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Transmission of periodontal bacteria and models of infection

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Abstract

Background: Bacteria play an essential role in the aetiology of periodontitis. Most bacterial species isolated from subgingival plaque are indigenous to the oral cavity. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* are detected infrequently in periodontal health, which makes these species prime candidates to study person-to-person transmission. The aim of the present study was to review the literature on transmission of these periodontal bacterial species.

Method: We review the literature on bacterial typing techniques and summarize the information on clonal distribution of *A. actinomycetemcomitans* and *P. gingivalis* in family units based on different typing techniques in order to establish the likelihood for person-to-person transmission of these periodontal pathogens.

Results: Vertical transmission of *A. actinomycetemcomitans* is estimated to be between 30% and 60%, whereas vertical transmission of *P. gingivalis* has rarely been observed. Horizontal transmission between spouses ranges between 14% and 60% for *A. actinomycetemcomitans* and between 30% and 75% for *P. gingivalis*. There is some evidence to show that cohabitation with a periodontitis patient influences the periodontal status of the spouse; however, substantially more information is needed to prove this hypothesis.

Conclusions: Transmission of putative periodontal pathogens between family members has been shown. The clinical consequences of these events have been poorly documented. Based on the current knowledge, screening for and prevention of transmission of specific virulent clones of *A. actinomycetemcomitans* may be feasible and effective in preventing some forms of periodontal disease. *P. gingivalis* is usually recovered from diseased adult subjects, and transmission of this pathogens seems largely restricted to adult individuals. Horizontal transmission of *P. gingivalis* may therefore be controlled by periodontal treatment involving elimination or significant suppression of the pathogen in diseased individuals and by a high standard of oral hygiene.

Key words: bacteria; transmission; periodontitis; infection; bacterial typing

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Epidemiology is the study of the factors that affect the distribution of diseases in populations, including infectious diseases that are spread by pathogenic organisms.

Microorganisms that are indigenous to the host and are referred to as commensal, or resident organisms, can cause infections. They can initiate disease when they disseminate from their normal ecological niche to sterile body sites as, for instance, is the case in urinary tract infection caused by *Escherichia coli* from the colon. Indigenous *Staphylococcus aureus* can cause hard- and soft-tissue infections when penetrating the natural barriers of the skin or the mucosa. Oral streptococci may cause transient bacteraemia following dental extraction or tonsillectomy and may cause subacute bacterial endocarditis in susceptible individuals. These microorganisms can survive in the new environment if they are able to attach to the surface of the host and are able to resist the local host immune responses. Often, a change of the innate immunity is necessary for commensal pathogens to cause opportunistic infections. When the composition of the normal microflora is disturbed, after or during antimicrobial therapy for instance, resistant microorganisms can emerge and cause infection (Helovuo & Paunio 1989, Helovuo et al. 1993). Infections caused by commensal microorganisms are called endogenous infections for which transmission of microorganisms from host to host is not needed. In the oral cavity an increase of the bacterial load and a concomitant shift in the microbial composition of the dental plaque can cause gingivitis.

Unlike exogenous bacterial disease, endogenous infections (1) have no definable incubation period, (2) are not communicable in the usual sense, (3) do not result in clinically recognizable immunity, and (4) involve bacterial agents found in the normal microflora. These agents are often characterized by a low intrinsic pathogenicity and cause disease when they appear either in unusual body sites or in greatly increased concentration in or near their usual habitats.

Microorganisms that do not normally occur in a healthy host and can cause disease upon infection in a significant proportion of the recipients are called exogenous pathogens (Salyers & Whitt 1994). Most human pathogens not only occur in diseased subjects, but also in healthy carriers, who provide a reservoir and serve as a focus. It is suspected that epidemics can be initiated by recombination between strains from different carriers. Exogenous pathogens need to overcome the conditions of the environment such as ultraviolet light, drying and temperature. To survive these conditions, they have developed different strategies such as spore formation (*Clostridium*, *Bacillus*), encapsulation (*Mycobacterium*) and survival in intermediate hosts such as the rabies virus in animals or *Legionella pneumophila* in amoebae. Organisms with moderate survival potential can be transmitted by spreading in air on droplet nuclei (viruses). Some bacterial pathogens are extremely delicate and cannot survive outside the host, e.g. *Neisseria gonorrhoea*. Transmission of this type of pathogen is achieved by direct mucosal contact between individuals.

Communicable diseases are infections that are capable of spreading from person to person. This is not the case with all infections. A subject with infective endocarditis caused by *Streptococcus sanguis* may suffer from an infection that is not able to spread from a patient to another person. Communicable microorganisms are not always pathogenic and not all pathogens are communicable. Prevention of transmission of an infectious agent is a major measure to prevent disease in a population. Therefore, the study of transmission of infectious agents is a relevant public health issue. In diseases that are caused by one microbial species (whooping cough, rabies) or a very limited number of related species (*Plasmodium* species all causing malaria), the rationale to study transmission is clear. In multifactorial diseases that are associated with a complex microflora such as infectious bowel diseases (Crohn and ulcerative colitis), vaginitis and periodontal disease, the matter of transmission is more complex.

First, some general principles of bacterial transmission are discussed. Then, the rationale for studying the transmission of two periodontal pathogens, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, is provided. Subsequently, studies on the distribution of these periodontal species in populations and in families are summarized. Different typing methods are briefly described, and data on distribution of genotypes among families are presented. Finally, routes of transmission of both pathogens and the possible clinical consequences of transmission are discussed.

Transmission

The behaviour of a pathogenic microorganism depends upon the interaction between the host, the pathogen and the environment. Changes in any of these factors will affect the likelihood of transmission. Host factors affect the chance of exposure to a microorganism, the possibility for colonization and the response to colonization, i.e. development of infection. Host factors include, among others, sexual behaviour, hygiene, occupation, nutrition, immunity and general health. Microbial factors include infectiousness, pathogenicity, virulence and survival in human and animal hosts, including resistance towards immune responses and drugs.

