

Presence of Periodontal Pathogens in a Lifetime

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The aim of this study was to determine the prevalence of four selected periodontal pathogens in a large population of patients with various periodontal conditions. The study was based on the databank of a commercial laboratory that provides diagnostic testing services. The study population consisted of 10,946 patients, aged 11–85 years (mean age: 46.2 years). Subgingival plaque samples were collected and analyzed for the presence of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis* (*B. forsythus*), and *T. denticola* using oligonucleotide probes. A total of 33,259 samples were included in the final analysis. Within this population, 97% of patients were found to harbor at least one of the four pathogens. *T. forsythensis*, *T. denticola*, and *P. gingivalis* were detected in 82–93% of the patients, whereas *A. actinomycetemcomitans* was found in only 33% of the patients. *A. actinomycetemcomitans* showed a skewed distribution with a higher prevalence of infected sites in patients ≤ 25 years (24–32% of sites). By contrast, the proportion of positive sites for the other pathogens increased with age to reach a maximum in adult populations ($\geq 74\%$ of sites). The prevalence of *T. forsythensis*, *T. denticola* and *P. gingivalis* increased significantly with increasing probing depth. These results confirm that *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, and *T. denticola* are frequently found in populations of patients with various periodontal conditions, and that the prevalence of *A. actinomycetemcomitans* is higher in younger patients.

Key words: periodontal pathogens, gingivitis, periodontitis, age

The colonization of the oral cavity by microorganisms begins at birth with the transmission of bacteria from mother to child. Using bacteriocin-typing as well as DNA fingerprint methods, identical clonal types have been found in mother and newborn (Alaluusua et al, 1993; Berkowitz and Jordan, 1975; Könönen et al, 1994). Transmission between spouses may also occur with some periodontal pathogens, such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (Saarela et al, 1993; von Troil-Linden et al, 1997). Thus, throughout life the familial environment plays an important role in the acquisition of bacteria and the establishment of the resident flora. The normal resident microflora consists of a variety of micro-organisms including viruses, bacteria, fungi, mycoplasma and, in some instances, protozoa. However, the equilibrium of the oral

ecosystem may be disturbed by ecological changes (Marsh, 1994). Events such as tooth eruption, hormonal unbalance, dental treatment, medication, and tooth loss can induce major shifts in the composition of the resident microflora.

It is generally accepted that, among the 500 species comprising the oral flora (Paster et al, 2001), only limited numbers of species play a role in the initiation and progression of periodontal destruction. Studies have shown that the microbial flora found in diseased sites is different from that in a healthy periodontium (Haffajee and Socransky, 1994; Loesche, 1976; Slots, 1986). In addition, it has been shown that the microflora from periodontal pockets is dominated by obligatory anaerobic Gram-negative bacteria with a high proportion of spirochetes (Moore et al, 1991; Socransky and Haffajee, 1992). The bacterial

Table 1 Distribution of patients according to the number of infected sites

Infected sites	<i>Actinobacillus actinomycetemcomitans</i>	<i>Porphyromonas gingivalis</i>	<i>Tannerella forsythensis</i>	<i>Treponema denticola</i>
1 site	49%	14%	6%	7%
2 sites	25%	15%	11%	11%
3 sites	15%	24%	23%	21%
4 sites	11%	47%	60%	61%

species commonly found in periodontal lesions include *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythensis* (*Bacteroides forsythus*), *Eikenella corrodens*, *Fusobacterium nucleatum*, *Campylobacter rectus*, and *Treponema* sp. However, some species such as *A. actinomycetemcomitans*, *T. forsythensis*, and *P. intermedia* are also present but in low numbers in patients with a healthy periodontium (Alaluusua and Asikainen, 1988; Gmür and Guggenheim, 1994). Therefore, periodontal diseases are essentially opportunistic polymicrobial infections.

The aim of the present investigation was to determine the prevalence of four selected bacterial species, *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis* and *T. denticola* in a large population of patients aged 11 to 85 years with various periodontal conditions.

