall CAL and the level of Pi. In the CP group, a positive correlation was found between the PI and the Bf level, as between the Pg level and the Bf level. In the same group, a significant positive correlation existed between the mean overall PD and the Td level.

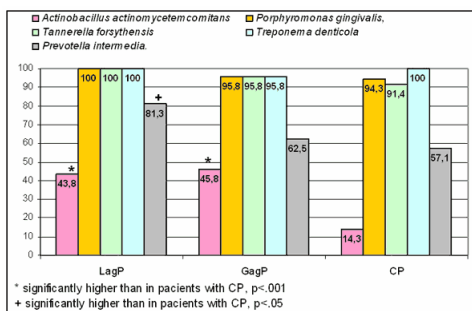


Fig.1 Subgingival bacterial species detected in patients with different periodontal conditions. Chi-square test.

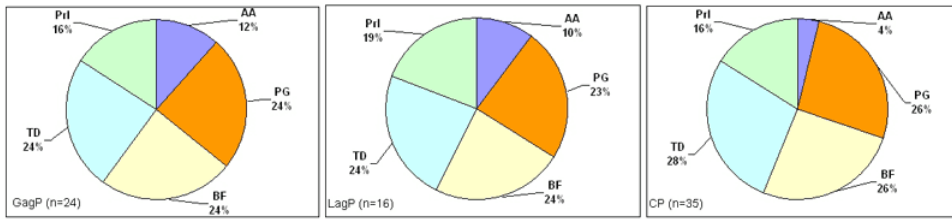


Fig.2 Mean proportions of the identified subgingival bacterial species in patients with different periodontal conditions

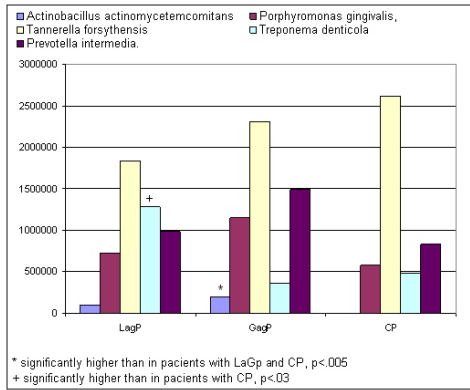


Fig.3 The mean proportions of Aa was higher in GagP than in LagP and CP respectively (p=0.005). The mean proportion of Td was higher in LagP than in CP and GagP respectively (p=0.028). Kruskal-Wallis test

Table 1. Correlation coefficients and their significance for the clinical parameters and identified species in all periodontal conditions

	PD_OVER	CAL_OVER	BOP	PI	AA	PG	BF	TD	PrI
<b>PD_OVER</b>	1,00								
<b>CAL_OVER</b>	0,86**	1,00							
<b>BOP</b>	0,30**	0,33**	1,00						
<b>PI</b>	-0,07	-0,03	0,07	1,00					
<b>Aa</b>	0,10	0,09	0,05	-0,12	1,00				
<b>Pg</b>	0,09	0,11	-0,08	-0,15	0,42**	1,00			
<b>Tf</b>	-0,04	-0,01	0,10	0,17	0,16	0,30**	1,00		
<b>Td</b>	0,05	-0,01	-0,22	0,04	-0,03	-0,12	0,00	1,00	
<b>Pi</b>	-0,02	0,06	0,20	0,03	0,16	-0,04	0,11	-0,02	1,00

\*\*Correlation is significant at the 0.01 level (2-tailed).

Table 2. Correlation coefficients and their significance for the clinical parameters and identified species in generalized aggressive periodontitis (GagP) patients

	PD_OVER	CAL_OVER	BOP	PI	AA	PG	BF	TD	PrI
<b>PD_OVER</b>	1,00								
<b>CAL_OVER</b>	0,84**	1,00							
<b>BOP</b>	0,46*	0,48*	1,00						
<b>PI</b>	0,14	0,33	0,18	1,00					
<b>Aa</b>	0,04	-0,04	-0,09	-0,27	1,00				
<b>Pg</b>	0,10	0,13	-0,29	-0,24	0,44**	1,00			
<b>Tf</b>	-0,18	-0,08	-0,15	0,01	0,39	0,49*	1,00		
<b>Td</b>	-0,41*	-0,43**	-0,10	0,04	0,00	-0,13	0,22	1,00	
<b>Pi</b>	0,07	0,01	0,30	0,15	0,22	-0,10	0,16	0,03	1,00

