Potential of Diagnostic Microbiology for Treatment and Prognosis of Dental Caries and Periodontal Diseases

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ABSTRACT: Most evidence suggests that only a finite number of bacteria are responsible for dental caries and periodontal diseases. This knowledge led to the development of microbial tests which can identify suspected pathogens. Current evaluation of the diagnostic power of microbial tests has shown that they have a low sensitivity and a low prognostic value. Despite these shortcomings, there are valid indications for microbiological-based diagnosis. Salivary microbial tests for the detection of mutans streptococci and lactobacilli may be useful, for example, in young children, oligosialic patients, and orthodontic patients. These tests can be used to monitor the success of chemopreventive measures or compliance with dietary recommendations. Microbial diagnosis may also be valuable in the treatment of early-onset periodontitis or in subjects who respond poorly to periodontal therapy. The use of microbial tests to monitor the efficacy of chemotherapy or mechanical treatment is of particular interest.

Key words. Dental caries, periodontitis, microbiology, diagnosis.

(I) *Introduction*

Dental caries and periodontal diseases are the most common diseases in the oral cavity. In industrialized countries, there are few adults who have not experienced dental caries. The Global Oral Data Bank of WHO shows that by the age of 12 only 15 to 30% of the population is caries-free. Epidemiological surveys have also shown that most adults present some mild form of periodontal disease, and that 5 to 15% suffer from severe periodontitis (Pilot *et al.*, 1986; Miller *et al.*, 1987). Both diseases are known to be associated with micro-organisms colonizing tooth surfaces (Socransky and Haffajee, 1993; van Houte, 1994). Dental caries can be prevented by adequate oral hygiene, reduced frequency in sugar consumption, and optimal use of fluoride. Prevention of periodontal diseases is mainly based on plaque control. Although such approaches have been proven to be effective in controlling these diseases, dental and periodontal diseases still remain a major public health problem in the general population, with substantial economic implications.

The core of traditional dentistry is based on the treatment of oral diseases. For dental caries, treatment is usually initiated when lesions are clinically detectable (evidence of tooth substance loss, radiotransparency in the tooth structure) and tissue damage is irreversible. Treatment usually involves the removal of the affected tissues and placement of a filling material. A similar approach is applicable to periodontal diseases. Mechanical debridement is initiated when lesions (presence of periodontal pockets, loss of attachment, radiographic evidence of bone loss, bleeding) are observed. Two comments should be made concerning this approach. First, clinicians lack diagnostic methods to detect early tissue changes when lesions are still at a reversible stage and can be treated in a non-invasive way. Second, as in the case of dental caries, the filling approach is not necessarily synonymous with control of the disease. The alternative would be to emphasize prevention, and treatment should be targeted at controlling the etiological agent(s). In this context, oral microbiology plays a major role not only in our understanding of the etiology but also in our ability to prevent and treat oral diseases.

During the past decade, remarkable advances have been made in the field of oral microbiology, particularly as it relates to diagnosis. Some of this newly gained knowledge can already be applied in the clinic today. The present review will discuss the role of microbiology in the diagnosis and treatment of dental caries and periodontal diseases. It will focus on several questions related to the use of microbiologic diagnosis in the clinic: Can microbiology tests be used to identify subjects at risk, help determine treatment strategies and modalities, as well as assist in determining treatment end-point?

(2) *Microflora of the Oral Cavity*

The mouth consists of numerous distinct habitats, each of which has its own ecological conditions. Each habitat supports the growth of certain populations of bacteria (defined bacterial species) and harbors a characteristic microbial community (Marsh, 1989). However, ecological conditions within the mouth are susceptible to environmental factors such as dietary habits, oral hygiene, and antimicrobial chemotherapy. These changes in the environment could affect the microbial community and produce shifts in the proportions of the resident species.

Where do the bacteria which colonize the oral cavity originate? Microbial colonization is known to begin at birth. A young child without any teeth usually does not carry any mutans streptococci (Carlsson *et al.*, 1975). Following eruption of teeth, the ecological conditions for colonization by mutans streptococci are established. It has been shown that children seem to acquire bacteria from their mother, as evidenced by studies involving the tracing of serotypes, bacteriocin patterns, or, more recently, by restriction endonuclease mapping (Berkowitz and Jordan, 1975; Kulkarni *et al.*, 1989). This concept of vertical transmission within families or populations has strong clinical implications. Some studies demonstrated that it was possible to delay the colonization and reduce the risk of caries by preventive measures aimed at decreasing mutans levels in mothers (Köhler and Andreen, 1994). Periodontal pathogens such as *Actinobacillus actinomycetemcomitans* or *Porphyromonas gingivalis* also seem to be transmitted among family members and acquired from the immediate environment (Alaluusua *et al.*, 1993; Petit *et al.*, 1993).

Dental caries and periodontal diseases are multifactorial diseases in which the bacteria play an important role. They are essentially opportunistic infections caused by bacteria residing in the oral cavity. However, there is evidence that only a finite number of the 200 to 300 distinct bacterial species comprising the oral flora play any role in the initiation of these diseases (Loesche, 1977; Tanner, 1991; Haffajee and Socransky, 1994). In caries lesions, the proportions of mutans streptococci and lactobacilli have been found to be elevated. The microflora at sites with periodontal destruction differs from that at healthy gingivae. The flora from periodontal pockets is dominated by anerobic Gram-negative bacteria as well as a high proportion of spirochetes.