Environmental factors such as temperature, humidity and oxygen tension are determinants of the survival of microbes outside the host.

Mechanisms of transmission

Many bacteria are transmitted from person to person primarily by direct contact, via the hands, mouth, or sexual organs. Some bacterial (e.g. syphilis) and viral diseases (e.g. rubella) can be transmitted transplacentally from mother to child. Indirect transmission occurs via intermediate agents. Enteric infections are mostly waterborne or foodborne and are because of faecal discharges. Respiratory infections are transmitted by contact and by droplets (aerosols) emitted during coughing and sneezing, which can produce as many as 20,000 contaminated droplets. Talking is also a source of airborne particles, although the number of organisms that is shed is significantly less. Whether periodontal bacteria can be transmitted via aerosols is currently not known. Staphylococci are often transferred by dust from contaminated clothing, beds and contaminated objects.

Some systemic infections are caused by transmission of pathogens from animals (zoonoses), which serve as a reservoir. Humans can be infected via milk (brucellosis, tuberculosis), carcasses (anthrax, tularaemia), contaminated swimming water (leptospirosis) or wounds.

Biting/sticking arthropods can serve as vectors and may transfer bacteria, rickettsiae and viruses from an animal reservoir by a cycle of infection (in lice, fleas or ticks). Often, these latter infections are not readily transmitted from person to person.

Vertical and horizontal transmission

When transmission is directly from parents to offspring (via ovum, placenta, milk, blood, saliva) it is referred to as vertical. Transmission is called horizontal when an individual infects unrelated individuals by contact, respiratory or faecal–oral spread. Examples of diseases that may spread by vertical transmission are rubella, cytomegalovirus, syphilis, toxoplasma (pre-natal), gonococcal/chlamydial conjunctivitis (perinatal) and hepatitis B (post-natal). Polio, influenza, tuberculosis and typhoid are examples of diseases that are horizontally transmitted.

Essential factors for transmission

The source

When the source of the infection is restricted to actively diseased individuals, isolation measures are effective in eradication of the infectious agents. This is not possible when pathogens occur in healthy carriers, who represent a reservoir. Eradication is also not feasible when pathogens are soilborne or animalborne.

Number of microorganisms shed

The more viruses, bacteria or protozoa are shed, the greater the chance they have of reaching a new host. The number of organisms required to infect a host varies greatly between microorgan-

isms. It takes approximately 10 *Shigella dysenteriae* bacteria (from a human host) to infect a new host, but 10^6 *Salmonella* sp. (from animals) to cause food poisoning. Also, the route of infection is important for the infection dose. As few as 10 gonocci bacteria are able to cause infection of the urethra, whereas thousands are needed to infect the mucosa of the oropharynx or rectum. Coughing, sneezing and spitting are activities that benefit the host by clearing contaminated (foreign) material from the upper respiratory tract and the mouth. Most of the shed microorganisms probably die in the environment; few are able to colonize a new host. Transmission also depends on genetic factors of the microorganisms, i.e. some strains of a given species are more readily transmitted than others. This property may vary independent of the pathogenicity and virulence of a species. Open and closed lesions can occur in various cases and stages of the same disease and may vary greatly in the shedding of organisms. The bubonic form of plague with closed infections of the lymph nodes and the blood stream is transmitted from rat to humans (by fleas) but not from person to person. However, open pulmonary lesions of the disease are highly contagious. The same is true for pulmonary (open) tuberculosis.

Frequency of effective contacts

Contacts between infected and susceptible individuals are an important factor in determining how fast an infection spreads. Crowding and limited airflow are critical terms in cross-infections, especially for respiratory diseases. One single contact can transmit gonocci and cause disease.

Survival in the environment

Drying is a major environmental factor that kills shed microorganisms. Staphylococci, tubercle bacilli and clostridia and anthrax spores are resistant to drying. Species such as *N. meningitidis*, gonococci and most viruses are sensitive to drying and will soon die in the environment.

Microbes Associated with Periodontitis

The oral cavity harbours several hundreds of bacterial species, most of which belong to the indigenous oral microflora (Moore & Moore 1994). It has been shown that a significant number of species that may be demonstrated have still not been cultured (Paster et al. 2001). The habitat of many of these species is restricted to the oral cavity. The majority of subgingival bacteria in periodontitis can also be isolated from the oral cavity in periodontal health and are therefore considered indigenous to the oral cavity. These indigenous, commensal bacteria are acquired from other individuals, such as family members.

Könönen et al. (1994) showed that *Prevotella melaninogenica* can be transmitted from the mother to the child, but sources other than the mother for this species were also noted. It has been speculated that maternal saliva may act as a source of oral anaerobes that are transmitted to young children (Könönen et al. 1992). The natural acquisition of the oral microflora is not the aim of this paper, and this review will focus on studies that have been published on vertical and horizontal transmission of selected periodontal pathogens.

Is periodontitis a transmittable disease?

It is well established that severe periodontitis clusters in families (van der Velden et al. 1993, 1996). This suggests that susceptibility factors for the onset and progression of the disease may be transferred from person to person in families. This can involve transfer of genetic susceptibility factors, common behavioural factors and exposure to environmental factors including transmission of putative pathogenic bacteria. It is commonly assumed that bacteria play an essential role in the pathogenesis of destructive periodontal disease. Therefore, the question of whether bacterial transmission plays a role in periodontitis is relevant. However, the classical meaning of transmission of a pathogen cannot be applied in periodontitis given the complexity of the bacterial component in this disease. This involves a different approach when the question of transmission of pathogenic agents in periodontitis is posed: Which bacteria represent relevant risk factors upon transmission, and how should these species, out of the several hundreds of subgingival species identified, be selected? In an early study, Offenbacher et al. (1985) have approached this issue by studying not a single species, but by analysing the similarity of the subgingival microflora between spouses using dark-field microscopy. With this technique, however, they were not able to demonstrate transmission of any morphotypes between spouses. More recent studies have focused on transmission of single, cultivable species. Most studies on bacterial transmission in periodontitis have focused on *A. actinomycetemcomitans* and *P. gingivalis*. These species have been selected on the basis of their strong association with different forms of destructive periodontal disease (Slots & Ting 1999) and their documented pathogenic potentials (Haffajee & Socransky 1994, Fives-Taylor et al. 1999, Holt et al. 1999). However, the possibility that other, and possibly even stronger, bacterial markers for disease exist in the non-cultivable part of the subgingival microflora cannot be ruled out.