MATERIALS AND METHODS

The present study was based on the databank of the Institute for Applied Immunology, IAI®, Zuchwil, Switzerland, a commercial laboratory that provides diagnostic testing services (IAI PadoTest 4.5®) for dental offices in several European countries. The population included in the study was composed of patients with various forms and severity of periodontal disease. The patient and site selection for microbial testing was based on practitioner's judgement and clinical experience. Subgingival plaque samples were collected from

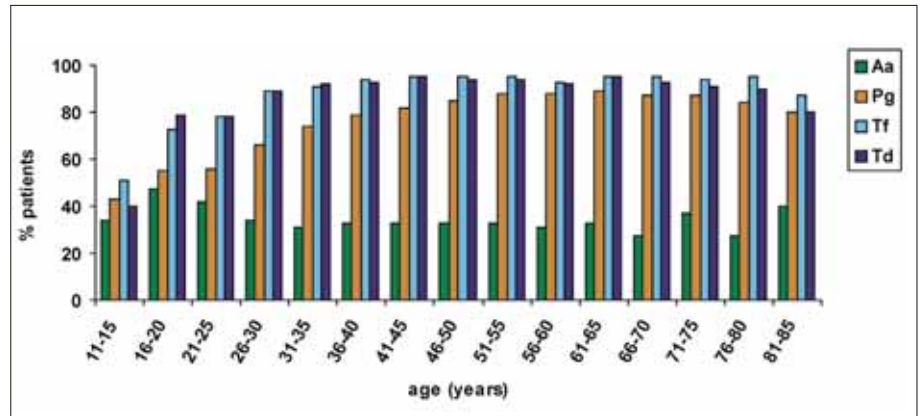
periodontally diseased sites using paper points according to the instructions provided by the laboratory. Samples were placed in screw-cap tubes containing a stabilizing buffer and sent to the laboratory together with relevant clinical information such as patient age, gender, smoking habits, and probing depth.

Samples were processed by standard procedures and hybridized with ³²P-labelled specific probes for the small subunit ribosomal RNAs (ssrRNAs) of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, *T. denticola* and a universal bacterial probe (Dix et al, 1990). Probes for *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis* and the universal probe were obtained from MicroProbe Corporation (Bothell WA, USA). *T. denticola* probe was synthesized by IAI and consisted of a mix of 3 oligonucleotides 22–25 bases long. The values for each bacterial species were computed by comparison with an homologous standard of each bacteria. The total bacterial count was determined using the universal probe. The results were translated in 'millions of bacteria' by arbitrarily deciding that one bacterium was equivalent to 10⁴ copies of ssrRNA.

The databank consisted of 71,573 plaque samples from 23,825 patients. Samples where there was no information on age or clinical status were excluded from the analysis. The analysis was limited to samples from patients aged 11 to 85 years. The study material was also limited to plaque samples collected before periodontal therapy and from sites with probing depth > 2 mm. Thus, a total of 33,259 samples from 10,946 pa-

Fig. 1 Prevalence of bacterial species among patients.

Aa = *A. actinomycetemcomitans*
Pg = *P. gingivalis*
Tf = *T. forsythensis*
Td = *T. denticola*



tients were included in the final analysis. The mean age of the population was 46.2 years; 43% were males and 36% were smokers. The mean probing depth at sampled sites was 6.8 mm, and 90% of the pockets were deeper than 4 mm. The number of samples per patient ranged from 1–4 samples.

Within this patient population, 97.3% of patients were found to harbor at least one of the four pathogens. *T. forsythensis* and *T. denticola* were the most frequently detected organisms, 93.2% and 92.8% respectively, whereas *P. gingivalis* was found in 81.9% of the patients. In contrast, *A. actinomycetemcomitans* was detected in only 32.7% of the patients. Fig. 1 shows the relationship between detection frequency of pathogens among patients and age. The prevalence of *P. gingivalis*, *T. forsythensis* and *T. denticola* increased with age whereas *A. actinomycetemcomitans* showed a skewed distribution with a higher prevalence in the 11–25 years age categories. Table 1 shows the frequency of infected sites among patients harboring the bacteria and for whom 4 samples were available. For *P. gingivalis*, *T. forsythensis* and *T. denticola*, the majority of patients were found to have 3–4 infected sites. In contrast, almost 50% of the patients had only 1 infected site for *A. actinomycetemcomitans*. In the population ≤ 25 years, 56% of the patients had 3–4 sites infected by *A. actinomycetemcomitans*.