Much has been learned about the micro-organisms that are associated with these diseases. Prevention should be aimed at maintaining the equilibrium between the host and the bacteria. As discussed below, microbial monitoring may be a valuable tool to screen the status of the oral ecology before and after treatment.

(3) *Identification of Oral Micro-organisms*

There are several ways of detecting and quantifying oral micro-organisms. For detailed information on this subject, the reader is refered to several reviews (Russell, 1991; Tanner *et al.*, 1991).

In vitro culturing methods have played a key role in the characterization of the microbiota of dental plaque. Current knowledge of the composition of the microflora associated with various forms of dental and periodontal diseases derives, for the most part, from cultural observations. In this approach, the plaque sample is plated on nonselective agar media, and the so-called "predominant cultivable flora" is identified. However, cultural methods require a high degree of expertise. They are difficult to perform and are technique-sensitive. Estimates suggest that less than 50% of the total flora from subgingival samples can be cultivated (Loesche *et al.*, 1992b). Nevertheless, this approach is still widely used to determine levels of bacteria associated with dental caries. Mutans streptococci and lactobacilli are usually detected in salivary samples cultured on *selective* media. These media are designed to increase the recovery species of interest by suppressing other organisms usually present in the sample. Cultures are still valuable as research tools to study micro-organisms associated with periodontal diseases, since parts of this flora remain unknown. However, this approach is not suitable for routine microbial screening in periodontal diagnosis, because it is time-consuming and expensive.

DNA probe methods, immunoassays, and micromethods used to determine phenotypic markers such as enzymes have recently been developed to identify a limited set of subgingival bacterial species. Genetic probe methods rely on DNA segments or sequences of nucleotides of 16S rRNA which are unique to each bacterial species. DNA probes prepared from a representative strain of the target micro-organism will hybridize to these sequences. The detection of target micro-organisms is therefore based on the binding of the DNA probe to the complementary strands of nucleic acids in these unique regions. Three types of DNA probes have been described: whole-genomic probes, cloned probes, and synthetic oligonucleotide probes (Dewhirst and Paster, 1991).

Whole-genomic and cloned probes are derived from chromosomal DNA, whereas oligonucleotide probes are constructed to hybridize hypervariable regions of 16S rRNA (Tanner *et al.*, 1991). Construction of whole-genomic probes is based on larger DNA fragments (23 kb or larger) than required for cloned probes (1 kb). For oligonucleotide probes, short segments of DNA (13-30 bp) are synthesized to hybridize with the 24 to 30 selected bases. With whole-chromosomal probes, cross-reactions may be observed with genetically related species.

Cloned probes are more specific than whole-genomic probes. As for oligonucleotide probes, they can be very specific. They may distinguish between closely related species, since they can differentiate among sequences that vary by only one base. After hybridization, the reaction is quantified by comparison of the signals of the probe with standards of pure DNA of the target species. The level of detection is estimated between 10^2 and 10^3 cells, depending on the type of probe, the labeling, and the detection method used. The polymerase chain reaction (PCR) method has been recently used to increase the sensivity of DNA probes. This technique permits replication and multiplication of the number of target DNA or RNA fragments present in a sample. Quantitative data can be obtained if the degree of amplification is known. These powerful tools can also be used to identify unknown organisms as well as for bacterial taxonomy.

Immunological methods have also been used to detect bacterial species that are genetically distinct (Tanner *et al.*, 1991). Immunoassays include agglutination, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and immunofluorescence. In these assays, polyclonal or monoclonal antibodies against species-specific surface antigens can be used to identify target bacteria. Generally, polyclonal antibodies comprise a mixed population of antibody molecules. They react with a set of different specific antigens of the target bacteria and may give cross-reactions with antigenically related species. This problem can be minimized, for example, by absorption of cross-reactive antibodies. Monoclonal antibodies are very specific and can be produced in potentially unlimited amounts (Gmür, 1995). One can argue that monoclonal antibodies are so specific, they may not recognize strains of the same species that present slightly different antigenic epitopes.

Identification and speciation of bacterial isolates can be based on phenotypic markers and key discriminatory properties, including enzyme profiles, sugar fermentation patterns, metabolic end-products, *etc.* For example, enzyme profile has been evaluated for the identification of Actinomycetaceae and related species (Kilian, 1978), the group of black-pigmented Bacteroides species (Slots, 1981), and oral spirochetes (Laughon *et al.*, 1982). Most of these bacterial species appear to possess a unique set of enzymes, which makes identification possible. Thus, a predetermined series of biochemical tests is used for a particular group of micro-organisms. Preformed enzymes can be detected by means of colorimetric and fluorimetric substrates that have been miniaturized and incorporated into kit form (Loesche, 1986). The advantage of enzymatic identification is the short incubation time of the test.