Detection of *A. actinomycetemcomitans* and *P. gingivalis*

The natural habitat of *A. actinomycetemcomitans* and *P. gingivalis* is the oral cavity. To date, there is no evidence that both species occur outside the human host. Animal isolates are phenotypically and genetically distinct from human isolates (Taichman et al. 1987, Beem et al. 1991, Karjalainen et al. 1993, Ménard & Mouton 1995).

The results of epidemiological studies depend on the sensitivity and specificity of the techniques used to detect a pathogen. In periodontal research, microscopy, culture methods, immunoassays and nucleic acid-based methods have been applied to detect and quantify microbial pathogens. Detection limits are different for the various methods. Also, selection of study populations affects the conclusions of epidemiological trails to a great extent. Tables 1 and 2 summarize the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* in relation to age and periodontal status based on the review by Slots & Ting (1999). *A. actinomycetemcomitans* is infrequently detected in periodontal health in children <19 years but was found in up to 78% in 14 Vietnamese subjects. In periodontally healthy subjects >19 years, it was frequently not detected but could be found in up to 33% of the subjects. *P. gingivalis* is usually not found in edentulous subjects and in periodontal health in persons under the age of 19 years, and it is infrequently isolated in periodontal health in persons above the age of 19 years.

Table 1. Occurrence (%) of subgingival *Actinobacillus actinomycetemcomitans* in periodontal health and disease in untreated individuals [number of studies]

	Edentulous	Periodontal health (%)	Gingivitis (%)	Periodontitis (%)
<11 years	0 (2)	0 [3] 3–78 [10]	14 [1]	20–100 [6]
12–18 years	Unknown	0 [2] 4–37 [3]	15–60 [2]	35–100 [12]
19–35 years	Unknown	0 [3] 17–33 [5]	0 [1] 3–14 [3]	0 [1] 19–94 [12]
>35 years	0	0 [1] 20 [1]	0 [1]	30–83 [15]

Adapted from Slots & Ting (1999).

Table 2. Occurrence (%) of subgingival *Porphyromonas gingivalis* in periodontal health and disease in untreated individuals [number of studies]

	Edentulous (%)	Periodontal health (%)	Gingivitis (%)	Periodontitis (%)
<11 years	0 [2]	0 [5] 4–70 [4]	14 [1]	0 [2] 7 [1]
12–18 years	Unknown	0 [1] 7 [1]	2 [1]	38–63 [4]
19–35 years	Unknown	0 [2]	0 [2] 10 [1]	8–100 [9]
>35 years	0 [1] 8 [1]	0 [1] 100 [1]	100 [1]	0 [1] 27–100 [13]

Adapted from Slots & Ting (1999).

Table 3 summarizes recent studies on the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* in periodontal health and disease. Boutaga et al. (2003) used a real-time PCR technique to detect *A. actinomycetemcomitans* and *P. gingivalis* in 111 periodontal healthy subjects and 259 adult patients with periodontitis. *A. actinomycetemcomitans* and *P. gingivalis* were detected in 18% and 9.9%, respectively, in subjects without periodontitis. In contrast, the prevalence of *A. actinomycetemcomitans* in Chinese adults without periodontitis amounted to 78%, exemplifying geographical differences in the prevalence of *A. actinomycetemcomitans*. In young Japanese children (2–12 years) without periodontitis, *A. actinomycetemcomitans* was found in 4.8% of the subjects. In Brazil, the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* appeared much higher in subjects without destructive periodontal disease (70% and 78%, respectively) in comparison with the Dutch population. It cannot be excluded that these different findings may have been caused by different techniques.

A relationship between age and distribution of *A. actinomycetemcomitans* and *P. gingivalis* has been observed. The prevalence of *A. actinomycetemcomitans* seems to decrease with age, whereas the prevalence of *P. gingivalis* increases with age (Rodenburg et al. 1990, Slots et al. 1990, Savitt & Kent 1991).

Intra-oral cross-contamination

A. actinomycetemcomitans and *P. gingivalis* are not restricted to the subgingival area in periodontitis patients. It has been shown that both species can also be found in saliva, supragingival plaque and on various mucous membranes in the oral cavity of patients with periodontal infections, although proportions are usually highest in subgingival plaque (Van Winkelhoff et al. 1988, Socransky et al. 1999). Supragingival plaque usually contains higher numbers of *P. gingivalis* in periodontitis patients compared with periodontally healthy subjects (Socransky et al. 1999). Current data indicate that both species spread intra-orally through contaminated saliva and colonize supragingival plaque before they colonize the subgingival area (Socransky et al. 1999). It has been shown that subgingival colonization of both pathogens not only occurs at sites with but also at sites without periodontal attachment loss (Van Winkelhoff et al. 1994). It has been speculated that deep pockets of diseased subjects act as reservoirs for spread of infection to healthy sites (Riviere et al. 1996), although an alternative hypothesis has also been proposed (Socransky et al. 1999).

Distribution within families

It has been shown that two major periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, cluster in families (Zambon et al. 1983, Slots & Listgarten 1988, Gunsolley et al. 1990, van der Velden et al. 1993, Tuite-McDonnell et al. 1997). These observations suggest that bacteria may be transmitted between family members and that they share susceptibility factors for colonization by these bacteria or clonal types of these species. Detection of the same bacterial species in family members does, however, not prove transmission. For further evidence, typing of the bacterial isolates is necessary.