In total, 11.8% of the sites harbored all four pathogens whereas 9.8% were negative for all bacteria. The relationship between the prevalence of bacteria and age as well as probing depth is shown in Fig. 2. *A. actinomycetemcomitans*

showed a skewed distribution with a higher prevalence of infected sites in patients ≤ 25 years (24–32% of sites). By contrast, the proportion of positive sites for the other pathogens increased with age to reach a maximum at the age of 31 years for *T. forsythensis* and *T. denticola*, and 51 years for *P. gingivalis* ($\geq 74\%$ of infected sites). The prevalence of all four bacterial species also increased significantly with increasing probing depth. Detection frequency of *P. gingivalis*, *T. forsythensis* and *T. denticola* comprised between 21–58% of shallow pockets and 42–93% of deep pockets. *A. actinomycetemcomitans* was found in 9–14% of shallow pockets and 14–23% of deep pockets. Up to the age of 46 years, the total bacterial count increased with age (Fig. 3), and was also found to increase with probing depth. Fig. 4 shows the estimated number of bacteria for each species in relation to probing depth. *P. gingivalis* was the most prominent species in pockets ≥ 5 mm whereas *A. actinomycetemcomitans* was found in low numbers. *P. gingivalis*, *T. forsythensis* and *T. denticola* increased as probing depth increased. The trend for *A. actinomycetemcomitans* was less clear although higher numbers were found in shallow sites and in pockets ≥ 11 mm.

DISCUSSION

The present investigation was based on the data-bank of a commercial laboratory which provides services for dental offices in several European countries.

Fig. 2 Prevalence of infected sites according to age and probing depth.

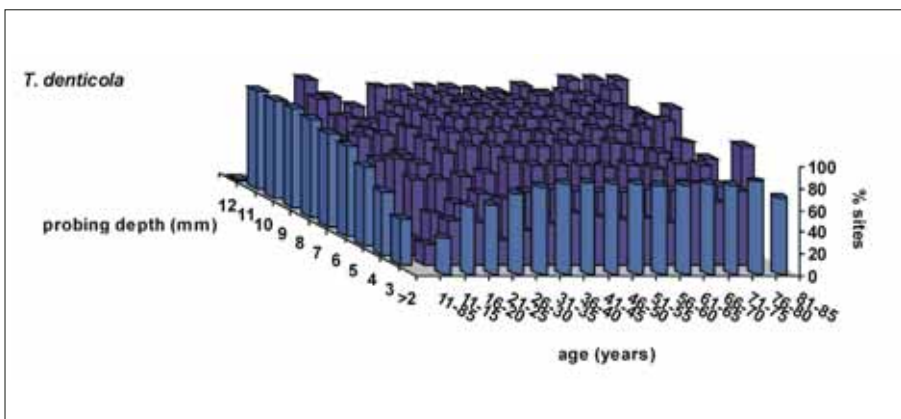
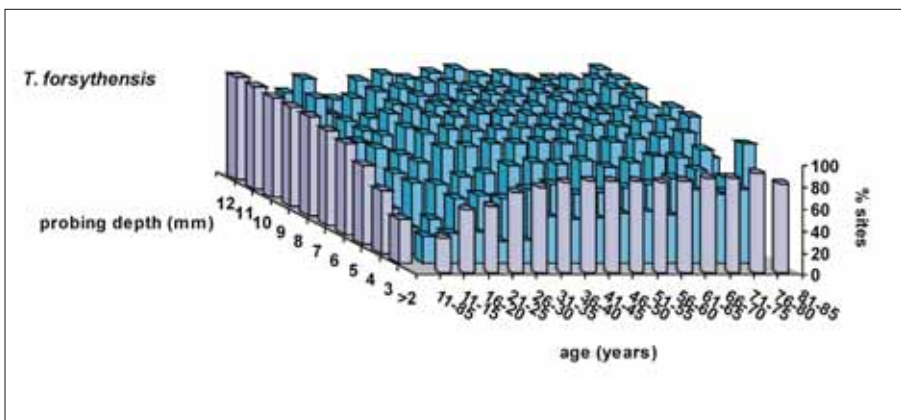
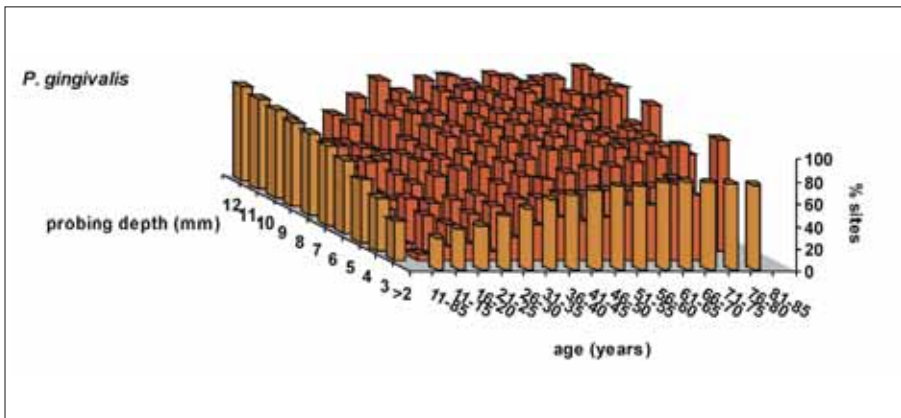
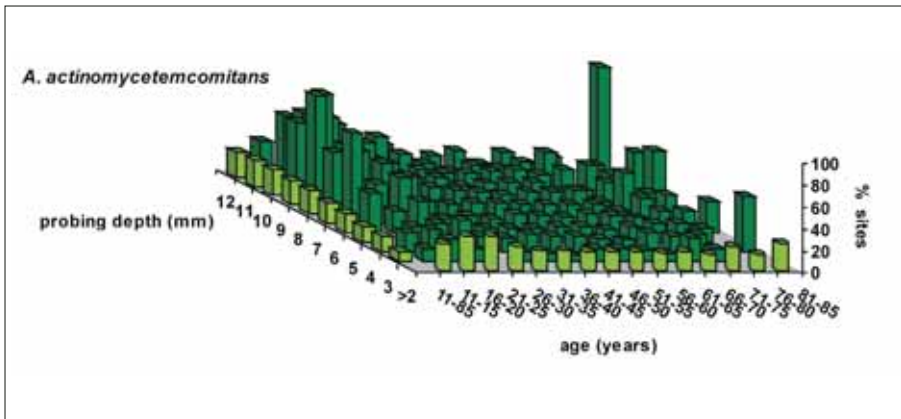