The recognition that dental and periodontal diseases are initiated by a limited number of species led to the development of microbiological diagnostic tests. Microbiological techniques primarily designed for research purposes were adapted to diagnosis. Both the academic community and industry played a major role in these developments. Extensive testing is still needed to determine the utility and implications of this technology in a clinical environment.

(4) *Bases and Perspectives of Salivary Microbial Tests to Predict Individual Future Dental Caries Activity*

(4.1) Current concepts of the etiology of dental caries

In modern textbooks of cariology (Newbrun, 1989; Thylstrup and Fejerskov, 1994), dental caries is still presented as a multifactorial disease. Prevalence and incidence are described as being dependent on the result of multiple interactions among host factors, diet, and microbiota. If this is indeed true, then any single test analyzing only one of these factors will almost certainly have a low predictive value for an individual's future caries activity.

Nevertheless, since W.D. Miller (1890) proposed the chemoparasitic caries theory, the development of tests to predict individual caries activity has attracted considerable interest. This is not astonishing, for it has always been the noble goal of responsible clinicians to prevent rather than to treat diseases. The state of the art can be fully comprehended only by taking into account the numerous efforts made in the past to predict individual caries activity. In restrospect, it is interesting to note that most caries activity tests used to date have utilized saliva and/or salivary bacteria as starting materials for analyses, as initiated by W.D. Miller more than 100 years ago.

(4.2) Basic etiologic findings and their relation to caries activity tests

In 1938, the American Dental Association made a worldwide enquiry among 195 institutions involved in caries research to elicit concise summary statements on basic findings of fact, conclusions, and references on dental caries. The results were compiled in a book, **Dental Caries, Findings and Conclusions on its Causes and Control** (ADA, 1939). This volume is an excellent source for information on both the development of basic etiologic concepts and on the accompanying development of tests that were used to predict future individual caries activity during the early 1920s up to 1939. Even though it is dated, this reference is the most comprehensive of its kind in the field of dental caries for this period. Major perceptive steps and the ensuing caries activity tests are compiled in Table 1. [Most of the respective references

TABLE 1

An Abbreviated Historical Review (1890-1939) of the Microbial Etiology of Dental Caries and Ensuing Caries Activity Tests

Major Perceptive Steps	Caries Activity Test
1890 Miller Chemoparasitic caries theory: Salivary bacteria produce acids from sticky dietary starch on teeth which dissolve enamel.	**1890 Miller** Artificial lesions in teeth in saliva-starch mixtures.
1897-1911 Williams, Black, von Beust (Ref. in Guggenheim, 1970) Formation of acids in saliva is of no relevance for the formation of lesions. Relevant are acids formed in gelatinous plaques on teeth.	**1929-1937 Hanke (Ref. in ADA, 1939)** *In situ* test for plaque acidity: Sucrose rinse followed by methyl-red test.
1915-1929 Kligler (1915), Howe and Hatch (1917), McIntosh et al. (1922), Rodriguez (1930, 1931), Morishita (1929), and others *Bacillus acidophilus (Lactobacillus acidophilus)* reportedly associated with dental decay.	**1930, 1931 Rodriguez** Selective agar for lactobacilli of oral flora used for susceptibility index for dental caries.
1924 Clarke *Streptococcus mutans* occurs in the front of dentinal lesions and lactobacilli in the body of lesions.	**1936 Blayney (Ref. in ADA, 1939)** Cultural and morphological plaque analyses for lactobacilli to assess future caries activity.
1930-1939 Belding, Bibby, Fosdick, Hearman, Lyons, and others (Ref. in ADA, 1939) Micro-organisms other than or in addition to *L. acidophilus* cause initial caries (streptococci, yeasts).	**1937-1938 Hansen (Ref. in ADA, 1939)** Rate of acid formation from sucrose in saliva as a measure for caries activity.
1936-1939 Dean, Cox (Ref. in ADA, 1939) Discoverer of the caries-preventive properties of F^-.	**1928-1937 Hatton (Ref. in ADA, 1939)** Saliva-glucose-enamel mixtures incubated for 4 hrs. Dissolution of Ca^{2+} and PO_4 as indices for caries activity.

may be found in the aforementioned book (ADA, 1939) and some of the earlier quotes in a review by Guggenheim (1970).] How very advanced were the insights into the etiology of dental caries almost fifty years ago is best documented by quoting the summary of Bunting and Russell (ADA, 1939) *verbatim*:

"1. No consistent relationship has been found between hardness or perfection of teeth, nor state of mouth hygiene, and activity of caries.
"2. No correlation has been demonstrated between amounts of salivary calcium, phosphorus, chlorides, pH, CO_2-capacity, total alkalinity, total solids, or ash, and activity of caries.
"3. No relationship has been demonstrated between intake of calcium, phosphorus, or acid/base dietary values, and activity of caries.
"4. Inherited tendencies or inherent individual characteristics, in a small percentage of cases, are more important determining factors in caries than ordinary dietary conditions. A great majority of caries-susceptibles, however, can be benefited apparently by adoption of very simple dietary measures.
"5. There is no evidence that caries is produced by malnutrition, or may be prevented by adequate diets.
"6. Sugar is a very important causative factor in caries. A remarkably low degree of caries was observed in children on a low-sugar diet deficient in calcium, phosphorus, and vitamin D. Active caries was induced in children by increasing the sugar intake while they were receiving a diet that nutritionally was adequate. Ingestion of low-sugar diets by children is conducive, as a rule, to freedom from caries.
"7. The most constant differential between caries-free and caries-susceptible persons, thus far demonstrated, is that of relative number of L. *acidophilus* organisms in the mouth. This correlation is approximately 90 percent positive."