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation coefficients and their significance for the clinical parameters and identified species in localized aggressive periodontitis (LagP) patients

	PD_OVER	CAL_OVER	BOP	PI	AA	PG	BF	TD	PrI
<b>PD_OVER</b>	1,00								
<b>CAL_OVER</b>	0,93**	1,00							
<b>BOP</b>	0,13	0,20	1,00						
<b>PI</b>	-0,35	-0,38	0,19	1,00					

<b>Aa</b>	0,21	0,15	0,20	0,14	1,00				
<b>Pg</b>	0,36	0,27	0,12	-0,55*	0,15	1,00			
<b>Bf</b>	-0,27	-0,30	0,22	-0,21	-0,04	-0,17	1,00		
<b>Td</b>	0,20	0,21	-0,51*	0,12	-0,06	-0,45	-0,08	1,00	
<b>Pi</b>	0,27	0,59*	0,30	-0,36	-0,03	0,10	-0,13	-0,05	1,00

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

Table 4. Correlation coefficients and their significance for the clinical parameters and identified species in chronic periodontitis (CP) patients

	<b>PD_OVER</b>	<b>CAL_OVER</b>	<b>BOP</b>	<b>PI</b>	<b>AA</b>	<b>PG</b>	<b>BF</b>	<b>TD</b>	<b>PrI</b>
<b>PD_OVER</b>	1,00								
<b>CAL_OVER</b>	0,86**	1,00							
<b>BOP</b>	0,22	0,21	1,00						
<b>PI</b>	-0,17	-0,28	-0,05	1,00					
<b>Aa</b>	-0,06	0,31	-0,01	-0,19	1,00				
<b>Pg</b>	-0,11	-0,20	0,02	0,10	-0,22	1,00			
<b>Tf</b>	0,12	0,12	0,17	0,40*	-0,07	0,33*	1,00		
<b>Td</b>	0,38*	0,29	0,28	0,12	-0,14	-0,14	0,16	1,00	
<b>Pi</b>	-0,26	-0,14	0,07	0,05	0,00	0,00	0,17	0,02	1,00

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

## Conclusions

Results demonstrate that the five periodontal pathogens analyzed are strongly associated with Romanian periodontitis. In particular, Aa are more significantly associated with generalized aggressive periodontitis, while Td are more significantly associated with localized aggressive periodontitis. Aa, Pg and Tf levels seemed to be correlated in all studied periodontal conditions, especially in generalized aggressive periodontitis. No characteristic pattern of correlation between the clinical parameters and the levels of studied pathogens was found.

## Abbreviations

AA - Actinobacillus actinomycetemcomitans  
 PG - Porphyromonas gingivalis,  
 TF - Tannerella forsythensis  
 TD - Treponema denticola  
 PrI - Prevotella intermedia.  
 GagP - generalized aggressive periodontitis  
 LagP - localized aggressive periodontitis  
 CP - chronic periodontitis  
 PD - pocket depth  
 BOP - bleeding on probing  
 PI - Plaque Index

*This Poster was submitted by Assist. Prof. Dr. Dr. Stefan-Ioan Stratul.*

## Correspondence address:

*Assist. Prof. Dr. Dr. Stefan-Ioan Stratul*  
 Victor Babes University of Medicine  
 Str.Em.Gojdu no.5  
 300176 Timisoara  
 Romania

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Stefan-Ioan Stratul\* - Victor Babes University of Medicine and Pharmacy, Timisoara, Romania  
Alin Dinca, Mirona Mesaros, Anca Benta - Dental Clinic Dr.Stratul, Timisoara, Romania

## ABSTRACT

No recent information exists on periodontitis-associated subgingival microflora of the Romanian population. The occurrence, the interspecies relationships and the relationship with the basic clinical parameters for a group of five periodontal bacteria were determined in a Romanian study population. Methods: Subgingival microbial pool samples were obtained from patients with localized (LAGP = 16), generalized (GAGP = 24) and chronic periodontitis (CP = 14). A polymerase chain reaction technique was used to detect *Akkermansia actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis* (formerly *Bacillus forsythus*), *Tronosema denticola* and *Prevotella intermedia*. The chi-square and Kruskal-Wallis non-parametric tests were used to assess the differences between the 3 diagnostic groups. Results: In all genera, excepted *Aa*, the detection frequency did not significantly differ between the CP, LAGP and GAGP groups. The detection frequency of *Aa* significantly differs, continuously increasing between the CP, LAGP and GAGP groups ( $p=0.016$ ). The mean proportions of *Aa* was higher in GAGP than in LAGP and CP respectively ( $p=0.005$ ). The mean proportion of *Td* was higher in LAGP than in CP and GAGP respectively ( $p=0.028$ ). Conclusions: Results demonstrate that the five periodontal pathogens analyzed are strongly associated with Romanian periodontitis. In particular, *Aa* are more significantly associated with generalized aggressive periodontitis, while *Td* are more significantly associated with localized aggressive periodontitis.