Fig. 3 Total bacterial counts according to age and probing depth.

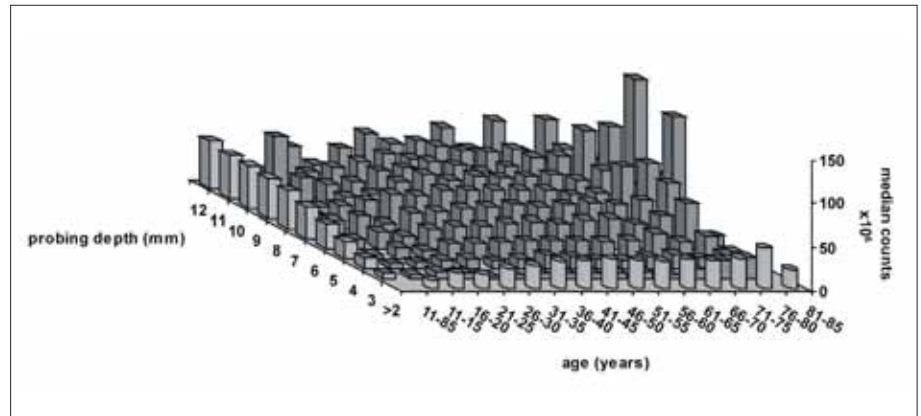
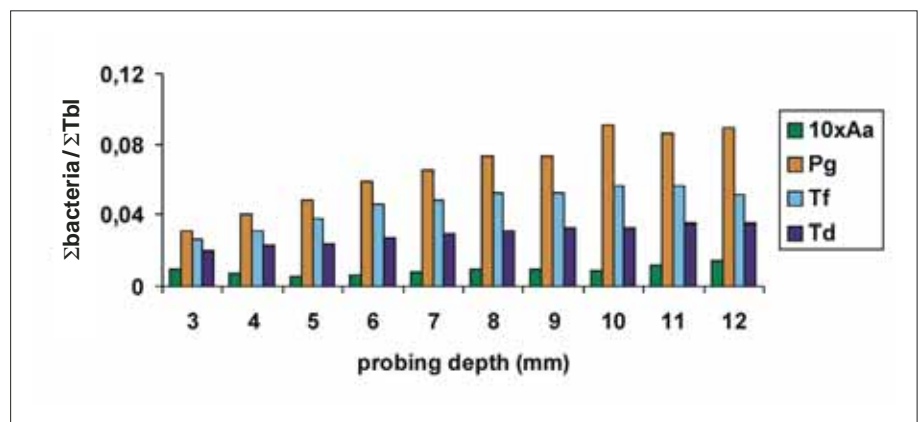


Fig. 4 Ratio of pathogens counts to total bacterial counts according to probing depth.