It is evident from Table 1 (left column) and especially from the summary of Bunting and Russell that fifty years after the conception of the chemoparasitic caries theory, the major insights into the etiology of dental caries had already been achieved. This includes the caries prophylactic action of fluoride and the discovery of *Streptococcus mutans*, the significance of which was not recognized by fellow researchers at that time.

Miller originally created artificial caries lesions to distinguish, *in vitro*, between the cariogenicity of different carbohydrates. All of the other researchers listed in Table 1 (right column) devised tests to evaluate individual caries susceptibility. Hanke proposed an *in vivo* plaque acidity test, while Hatton introduced an enamel dissolution assay for calculating a caries activity index. On the basis of microbial analyses of carious dentin, especially salivary *Lactobacillus* counts, a specific etiologic role of *Lactobacillus acidophilus* in the development of caries lesions emerged between 1915 and 1929. This period is covered in an excellent review by Davis (1959). It is therefore not surprising that forerunners of today's salivary lactobacilli tests were established in the early 1930s. Credit for improved culture techniques for lactobacilli and the first systematic investigation of the quantitative incidence of L. *acidophilus* and caries goes to Rodriguez (1930, 1931). Two years later, Blayney (ADA, 1939) combined cultural and morphologic plaque analyses to determine the presence or absence of a clinically undetectable caries process at the sample site. An additional morphological analysis was included because of the discrepancy between the number of cultivable lactobacilli and the number of Gram-positive rods found in plaque with the morphological characteristics of lactobacilli. Although lactobacilli were considered as prime etiologic agents for dental caries during that period, there were at that time (Table 1, left column)—and even before—researchers who questioned this concept, most notably, Clarke (1924), who detected S. *mutans* at the front of the histological lesion of dentinal caries, whereas lactobacilli were isolated from the body of a lesion.

In 1940, Snyder described a simple colorimetric test for estimating the relative number of lactobacilli in saliva. Saliva (0.2 mL) was added to tubes of a selective (pH 5.0) liquefied agar medium. A change in the color of the indicator brom-cresol-green from green to yellow after 48 hours' incubation was indicative of $> 10^3$ lactobacilli *per* mL of saliva. A further refinement in the cultivation of lactobacilli was an improved selective medium, introduced by Rogosa *et al.* in 1951, which allowed for growth of an extended spectrum of oral lactobacilli. This medium is still the basis of modern diagnostic salivary lactobacilli tests. In the 1950s, the pendulum started to swing in another direction. Several factors were responsible for a gradual shift in the perception of the microbial etiology of dental caries (Table 2, left column). First was the classic experiment of Orland *et al.* in 1955 in which caries was induced in rats mono-associated with a *Streptococcus* strain. This was soon followed by a series of experiments by Fitzgerald and Keyes at the NIH (van Houte, 1980) which showed that caries in rodents was a transmissible disease caused by streptococci that were believed to be members of a heretofore-undescribed species. Similar strains were subsequently isolated from man and were found to be identical with the species S. *mutans* previously described by Clarke (1924). Besides its strong acidogenic potential, mutans streptococci produce mutan, a predominantly α-1,3-linked glucan and a major virulence factor not synthesized by other oral streptococci. The importance of this glucan (as well as the glucosyltransferase responsible for its synthesis) for the formation of a water-insoluble plaque matrix in promoting the accumulation of mutans streptococci on human teeth and colonization of other organisms in supragingival plaque has been reviewed by Guggenheim (1970) and van Houte (1983). Another line of evidence which helped demolish the credibility of the lactobacillus theory came in the form of reports that the number of lactobacilli in plaque covering sound enamel never exceeded 0.1% of the cultivable microbiota, which excluded a significant role for these bacteria in the etiology of incipient lesions. In contrast, a close association of streptococci of the mutans group with incipient enamel lesions was demonstrated in cross-sectional and longitudinal studies (Table 2, left column). This table contains the names of only a few of the pioneers who first addressed this question. The *coup de grâce* to the lactobacillus theory of dental caries had already been given in 1963 by Snyder *et al.*, who showed that neither the colorimetric lactobacillus (LB) test, the exact enumeration of lactobacilli in saliva, nor other salivary tests had a reliable predictive value for future caries activity on an individual basis. Thus, within fewer than 20 years, the concept of the etiology of caries had swung from lactobacilli to S. *mutans* theory. Following this development, Gold *et al.* (1973) described a selective medium based on the Mitis-Salivarius agar (MS) for mutans streptococci, which were found to be resistant to bacitracin. Based on this medium, Alaluusua *et al.* (1984) introduced the Dentocult® Strip Mutans Test, a simple diagnostic test allowing the gross enumeration of salivary mutans streptococci to be done outside a bacteriology laboratory under both clinical and field conditions. A similar dip slide test for salivary lactobacilli based on Rogosa's medium, the Dentocult® LB-test, had been previously devised by Larmas (1975).