Methods of Typing Microorganisms

Bacterial strain typing is an integral part of epidemiological investigations. Methods of bacterial typing range from simple biotyping to complex genetic characterization. It is common to use more than one typing method, e.g. *L. pneumophila* is first serotyped into nine groups and then further biotyped for final identification. Molecular typing systems are able to distinguish among epidemiologically unrelated isolates because of the genetic variation in the chromosomal DNA of a bacterial species.

Phenotypic typing methods

These methods are based on phenotypic characteristics of the bacterial species and can, for instance, be used to type successive isolates of the same organism from an individual patient.

Biotyping

Biotyping uses the results of biochemical tests to assign members of the same species into different groups. In *A. actinomycetemcomitans*, six biotypes have been described based on sugar fermentation, but the method has a low discriminatory potential in comparison with other methods (van Steenbergen et al. 1994). A useful biotyping method for human *P. gingivalis* strains is not available.

Serological typing

Serotyping uses a series of antibodies, raised in animals, to detect different

Table 3. Studies on the frequency of detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in healthy subjects and adult patients with periodontitis by culture and PCR techniques

Reference	Study group	Technique used	Prevalence of bacteria (%)			
			A. actinomycetemcomitans		*P. gingivalis*	
			periodontitis	healthy	periodontitis	healthy
Van Winkelhoff et al. (2002)	210 adult subjects (> 25 years old): 94 healthy, 116 periodontitis, the Netherlands	Culture	31	12.8	59.5	10.6
Boutaga et al. (2003)	370 adult subjects (> 25 years old): 111 healthy, 259 periodontitis, the Netherlands	Real-time PCR	27	18	43	9.9
		Culture	19.7	4.5	53	7.2
Griffen et al. (1998)	311 adult subjects (49.2–51.4 years old): 181 healthy, 130 periodontitis, USA	PCR	ND	ND	79	25
Riggio et al. (1996)	43 periodontitis patients, UK	PCR	15	ND	11	ND
		Culture	24		24	
Ashimoto et al. (1996)	49 subjects (15–75 years old): 49 advanced periodontitis, Japan	PCR	26.5	ND	70	ND
		Culture	22.4		18.4	
Lau et al. (2004)	92 subjects (30–71 years old): 30 healthy, 30 gingivitis, 32 periodontitis, Spain	Culture	6.3	0	84.4	20
		PCR	18.8	6.7	81.3	13.3
Takeuchi et al. (2003)	103 (412 sites) subjects (15–70 years old): 50 aggressive periodontitis, 35 generalized chronic periodontitis, 18 healthy, Japan	PCR	8.6	ND	97.1	ND
		Culture	1.33		16.10	
Avila-Campos & Velasquez-Melendez (2002)	100 subjects: 50 healthy, 50 periodontitis, Brazil	PCR	90	70	66	78
Okada et al. (2004)	104 subjects (2–12 years old): 10 healthy, 73 gingivitis, 21 periodontitis, Japan	PCR	20	4.8	20	4.8
Tan et al. (2001)	92 adult subjects (25–65 years old): 50 healthy, 15 moderate periodontitis, 27 severe periodontitis, China	PCR	69	78	ND	ND

antigenic determinants on the bacterial cell surface. It remains a valuable method for typing isolates of *P. gingivalis* (Van Winkelhoff et al. 1993, Laine et al. 1996) and *A. actinomycetemcomitans* (Zambon et al. 1983).

Phage typing

Different strains within a species have different sensitivities for the lytic activity of bacteriophages. Phage typing is not available for *A. actinomycetemcomitans* and *P. gingivalis*.

Molecular typing methods

Protein typing

This is a relatively crude method of typing in which the proteins of an organism are simultaneously extracted. The patterns of proteins are demonstrated by polyacrylamide gel electrophoresis (PAGE). The SDS-PAGE typing technique has a low discriminatory potential.

Multilocus enzyme electrophoresis (MLEE)

MLEE is based on metabolic enzymes that are highly conserved and unlikely to change quickly in a given species. The method is highly reproducible with a moderate level of discriminatory power. It is useful for epidemiological studies that compare isolates from geographically distant areas over time.

Restriction enzyme analysis (REA)

REA uses endonucleases to cleave DNA at a particular sequence of nucleotides that may be present on different places on the bacterial chromosome. The number in size of the generated restriction fragments reflects the frequency and distribution of the restriction sites. Fragments are separated by size using agarose gel electrophoreses. Different strains of the same bacterial species have different REA profiles because of variations in their DNA sequences. This technique has been applied to type *A. actinomycetemcomitans* (Petit et al. 1993a, b) and *P. gingivalis* (van Steenbergen et al. 1993).

Restriction fragment length polymorphism (RFLP)

With RFLP, chromosomal DNA is digested by frequent cutting enzymes and separated by agarose gel electro-

phoresis. The DNA fragments are transferred onto a membrane. The DNA on the membrane is then hybridized with a specific labelled probe, which binds to a limited number of fragments on the membrane that have complementary nucleotide acid sequences. Variations in the number and size of the fragments detected by hybridization are referred to as RFLPs. This method has been successfully used to type *A. actinomycetemcomitans* (DiRienzo & Slots 1990, DiRienzo & McKay 1994, DiRienzo et al. 1994).

Ribotyping

This method uses chromosomal DNA and a ribosomal RNA probe to provide different profiles. Because all bacterial isolates have one or more chromosomal rRNA operons distributed on the chromosome, and because these sequences are highly conserved, essentially all bacterial isolates can be typed using probes directed to the DNA sequences that encode the rRNA loci using a single rRNA probe. This method has been successfully used to type *A. actinomycetemcomitans* (Alaluusua et al. 1993, Saarela et al. 1993a).