## INTRODUCTION

Large amounts of information on the periodontitis-related germs are available in the literature, from data originating in various parts of the world. Studies demonstrate a lower prevalence of *Aa* in European and American (Hanson-Beggs et al., 2003) and subjects with periodontitis harbor *Aa* more than twice as frequently in Thailand as in Romania or Holland (Bakik et al., 2003). To date, except for one prevalence study (Ali et al., 1996), no recent information exists on the characteristics of periodontal microflora in Romanian subjects.

## OBJECTIVE

To determine, by PCR technique the occurrence of the most common periodontopathogenic species in Romanian subjects with various periodontal conditions, the interspecies relationship and the relationship with the basic clinical parameters.

## MATERIALS & METHODS

The study population consisted of 54 patients referred with the clinical diagnosis of localized aggressive periodontitis (LAGP = 16), generalized aggressive periodontitis (GAGP = 24) and chronic periodontitis (CP = 14), during May through September 2004. Periodontitis (SS, AD, MM, AB) assessed the Pocket depth (PD) and the Clinical attachment level (CAL) with a PCR 15 probe (H-Fredy Co., Chicago, USA) the gingival index (GI, Silness and Loe), the bleeding on probing (BOP) and performed radiographic periodontal examinations. The criteria for inclusion in the study were: 1) patients were Romanian and long-term inhabitants of the city of Timisoara and surrounding regions; 2) they were free from systemic diseases; 3) they had never received periodontal surgery; 4) they have not received SRP or antibiotics within the preceding 6 months; 5) they had at least 20 natural teeth; 6) patients had at least one molar with PD > 4mm and CAL > 5 mm in 2 quadrants. Subgingival microbiological samples were collected from all patients with sterile paper points (Hain Lifescience GmbH, Nehren, Germany). In each patient, a total of 5 pockets with PD > 5 mm and CAL > 6 mm were chosen for sampling. At least half of the pockets were from different quadrants. The samples were pooled in a single vial for each patient. The samples were subject to PCR analysis by the Hain Lifescience GmbH (Nehren, Germany). The samples were processed within 4 days after sampling. All samples were tested for the presence of *Aa*, *Pg*, *Td* and *Pi*. The chi-square and Kruskal-Wallis non-parametric tests were used to assess the differences between the 3 diagnostic groups.

## RESULTS

In all genera, excepted *Aa*, the detection frequency did not significantly differ between the CP, LAGP and GAGP groups. The detection frequency of *Aa* significantly differs, continuously increasing between the CP (14.3%), LAGP (43.8%) and GAGP (45.8%) groups ( $p=0.016$ ). Additionally, the detection frequency of *Pi* was significantly higher in patients with LAGP (61.3%) than in patients with CP (14.3%). The mean proportions of *Aa* was higher in GAGP than in LAGP and CP respectively ( $p=0.005$ ). The mean proportion of *Td* was higher in LAGP than in CP and GAGP respectively ( $p=0.028$ ). Significant positive correlations were found between the levels of *Aa*, *Pg*, and *Td* and the levels of *Pi* and *Ti* in all periodontal conditions. Some positive correlations between the gums were found in patients with GAGP, with additional positive correlations between the mean overall PD, CAL and *Td*. In LAGP patients, significant positive correlations were found between *Pi* and the level of *Pg* between the BOP and the level of *Td*, and a strong positive correlation between the mean overall CAL and the level of *Pi*. In the CP group, a positive correlation was found between the *Pi* and the BOP level, as between the *Pg* level and the BOP level. In the same group, a significant positive correlation existed between the mean overall PD and the *Td* level.