(4.3) The value of caries susceptibility tests in the prediction of future caries activity, more recent findings

In 1968, Socransky assessed the state of the art in a very competent and critical manner. He screened the litera-

TABLE 2

An Abbreviated Historical Review (1946-1985) of the Microbial Etiology of Dental Caries and Ensuing Caries Activity Tests

Major Perceptive Steps		Caries Activity Test	
1946	**McClure and Hewitt** Addition of penicillin to the diet of rats prevents caries.	**1940**	**Snyder** Colorimetric method for the estimation of relative numbers of lactobacilli in saliva.
1955	**Orland *et al.*** Germ-free rats remain caries-free on a sucrose diet: when mono-associated with an *Enterococcus*, caries lesions form.	**1951**	**Rogosa *et al.*** Improved selective medium for lactobacilli.
1958-1962	**Winkler and Backer-Dirks, MacDonald, Gibbons, and others (Ref. in van Houte, 1980)** Lactobacilli in smooth-surface plaque constitute a minor proportion of the microbiota. Role of lactobacilli in initiating caries questioned; salivary microbiota not representative for plaque.	**1963**	**Snyder *et al.*** Comparison of *Lactobacillus* fermentation test with *Lactobacillus* count, buffering capacity, amylase test, etc., showed that a reliable prediction of future caries activity on an individual basis was not possible with these tests.
1960-1962	**Keyes and Fitzgerald (Ref. in van Houte, 1980)** Caries transmissible in rats and hamsters. Etiologic agents *Streptococcus* FA and HS believed to be so far undescribed.	**1973**	**Gold *et al.*** A selective medium for mutans streptococci based on MS-agar plus bacitracin devised.
1965-1967	**Carlsson, Guggenheim (Ref. in Guggenheim, 1968)** *Streptococcus mutans* isolated from human plaque shows identical HS and FA strains of Keyes and Fitzgerald.	**1975**	**Larmas** A dip-slide method for counting lactobacilli based on Rogosa medium developed (Dentocult-LB).
1967-1972	**Carlsson, Gibbons, Guggenheim, Newbrun, and others (Ref. in Guggenheim, 1970)** Investigated extracellular polysaccharide formation of *Streptococcus mutans* and *sanguis* from sucrose. Mutan α-1,3-linked glucan designated major "virulence factor" of mutans streptococci.	**1984**	**Alaluusua *et al.*** Slide scoring method for estimation of *Streptococcus mutans* levels in saliva based on the medium of Gold (Dentocult-SM).
1967-1975	**de Stoppelaar, Bowden, Hardle, Loesche, van Houte, and others (Ref. in van Houte, 1980, 1994)** In cross-sectional and longitudinal studies, the close association of mutans streptococci with incipient caries lesions was shown.		
1973-onward	**Gibbons, van Houte, Cisar, Kolenbrander, and many others (Ref. in Kolenbrander, 1988)** Specific adherence/co-aggregation concept of oral bacterial colonization.		

ture of over 40 years, covering more than 160 papers. All caries susceptibility tests so far proposed were divided into three categories:

(1) Relation of physiological and biochemical properties of saliva to caries activity;
(2) Relation of enzyme activity to caries activity; and
(3) Relation of acidogenic potential of salivary constituents to caries activity (microbiota).

He drew the following sobering conclusions: "None of the caries activity tests currently available are adequate

TABLE 3

Definitions and Calculations

Screening Criteria	Validating Criteria: Positive	Validating Criteria: Negative	Total
Positive	a (true positive)	b (false positive)	a + b
Negative	c (false negative)	d (true negative)	c + d
Total	a + c	b + d	n

TABLE 4

Definitions of Sensitivity, Specificity, and Positive and Negative Predictive Values (Krasse, 1988)

Sensitivity: The probability that a screening procedure will give a positive finding which is in agreement with the validating criteria.

$$= \frac{a}{a + c} \times 100$$

Positive predictive value: The proportion of true positives among recorded positive cases:

$$= \frac{a}{a + b} \times 100$$

Specificity: The probability that a screening procedure will give a negative finding which is in agreement with the validating criteria.

$$= \frac{d}{b + d} \times 100$$

Negative predictive value: The proportion of true negatives among recorded negative cases:

$$= \frac{d}{c + d} \times 100$$

for the evaluation of caries activity of a patient." He regarded the two major reasons for the failure of these tests to be: (1) the multifactorial etiology of the disease and (2) the use of saliva as a test material because salivary micro-organisms are not representative of plaque microbiota (van Houte, 1980; Table 2). With regard to the salivary lactobacilli tests (Snyder test and *Lactobacillus* count), Socransky (1968) concluded: "Different investigators have found the test to vary from essentially useless in the prediction of caries to a low or moderate correlation coefficient that is significant at a group level, but of no value for individual prediction."