Pulse field gel electrophoresis (PFGE)

With the PFGE method, the bacterial genome is digested with a restriction enzyme that has relatively few recognition sites and thus generates $\sim$10–30 restriction fragments and then is separated by PFGE to produce a pattern of bands. This method has been applied to *A. actinomycetemcomitans* (Valcarcel et al. 1997).

Arbitrarily primed (AP)-PCR

This is a kind of PCR technique using a short primer that is not targeted to amplify any specific bacterial DNA sequence. The resulting AP-PCR products will represent a variety of different-sized DNA fragments that are visualized by agarose gel electrophoresis. This method has been applied to *A. actinomycetemcomitans* and *P. gingivalis* (Dogan et al. 1999a).

Amplified fragment length polymorphism (AFLP)

AFLP is a PCR-based fingerprinting technology. AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. Compared with other marker technologies, AFLP provides equal or greatly enhanced performance in terms of reproducibility and resolution. Probably the single greatest advantage of the AFLP technology is its sensitivity to polymorphism detection at the total genome level. This sensitive technique has occasionally been applied to type *A. actinomycetemcomitans* and *P. gingivalis* (Fig. 1; Van Winkelhoff et al. 2000).

Genetic diversity of *A. actinomycetemcomitans* and *P. gingivalis* between individuals

Unique genetic or phenotypic markers between independent bacterial isolates are a prerequisite to studying the spread of distinct bacterial strains among individuals. Strains within a bacterial species may display variable virulence, and typing of these strains may distinguish between pathogenic and less pathogenic representatives of a species.

Using the AP-PCR method, 95 *A. actinomycetemcomitans* strains from 92 unrelated Finnish subjects yielded 17 genotypes, and 29 *P. gingivalis* isolates from 29 unrelated subjects yielded 27 genotypes (Asikainen et al. 1996). van Steenbergen et al. (1993) used the REA method to type *P. gingivalis* and found, with one exception, that each individual was colonized with one clonal type of *P. gingivalis* only. The DNA patterns of all *P. gingivalis* isolates from unrelated individuals were found to be distinct. This was also found for *A. actinomycetemcomitans* using the REA technique (Petit et al. 1993a, b).

Genetic diversity of *A. actinomycetemcomitans* and *P. gingivalis* within individuals

Asikainen & Chen (1999) have recently analysed all studies dealing with the number of clonal types of *A. actinomycetemcomitans* and *P. gingivalis* within individuals (Table 4). They found differences between studies performed in Europe and the United States on the one hand and Asian studies on the other. In Europe and the USA, the majority of patients studied harboured one single genotype of *A. actinomycetemcomitans* (75%, range 67–80%), and 80% (range 75–85%) of the tested patients harboured one genotype of *P. gingivalis*. These observations confirmed earlier observations of Loos et al. (1990), Petit et al. (1993b) and van Steenbergen et al. (1993), who found only one clonal type of *A. actinomycetemcomitans* and *P. gingivalis* in most study subjects using the REA typing method.

Recently, *P. gingivalis* strains, isolated from 106 Indonesian subjects were typed using the AFLP method. The number of subjects with one single genotype amounted to 65%. In 27% of the subjects two genotypes were detected and 7.5% (eight subjects) harboured three clonal types. The presence of >2 clonal types of *P. gingivalis* has not been observed so far in any Western study population (Table 5, Van Winkelhoff et al. unpublished data, Timmerman et al. 1998). This exemplifies that the distribution of clonal types of *P. gingivalis* in Eastern populations is

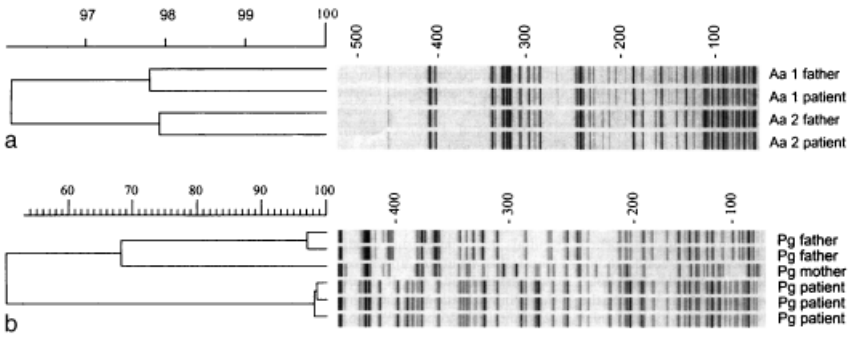

Fig. 1. Example of amplified fragment length polymorphism (AFLP) typing profiles of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* isolates from a 13-year-old Turkish patient with congenital periodontitis and their mother and father. (a) The *A. actinomycetemcomitans* isolates from the patient ($n = 2$) and the father ($n = 2$) were indistinguishable, suggesting transmission. (b) The *P. gingivalis* isolates from the patient ($n = ...$), the mother ($n = 1$) and the father ($n = 2$) show different AFLP profiles (similarity $<70\%$) (Van Winkelhoff et al. 2000).

Table 4. Number of genotypes of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in subjects with periodontitis

Species	Total # of subjects	Mean number of strains typed/subject	Number of clones			
			$n = 1$	$n = 2$	$n = 3$	$n = 4$
A. actinomycetemcomitans	120	10.3 (range: 1–115)	75%	19%	5%	0.8%
P. gingivalis	41	5.6 (range: 1–15)	80%	20%	0	0

Adapted from Asikainen & Chen (1999).

Table 5. Number of genotypes of *Porphyromonas gingivalis* in an Indonesian study population

Species	Total # of subjects	Mean number strains typed/subject	Number of clones			
			$n = 1$	$n = 2$	$n = 3$	$n = 4$
P. gingivalis	106	3.4 (range: 1–6)	65%	27%	7.5%	0

different in comparison with Western populations. The phenomenon of polyclonal colonization has also been found for *A. actinomycetemcomitans* in non-Western populations. Using the serotyping method, it has been found that, in contrast to European and USA studies (Zambon et al. 1983, Ebersole et al. 1994, Dogan et al. 1999), subjects from Eastern countries may frequently harbour more than one serotype of *A. actinomycetemcomitans* (Chung et al. 1989, Höltta et al. 1994). This discrepancy in intra-individual colonization by periodontal pathogens may be caused by socio-economic status, higher frequency of contacts with infected individuals and individual oral hygiene level.