In the present study only samples collected prior to treatment were extracted from the database. The sample size was robust given that 33,259 plaque samples from 10,946 patients were included in the analysis. This approach differs from other studies performed in university settings, or using limited numbers of patients. Our sampling allowed us to analyze a geographically large and diverse population of patients with varied socio-economic status. The study population is probably representative of patients found in private dental practices. The lack of calibration for clinical measurements, diagnosis and sampling site selection represents a limitation of the study. No attempt was made to classify the study population into different disease categories. Parameters included in the analysis were limited to age and probing depth.

There is evidence that the distribution of periodontal pathogens within the population is related to

age. Indeed, a high prevalence of *A. actinomycetemcomitans* was reported among children and young adults with no or minimal periodontal disease (Alaluusua and Asikainen, 1988; Gmür and Guggenheim, 1994). *A. actinomycetemcomitans* was also found to be strongly associated with periodontitis in adolescents and young adults (Asikainen et al, 1991; Lamell et al, 2000; Müller et al, 1996). Furthermore, several studies have shown that the occurrence of *A. actinomycetemcomitans* in plaque decreases with increasing age (Hamlet et al, 2001; Rodenburg et al, 1990; Savitt and Kent, 1991; Slots et al, 1990). In addition, these studies showed that the prevalence of *P. gingivalis*, *T. forsythensis* and *P. intermedia* increases with age. Our observations derived from a large patient population are in agreement with these findings. In our study, *A. actinomycetemcomitans* showed a skewed distribution with a

higher prevalence in patients \leq 25 years. In contrast, the prevalence of *P. gingivalis*, *T. forsythensis* and *T. denticola* increased with age before reaching a plateau in adults. It is worth noting that *T. forsythensis* and *T. denticola* were the most frequently detected species in patients \geq 16 years. The present results were based on populations from several Central European countries mainly from Germany and Switzerland. The epidemiology of subgingival periodontal pathogens may be significantly different in other countries. Indeed, several studies showed that the prevalence of periodontal pathogens varies among ethnic populations (Al-Yahfoufi et al, 2003; Dahlén et al, 1989; Dahlén et al, 1995; Papapanou et al, 2002; Yano-Higuchi et al, 2000; Zambon et al, 1994).

The periodontal pocket is made up of numerous micro-habitats, each of which associated with distinct microbial communities. Different gradients of redox potential, pH, and nutrients are also found in periodontal pockets. As a result, different micro-colonies, spatially organized into a three-dimensional structure, can be identified within the subgingival biofilm (Listgarten, 1999). Shallow pockets may be expected to harbor capnophilic species such as *A. actinomycetemcomitans*, whereas *P. gingivalis*, *P. intermedia* and *E. corrodens* are likely to be found in deeper pockets. In our study, a relationship was found between probing depth and the frequency of detection of all four bacterial markers. A relationship was also observed between probing depth and the levels of *P. gingivalis*, *T. forsythensis* and *T. denticola*. The trend was less clear for *A. actinomycetemcomitans* although higher levels were detected in shallow sites and deep pockets. The total number of bacteria was also correlated with probing depth. Our observations are consistent with the results from a number of other studies (Hamlet et al, 2001; Savitt and Kent, 1991; Savitt and Socransky, 1984; Socransky et al, 1991; Wolff et al, 1985; Wolff et al, 1993). These observations support the concept that environmental conditions in deep periodontal pockets favor the growth and the development of fastidious microorganisms.

Our study focused on the prevalence of single bacterial species in periodontal patients. However, associations between different bacterial species have been identified in subgingival plaque (Socransky et al, 1988). Among the vari-

ous associations described by Socransky et al (1998) the complex *P. gingivalis*, *T. forsythensis* and *T. denticola* was found to be strongly associated with periodontal destruction. The distribution of these various bacterial associations in different age populations still remains to be determined.

These data may have clinical implications and may serve as a framework in the management of periodontal patients. Indeed, therapy should be designed according to microbial profiles and targeted at specific periodontal pathogens. In young populations, where *A. actinomycetemcomitans* is present more frequently, one might consider mechanical treatment combined with the use of systemic antimicrobials. Studies have shown that scaling and root planing alone do not reliably eliminate *A. actinomycetemcomitans*. On the other hand, mechanical treatment alone might be the therapy of choice in older populations as *P. gingivalis* is very susceptible to ecological changes. In this context, microbial diagnosis may prove to be a valuable tool in targeting periodontal therapy.

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