After 1970, some major trends related to the microbial etiology of dental caries as well as periodontal diseases became evident, trends which were substantially intertwined with changes occurring in the socio-economic conditions of oral research. The specific plaque hypothesis emerged, which addressed dental caries as an infectious disease caused by mutans streptococci (reviewed by van Houte, 1980, 1994), and the different forms of periodontal disease as a result of infections by exogenous micro-organisms not associated with gingival and periodontal health were identified (Slots, 1986; Genco *et al.*, 1988; van Winkelhoff *et al.*, 1988a). Indeed, there is no longer any doubt that incipient caries lesions are strongly associated with mutans streptococci (for review, see van Houte, 1980, 1994). The occurrence of lactobacilli as invaders of carious crown (Edwardsson, 1984) and root dentin (Schüpbach *et al.*, 1995) was confirmed. In addition, these bacteria were also found in the crypts of the tongue in subjects consuming sucrose with high frequency (van Houte *et al.*, 1972). The wide dissemination of fluoride prophylaxis led to a declining caries prevalence and incidence in industrialized countries. Health costs exploded and a decrease of public support for oral sciences had to be endured. In countries where research was largely dependent on public grant support, this led to the formulation of simplistic but promising hypotheses such as the abovementioned specific plaque hypothesis. In addition, many researchers became increasingly associated with and dependent on industry. Oral microbial diagnostic tests were vigorously promoted with increasing success by both industry and their contract scientists. Conceptional studies in which the level of infection with mutans streptococci and/or lactobacilli was correlated with the incidence of caries have been excellently reviewed by Krasse (1988) and Pienihäkkinen (1988). In both reviews, more than 20 studies, in part identical, were evaluated according to the following definitions and criteria (Carlos, 1978): sensitivity, specificity, and positive and negative predictive values. These terms are explained and mathematically defined in Tables 3 and 4. They were used to assess the diagnostic power of microbial tests.

Sensitivity, specificity, and positive predictive value are the determinants by which the diagnostic power of a microbial test is evaluated. If the same test, *e.g.*, the Strip Mutans test, is used in several studies, the values of these determinants are strictly comparable between studies only if the validation criteria are identical. As shown by Krasse (1988), this is often not the case. There were great differences in the validating criteria (from 1 to 4 new decayed surfaces *per* year) as well as considerable differences in sampling and test methods in the different studies reviewed by Krasse (1988) and Pienihäkkinen (1988). It is therefore not surprising that the results show a considerable degree of variation despite these clear definitions. Sensitivity, *i.e.*, the probability that caries-active individuals have high values for S. *mutans* or lactobacilli, varied from 44% to 71% in the studies analyzed by Krasse (1988). Specificity, *i.e.*, the probability that sub-

TABLE 5

Caries (DT) and Restorations (FT) in Selected Age Groups in the Canton of Zürich in 1991

8-9 Years Old	13-14 Years Old	35-54 Years Old
8% have 100% of DT	11% have 100% of DT	20% have 70% of DT
13% have 100% of FT	20% have 66% of FT	31% have 45% of FT

(Menghini, pers. comm. 1991)

jects without new caries or a low caries incidence had low values for mutans streptococci or lactobacilli, ranged from 56% to 100%.

Strains of *Streptococcus mutans* were previously subdivided into eight serotypes (a-h). DNA hybridization studies showed enough diversity among these strains and serotypes to recommend their classification first into four and later into seven separate species. Humans harbor S. *mutans* and S. *sobrinus* in dental plaque. Occasionally, S. *rattus* and S. *cricetus* have also been isolated from man. These strains are predominantly found in rodents (Coykendall, 1989). Since the human and animal strains are physiologically very similar, the differentiation into these species, although accepted in principal, is, however, often circumvented by use of the term "mutans streptococci".

Almost all study groups included a considerable number of subjects with high numbers of mutans streptococci who did not develop lesions (false-positives), which explains the resulting modest positive predictive values. More accurate predictive values were obtained when results of different tests were combined—for example, the SM-test and LB-test—with incipient lesions used as the validating criterion (ref. in Krasse, 1988). In populations with high caries prevalence (*e.g.*, Third World countries), the value of these tests (sensitivity, positive predictive value) is much higher than in populations with low caries prevalence (Krasse, 1988). However, in the former countries, almost the entire population is at risk, and the modest financial resources available would be better spent on group prophylactic programs than on futile tests. In industrialized countries where fluoride prophylaxis is practiced, sensitivity and predictive value of microbial diagnostic tests were seen to decrease with age. Excellent positive predictive values were found for young children in the age group 2 to 4 years (Alaluusua and Renkonen, 1983) and for children in the age group 12 to 13 years (Krasse, 1988); when a large number of teeth erupt, predictive values were below the modest expectations in older age groups.

(4.4) Perspectives and Alternatives for Salivary Microbial Tests

Salivary microbial tests in industrialized countries are of little value for predicting the individual future caries activity of subjects older than 4 years. However, these tests are still widely recommended to identify the 25-35% of children who are most prone to develop caries, so that they might be included in special prophylaxis programs. To illustrate this point, the distributions of carious teeth and restorations in some age groups in Switzerland are provided in Table 5. It is immediately evident that under the prevailing epidemiological conditions, prophylactic programs addressing the population at large are no longer cost-effective. An early identification of the "caries risk" groups would therefore be highly desirable. As already mentioned, this does not apply to Third World countries, where caries prevalence is still high and where the capacity for community dental health care is rather limited. In these countries, the very scarce professional dental infrastructure and funds should be invested in public health programs that include prophylactic measures. There is absolutely no need for microbial diagnostic tests in these countries.