## CONCLUSIONS

Results demonstrate that the five periodontal pathogens analyzed are strongly associated with periodontitis in Romanian population. In particular, *Aa* are more significantly associated with generalized aggressive periodontitis, while *Td* are more significantly associated with localized aggressive periodontitis. *Aa*, *Pg* and *Td* levels seemed to be correlated in all studied periodontal conditions, especially in generalized aggressive periodontitis. No characteristic pattern of correlation between the clinical parameters and the levels of studied pathogens was found.

## Contact the authors:

Dr. Dr. Stefan-Ioan Stratul, DMD, PhD, MD, MSc, Medico Primarius, ResAsoc  
Lithuania, Gaziurny University of Maria, Germany, Assoc.Prof. (Victor Babes University of Medicine, Timisoara, Romania).  
stia@scdntim.ro

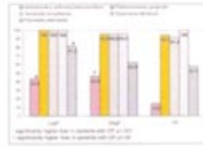


Fig 1 Subgingival bacterial species detected in patients with different periodontal conditions. Chi-square test.



Fig 2 Mean proportions of the identified subgingival bacterial species in patients with different periodontal conditions.

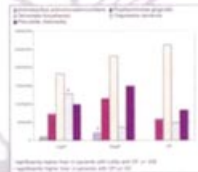


Fig 3 The mean proportions of *Aa* was higher in GAGP than in LAGP and CP respectively ( $p=0.005$ ). The mean proportion of *Td* was higher in LAGP than in CP and GAGP respectively ( $p=0.028$ ). Kruskal-Wallis test.

Table 1. Correlation coefficients and their significance for the clinical parameters and identified species in all periodontal conditions.

Species	Parameter	Correlation Coefficient	Significance
<i>Aa</i>	PD	0.12	
	CAL	0.15	
	BOP	0.18	
	GI	0.21	
	TI	0.24	**
<i>Pg</i>	PD	0.10	
	CAL	0.12	
	BOP	0.15	
	GI	0.18	
	TI	0.21	**
<i>Td</i>	PD	0.11	
	CAL	0.13	
	BOP	0.16	
	GI	0.19	
	TI	0.22	**
<i>Pi</i>	PD	0.14	
	CAL	0.16	
	BOP	0.19	
	GI	0.22	
	TI	0.25	**

\*\*Correlation is significant at the 0.01 level (2-tailed).

Table 2. Correlation coefficients and their significance for the clinical parameters and identified species in generalized aggressive periodontitis (GAGP) patients.

Species	Parameter	Correlation Coefficient	Significance
<i>Aa</i>	PD	0.15	
	CAL	0.18	
	BOP	0.21	
	GI	0.24	
	TI	0.27	**
<i>Pg</i>	PD	0.12	
	CAL	0.15	
	BOP	0.18	
	GI	0.21	
	TI	0.24	**
<i>Td</i>	PD	0.13	
	CAL	0.16	
	BOP	0.19	
	GI	0.22	
	TI	0.25	**
<i>Pi</i>	PD	0.16	
	CAL	0.19	
	BOP	0.22	
	GI	0.25	
	TI	0.28	**

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation coefficients and their significance for the clinical parameters and identified species in localized aggressive periodontitis (LAGP) patients.

Species	Parameter	Correlation Coefficient	Significance
<i>Aa</i>	PD	0.18	
	CAL	0.21	
	BOP	0.24	
	GI	0.27	
	TI	0.30	**
<i>Pg</i>	PD	0.15	
	CAL	0.18	
	BOP	0.21	
	GI	0.24	
	TI	0.27	**
<i>Td</i>	PD	0.16	
	CAL	0.19	
	BOP	0.22	
	GI	0.25	
	TI	0.28	**
<i>Pi</i>	PD	0.19	
	CAL	0.22	
	BOP	0.25	
	GI	0.28	
	TI	0.31	**

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

Table 4. Correlation coefficients and their significance for the clinical parameters and identified species in chronic periodontitis (CP) patients.

Species	Parameter	Correlation Coefficient	Significance
<i>Aa</i>	PD	0.10	
	CAL	0.12	
	BOP	0.15	
	GI	0.18	
	TI	0.21	
<i>Pg</i>	PD	0.08	
	CAL	0.10	
	BOP	0.13	
	GI	0.16	
	TI	0.19	
<i>Td</i>	PD	0.09	
	CAL	0.11	
	BOP	0.14	
	GI	0.17	
	TI	0.20	
<i>Pi</i>	PD	0.11	
	CAL	0.13	
	BOP	0.16	
	GI	0.19	
	TI	0.22	

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).