Distribution of clonal types of *A. actinomycetemcomitans*

Not all representatives of a species may display identical pathogenic properties. Within a species, special pathogenic clones can be present (Selander et al. 1987, Musser 1996). Population studies on *A. actinomycetemcomitans* using MLEE revealed that the species comprises distinct genetic clusters that correlate with serotype distribution, but an association between genetic clusters and type of disease was not found (Poulsen et al. 1994, Haubek et al. 1995). In contrast, DiRienzo et al. (1994), using the RFLP method, have identified a specific *A. actinomycetemcomitans* genotype that appeared to be associated with juvenile periodontitis and was associated with conversion from periodontal health to disease. They also identified two genotypes that were exclusively isolated from periodontally healthy subjects. The disease-associated genotypes showed a deletion in the promoter region (Bueno et al. 1998), which may be associated with a high leucotoxine production (Brogan et al. 1994). This type of *A. actinomycetemcomitans* seems to occur specifically in patients originating from the Northern part of the African continent (Haubek et al. 1996).

Specific AP-PCR *A. actinomycetemcomitans* genotypes have been identified in periodontitis patients in the USA (Asikainen et al. 1995) and in Finnish localized juvenile periodontitis patients, which were rarely found in Finnish adult patients with periodontitis and periodontally healthy subjects (Asikainen et al. 1997). Recently, a study suggested increased periodontal destruction among Moroccan early-onset periodontitis patients who were culture positive for *A. actinomycetemcomitans* of the highly leucotoxic JP2 type compared with EOP patients without the JP2 clone (Haubek et al. 2002).

MLEE has been applied to study the population genetic structure of *P. gingivalis* (Loos et al. 1993). A total of 78 distinct electrophoretic types (ETs), representing multilocus genotypes, were identified, and cluster analysis identified three major phylogenetic divisions. Division I (71 ETs) included all 88 human isolates examined. The strains in division II and division III, isolated from different animal species, were strongly differentiated from those in division I. They found no evidence of association between specific genetic lineages or clusters and the type of disease (periodontitis or root canal infections), invasive potential, serogroup or fimbrial RFLP group. Frandsen et al. (2001) studied the population structure of *P. gingivalis* by comparing phylogenetic trees derived from sequencing fragments of four housekeeping genes. They concluded that *P. gingivalis* has a non-clonal population structure characterized by frequent recombination. Their results also suggest that particular genotypes with possible increased pathogenic potential may spread in the human population.

AP-PCR genotyping of *P. gingivalis* has also not been able to associate specific genotypes and disease status (Ménar & Mouton 1995). Serotyping of *P. gingivalis*, based on capsular polysaccharide antigens, has identified at least six different serotypes, of which the uncapsulated variant (K-) appeared less pathogenic in an animal model (Laine & Van Winkelhoff 1998). Whether the different K serotypes of *P. gingivalis* are associated with different forms or variation in progression of periodontitis in humans is currently unknown.

Distribution of *A. actinomycetemcomitans* and *P. gingivalis* among family members

One important study on the distribution of *A. actinomycetemcomitans* and *P. gingivalis* among family members is from Asikainen et al. (1996). They studied 47 families for the occurrence of *A. actinomycetemcomitans* and 31 families for *P. gingivalis*. Probands were selected on the basis of the presence of periodontal diseases (adult periodontitis) or on the presence of *A. actinomycetemcomitans*-positive children ($n = 6$). They found that spouses of periodontitis patients with *A. actinomycetemcomitans* were seven times as frequently infected with the organism in comparison with spouses of bacterium-negative patients (Table 6). Spouses of patients culture positive for *P. gingivalis* were twice as frequently positive compared to spouses of patients negative for this species. Thirty-two percent of the children with a proband culture positive for *A. actinomycetemcomitans* also had detectable levels of this species (Table 6). Only one of 19 children with a *P. gingivalis*-positive proband had detectable *P. gingivalis*.

In a large study on the transmission of *P. gingivalis* within families, Tuite-McDonnell et al. (1997) used the PCR technique to detect *P. gingivalis* in 564 members of 104 multigeneration families recruited from church and community organizations in Columbus, OH,

Table 6. Occurrence of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in family members of adult patients with periodontitis

Proband	Family member	OR	*p*-value	% of positive family members when proband is positive	% of positive family members when proband is negative
Adult patient with *A. actinomycetemcomitans*	Spouse: 7/11	17.5	0.024	64	9
Parent with *A. actinomycetemcomitans* periodontitis	Child: 6/19	8.2	0.14	32	0
Adult patient with *P. gingivalis*	Spouse 9/12	5	0.17	75	37.5

From Asikainen et al. (1996).