Büttner (1993) published data derived from 5743 children cared for by the schoolchildren's service in the city of Basel (Switzerland). The percentage of caries-free children with different degrees of salivary mutans counts was plotted for three different age groups (Fig. 1). In all age groups, from 28 to 40% of the children with $\geq 10^6$ mutans streptococci/mL saliva were caries-free (low sensitivity). However, in the age groups 7 to 12 years and 13 to 16 years, over 30% and 50%, respectively, of the children with less than 10^5 mutans streptococci/mL saliva (scores 0 and 1) exhibited caries lesions. The cost-benefit ratio of salivary microbial diagnostic tests can be calculated as follows: A prophylactic measure is cost-effective if the avoided therapy costs divided by prophylaxis expenditures is ≥ 1. Assuming that under the prevailing epidemiological situation in Switzerland, 25% of the younger population is at risk and that the costs for both a Dentocult® Strip Mutans and a Dentocult-LB test will total $35 including labor, the selection of one subject at risk will cost $140. Even if the predictive value of the combination of these tests were 100%, which it is not, it becomes clear that salivary microbial tests are not a cost-effective way of selecting subjects for intensive prophylactic programs. The same conclusion had been reached by Isokangas *et al.* (1993). In this study, 15 dentists made caries activity predictions in groups of children aged 3 to 4 and 5 to 16 years on the basis of clinical examinations only, with astonishing results. The sensi-

tivity of their predictions verified one year later was 58%. Specificity was 84%, and the positive predictive value 56%. They predicted that 26.9% of the children would show dentinal caries, and subsequently found that 26.6% of the children did indeed show dentinal caries. Furthermore, the clinicians predicted more than one carious surface for 9.2% of the cases, and 10.7% were subsequently observed.

Helfenstein *et al.* (1991) and Steiner *et al.* (1992) described a method, named "Dentoprog", for predicting high caries increments in children based on clinical parameters only. They sought to answer the question: Is it possible to predict future high caries increments in schoolchildren, using clinical parameters only and no microbial, salivary, socio-economic, or other data? In the first stage, possible dental predictors were selected. Clinical and radiographic data (dmfs/DMFT) collected at four-year intervals since 1972 in 16 communities around the city of Zürich were systematically tested by pilot statistical models to determine whether any of the clinical parameters could be related to future caries activity. In the second stage, 46 clinical variables were chosen and tested as individual predictors using data collected in 1980, 1984, and 1988. These data were subjected to simple logistic regression for two age groups (7 and 10 years) according to three definitions of high caries increment. In the final phase, the 22 most promising variables, which were significant for all data sets, were further analyzed by multiple logistic regression. The following three clinical parameters proved most suitable for predicting future high caries increments in children: (1) the number of sound primary molars [SOU prm, 0-8]; (2) the number of discolored pits (palatal in maxilla, buccal in mandible) and the number of discolored fissures on first permanent molars [DISC pifi, 0-8]; and (3) the number of buccal and lingual/palatal smooth surfaces of first permanent molars with white spots [SPOT sm, 0-8]. The formula of the Dentoprog value (DPV) for 9.5-11.5-year-old children, for example, is:

$$DPV = 0.27\,[SOU\ prm] + 0.24\,[DISC\ pifi] + 0.34\,[SPOT\ sm]$$

By this formula, the DPV of clinically examined individuals was calculated. The cohort could be ranked according to the results. The risk group could be selected according to available capacity and means for intensive prophylactic programs, e.g., 30% of the cohort with the highest DPV. The precision of the predictions was calculated for periods of four, five, and eight years. A specificity of 71-79% and a sensitivity of 67-70% was observed, which is, compared with microbial salivary tests, in the upper range of what can be achieved. This method is almost cost-neutral, because these parameters are scored anyway during regular clinical examinations by the school dental service.

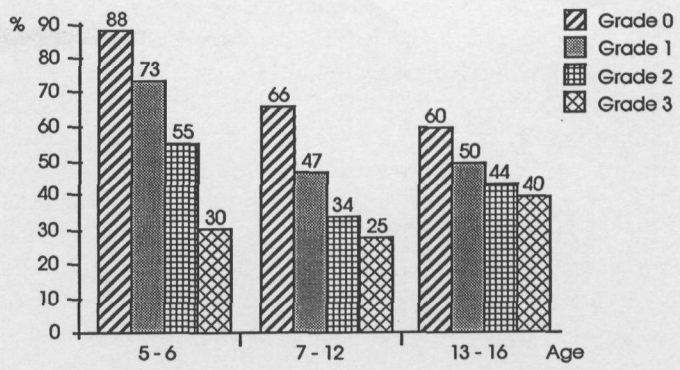

Figure 1. Percentage of caries-free children and adolescents (3 age groups) with grades 0-3 in the Dentocult®-Strip Mutans Test. N = 5743.