USA. One hundred and one of the families contained two parents, at least one child and at least one grand parent. The child had lived with the parents at the time of sampling, the parents had lived together for at least 2 years and the parents had lived with the grand parents at least up to the age of 15 years. 53.4% were females, 98.2% were white subjects and 1.8% were African Americans. The mean age of the 564 subjects was 37.5 years (SD 24 years, range 0.7–95.4 years). The overall prevalence of *P. gingivalis* was 37.1%, 46.1% in the grand parents, 42.3% in the parents and 28.2% in the children (children *versus* grand parents $p<0.02$). Table 7 summarizes the relative risks for colonization of *P. gingivalis* within pairs of family members. Individuals whose spouses were colonized were four times more likely to be colonized than those married to persons who were not colonized. No relationship was found between the length of time a couple had been married (mean 14.1 years) and their concordance of colonization ($p = 0.61$ by logistic regression). Children and mothers and their fathers were also significantly more frequently concordant in colonization than would be expected if *P. gingivalis* were randomly distributed in the study population (Table 7). A *P. gingivalis*-colonized mother would give a child a risk for colonization that was 4.7 times greater than a child with a mother who was not colonized. This risk was 2.98 for colonized fathers and a child. The risk for colonization was highest when a child had both parents colonized by *P. gingivalis*. The likelihood of whole families (grandfather/mother, both parents and a child, $N = -101$ with 404 subjects) was tested. Concordance in colonization status was highly significantly more frequent among family members than would be expected if *P. gingivalis* were randomly distributed in the population studied ($p = 0.0000$). Based on these observations, Tuite-McDonnell et al. concluded that ''contact with an infected family member substantially increased the relative risk of colonization for spouses, children and their parents, adults and their mother, and siblings'' (Table 8).

Table 7. χ^2 test results for observed and expected concordances of colonization by *Porphyromonas gingivalis* of pairs within families according to Tuite-McDonnell et al. (1997)

Pair	No. of pairs	% concordance		Probability
		observed	expected*	
Parental spouses	102	78	51	< 0.001
Grandparental spouses	62	73	51	< 0.001
Oldest, second oldest children	63	71	59	< 0.05
Mother, oldest child	103	72	54	< 0.001
Father, oldest child	101	76	54	< 0.01
Adults, grandmother	98	76	51	< 0.005
Adults, grandfathers	69	64	53	< 0.10

*Calculated based on the prevalence of *P. gingivalis* in the group being considered.

Table 8. Relative risk for colonization with *Porphyromonas gingivalis* for pairs within families according to Tuite-McDonnell et al. (1997)

Pairs	Generation	*N*	RR (95% confidence interval)
Wife–husband	F2–F2	102	4.0 (2.3–7)
Mother–oldest child	F2–F3	103	4.7 (3.1–7)
Grandmother–adult	F1–F2	98	2.4 (1.5–3.7)
Oldest child–second child	F3–F3	63	2.4 (1.1–5.3)
Father–oldest child	F2–F3	101	3 (2–4.5)
Grandfather–adult	F1–F2	69	1.8 (1–3.3)

RR, relative risk calculated χ^2 with Bonferroni's correction for multiple testing.

Genotyping of *A. actinomycetemcomitans* and *P. gingivalis* within families

Asikainen et al. (1996) used AP-PCR to genotype *A. actinomycetemcomitans* isolates from family members. In 11 of 12 families, they found identical *A. actinomycetemcomitans* genotypes among family members, which could not be explained by chance alone. This suggests transmission of this microorganism among family members. Four of 11 couples with an *A. actinomycetemcomitans*-positive proband showed identical genotypes (36% probability for transmission between spouses) and 32% of the children harboured *A. actinomycetemcomitans* that was always of the same genotype as the parent. Petit et al. (1993a, 1993b), using REA typing and an *A. actinomycetemcomitans*-positive periodontitis patient as the proband, observed that *A. actinomycetemcomitans* can be transmitted among family members but at low occurrence (15% for children, 8% for spouses). Preus et al. (1994) found essentially the same using AP-PCR genotyping of isolates from family members of which one parent suffered from periodontitis. They found that in six of seven families, the husband and wife did not harbour the same *A. actinomycetemcomitans* genotype. Furthermore, most often, children carried a genotype identical to one of the parents, indicating vertical trans-

mission (Alaluusua et al. 1991). The phenomenon of vertical transmission of *A. actinomycetemcomitans* was not observed in a study with Brazilian patients with juvenile periodontitis, who served as probands (Tinoco et al. 1998). None of the parent–child pairs showed identical *A. actinomycetemcomitans* genotypes. The presence of multiple clones in the study subjects and social–economic situation may explain these discrepant results.

P. gingivalis was isolated from both spouses in 10 couples, two of which showed identical AP-PCR profiles (20% probability for sharing the same genotype, Asikainen et al. 1996). This was significantly lower in comparison with the findings of van Steenbergen et al. (1993), who found identical REA profiles in six of eight couples positive for *P. gingivalis* (75% probability of sharing the same genotype). Saarela et al. (1993b) found two of four couples sharing the same serotype and ribotype of *A. actinomycetemcomitans* and in two of four couples the same ribotype of *P. gingivalis*. These findings support the phenomenon of transmission of periodontal pathogens among family members. Both different and identical genotypes of *A. actinomycetemcomitans* and *P. gingivalis* were found among spouses with a probability of 20–75% of transmission (identical clones). Children of a parent with an *A. actinomycetemcomitans*-associated form of periodontitis had an 8–30% probability of acquiring the *A. actinomycetemcomitans* from the proband. Vertical transmission of *P. gingivalis* has rarely been observed.

Possible ways of transmitting periodontal bacteria

Nearly all microorganisms are shed from human body surfaces and disseminated into the outside world. Most of these microbes will not survive in the environment; few can find a next host. There is no evidence that periodontal bacteria can be transmitted via aerosols, and it seems likely that saliva and direct mucosal contact are the transmission routes of periodontal bacteria. Also, inanimate objects (dental probes, tooth brushes) may serve as a vehicle for transmission. *A. actinomycetemcomitans* and *P. gingivalis* can be cultured from saliva from periodontitis patients (Van Winkelhoff et al. 1988, Asikainen et al. 1991). Van Winkelhoff et al. (1988) found *P. gingivalis* in six of eight saliva samples from untreated patients with periodontitis with numbers ranging from 6 to 20×10^6/ml. Kissing may therefore be one possible way of direct inoculation of this pathogen into another person. The survival of periodontal pathogens in saliva outside the oral cavity is not known. Indirect inoculation may occur when a contaminated toothbrush is used within several hours after use (Müller et al. 1989, Quirynen et al. 2001). The minimal infection dose and the required number of exposures to transfer *A. actinomycetemcomitans* or *P. gingivalis* from one person to another are unknown, but it seems likely that both parameters will influence the odds for transmission. Periodontal treatment reduces the salivary load of both species significantly (von Troil-Lindén et al. 1995a). Periodontally healthy subjects may have detectable salivary *A. actinomycetemcomitans* and may therefore remain a source of transmission (Asikainen et al. 1991). Salivary *A. actinomycetemcomitans* and *P. gingivalis* have shown to be of the same clonal type as those found in the subgingival plaque in the majority of patients (Petit et al. 1993a, 1993b, van Steenbergen et al. 1993, von Troil-Lindén et al. 1996, Asikainen et al. 1997).

Consequences of transmission

The clinical consequence of transmission of *A. actinomycetemcomitans* or *P. gingivalis* from a diseased person to another person is not known. It is evident that both bacterial species can be found in subjects without overt periodontal breakdown (Petit et al. 1993a, 1993b, van Steenbergen et al. 1993, Griffen et al. 1998, Van Winkelhoff et al. 2002). These species can probably persist in equilibrium with the host and the resident microflora for years. Strikingly, van Steenbergen et al. (1993) found high percentages of *P. gingivalis* not only in the periodontitis probands (ranging from 5% to 48% of the cultivable subgingival microflora) but also in the spouses (0.2–61%). Van Winkelhoff et al. (2002) also found only a marginal difference in the percentage of *P. gingivalis* between periodontitis patients and subjects without periodontitis (28.7% *versus* 17.4% of the cultivable subgingival microflora, $p = 0.052$), although the absolute numbers were significantly higher in patients. The same phenomenon was found for *A. actinomycetemcomitans*. It is not as clear which microbial and host factors determine the onset of periodontal breakdown upon infection with these pathogens.

One study has dealt with the issue of bacterial transmission between spouses and the clinical status of the spouse (von Troil-Linden et al. 1995b). In this study, two groups of married couples were involved to assess whether spouses show similarities in their periodontal status. One group consisted of probands with periodontitis and their spouses ($n = 10$); a second group consisted of periodontally healthy proband and their spouses. Clinical, radiographic and microbiological data were obtained from all subjects. It was found that spouses of the diseased probands had a significantly lower percentage of $\leqslant 3$ and 4–5 mm pockets and more sites with alveolar bone ($< 1/3$ of root length) than spouses of the healthy probands. Microbiological analysis showed that in the diseased group, four couples (proband and spouse) were infected with *A. actinomycetemcomitans*, and six couples with *P. gingivalis*, whereas in the healthy group no *A. actinomycetemcomitans*-positive couple (proband and spouse) and one *P. gingivalis*-positive couple was found. These observations suggest that cohabitation with a person with periodontitis may influence the microbiological and the periodontal status of the spouse. One factor that may explain these observations is the higher occurrence of *A. actinomycetemcomitans* and *P. gingivalis* in subjects in the diseased group, suggesting person-to-person transmission of these pathogens. More of these studies, with larger numbers of couples and genotyping of the bacterial isolates, are needed to provide further evidence for a possible transmission of destructive periodontal disease among spouses.

Summary and conclusions

Periodontitis is a collection of clinically different diseases associated with a subgingival microflora that significantly differs among different patient groups. The majority of subgingival bacterial species are indigenous to the oral cavity, and periodontitis associated with indigenous bacteria can be looked upon as an opportunistic infection. *A. actinomycetemcomitans* and *P. gingivalis* are infrequently detected in periodontal health in Western populations and have characteristics of exogenous pathogens.

Therefore, periodontitis associated with these pathogens can be looked upon as exogenous infections. In *A. actinomycetemcomitans*, but not in *P. gingivalis*, special clones associated with localized juvenile periodontitis have been identified. Vertical transmission of *A. actinomycetemcomitans* but not of *P. gingivalis* has been established. Most studies have shown that if children harbour *A. actinomycetemcomitans*, usually one or two parents harbour the same genotype. From these observations, it is assumed that the parent is the source of transmission. However, identical genotypes in family members are not 100% proof of transmission, as there is no infinitive number of genotypes and finding identical genotypes may have occurred by chance (Asikainen et al. 1996). The frequency of vertical transmission of *A. actinomycetemcomitans* is estimated to be between 30% and 60% based on detection of identical genotypes in children and parents. This pattern of vertical transmission may not occur as frequently in Eastern and Southern American countries. Vertical transmission of *P. gingivalis* may occur but has only rarely been observed based on genotyping of the isolates from parents and children. The Tuite-McDonnell et al.'s (1997) study has suggested that vertical transmission of *P. gingivalis* can occur, but this study does not provide conclusive evidence as genotyping has not been performed.

Horizontal transmission of *A. actinomycetemcomitans* and *P. gingivalis* between spouses has been documented and may range between 14% and 60% for *A. actinomycetemcomitans* and between 30% and 75% for *P. gingivalis*. Transmission of *A. actinomycetemcomitans* between siblings has been suggested, but infection by the same source cannot be ruled out. Frequency of contact, number of organisms, oral health status, the resident microflora and immunological and genetic factors may determine whether a person will be permanently colonized by periodontal pathogens upon challenge. Although there is some limited evidence to show that cohabitation with a periodontitis patient influences the periodontal status of the spouse, substantially more information is needed to prove this hypothesis. The effects of prevention of transmission of *A. actinomycetemcomitans* and *P. gingivalis* have not been studied so far. For *A. actinomycetemcomitans*, screening for and prevention of transmission of specific virulent clones may be feasible and effective in preventing some forms of periodontal disease. *P. gingivalis* is usually recovered from diseased adult subjects and transmission of this pathogen seems largely restricted to adult individuals. Horizontal transmission of *P. gingivalis* may therefore be controlled by periodontal treatment involving elimination or significant suppression of the pathogen in diseased individuals and by a high standard of oral hygiene.

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