The Dentoprog method has been criticized because it selects children at risk only on the basis of already experienced damage. However, this is a specious argument. It is indeed true that the Dentoprog method can be applied only when children enter kindergarten at four years of age. However, there is no country in the world which has an established collective control of children younger than four. If such young children are brought to a dentist, it is because of severe caries, mostly in their anterior teeth. The results of microbial tests can then be predicted without performing them.

In adults, the situation is similar to that in children. Adults visit dentists at their own discretion. If they manifest a high caries activity, the results of salivary microbial tests can be anticipated. For any experienced dentist, future caries activity can be estimated by the present clinical status, salivary flow parameters, amount of plaque, rapidity of plaque formation, and oral hygiene habits. The following clinical parameters seem to be of particular importance: crowding of teeth, deep fissures, caries at non-predilection sites, unstained white spots, retentive restorations, and exposed root surfaces. With regard to oral hygiene, techniques of toothbrushing and interdental cleaning habits have to be assessed. In addition, a careful history should provide a clear picture of the application of fluorides in oral care products and in salt or water, and the influence of dietary habits, in particular the frequency of sucrose consumption. Other factors—like the presence or absence of chronic disease, stress, work shift, congenital tooth defects, the regular intake of medications, etc.—must be thoroughly investigated. Combining the results of clinical findings with those of careful history should allow a very precise estimation of future caries risk to be drawn. Evident risk factors should, if possible, be eliminated by appropriate counseling.

(4.5) Legitimate indications for salivary microbial tests

There are several valid indications for the use of salivary

microbial tests. Pediatricians should be convinced to use these tests regularly on children under the age of four because they have a greater chance to see infants before damage occurs. Ideally, they should be trained to provide appropriate counseling to the parents, or else refer the children to a prophylactically minded pediatric dentist. The success of chemopreventive measures such as chlorhexidine varnish can be accurately monitored by these tests. Compliance with dietary recommendations may also be controlled. Salivary microbial tests are of value in the surveillance of oligosialic and xerostomic patients. As a guide, these tests may also be useful in the dentist's deciding whether expensive restorations are advisable. When fixed bands are used by orthodontists, these tests may provide precautionary signals. Last but not least, the cooperation and motivation of a caries-active individual toward preventive measures may be increased if the results of such tests are shown and their clinical significance explained to the individual.

(5) *Bases and Perspectives of Microbial Tests in Relation to Periodontal Diseases*

(5.1) Current concept of the etiology of periodontal diseases

Periodontal diseases represent a group of pathologic conditions affecting the periodontium. Although different forms of periodontitis have been recognized, their classification still remains unclear (American Academy of Periodontology, 1992; Attström and van der Velden, 1993). These diseases are distinguished from one another primarily by differences in their clinical features. In addition, much of today's knowledge suggests that they vary with respect to the composition of the subgingival microbial flora, host responses, and susceptibilities. It is not the purpose of this paper to review the bacterial flora associated with the various forms of periodontal diseases (Slots, 1986; Haffajee and Socransky, 1994). The discussion will be limited only to micro-organisms that are most commonly identified in diagnostic tests.

It is important to stress that these bacterial species are currently considered as suspected or putative periodontal pathogens. Whether these "pathogens" are true etiologic agents remains to be proven. Nevertheless, these bacterial species can be used as diagnostic markers as defined by Burt (1991): "an attribute or exposure associated with the increased probability of occurence of disease, and which can be used as an indicator of the disease."

Actinobacillus actinomycetemcomitans

Increased frequency of detection of this organism was first described in localized juvenile periodontitis (Slots *et al.*, 1980; Zambon, 1985). This species produces a number of virulence factors, including a leukotoxin which has drawn considerable interest (Baehni *et al.*, 1979, 1981; for review, see Ohta and Kato, 1991). A. *actinomycetemcomitans* isolates vary in their ability to produce leukotoxin *in vitro*. Tsai and Taichman (1986) recovered the highest proportion of leukotoxin-producing strains from young localized juvenile periodontitis (LJP). Higher prevalence of leukotoxic A. *actinomycetemcomitans* strains was found in periodontal lesions than in healthy sites (Zambon *et al.*, 1983). However, many subjects with LJP were found to have elevated serum antibodies to A. *actinomycetemcomitans* and its leukotoxin (Tsai *et al.*, 1981). The role of the leukotoxin in the pathogenesis of A. *actinomycetemcomitans*-associated periodontitis is therefore not clear. More recent studies on LJP have reported higher numbers of A. *actinomycetemcomitans* in lesions than in non-diseased sites (van Winkelhoff *et al.*, 1994). In addition, these studies showed that the species was elevated in active sites compared with inactive sites (Haffajee *et al.*, 1984; Mandell, 1984). A. *actinomycetemcomitans* has also been implicated in adult forms of periodontal destruction (Gmür and Guggenheim, 1990; Rodenburg *et al.*, 1990; Skaar *et al.*, 1992). Recent reports indicate that the species is also present but in low numbers in subjects with a healthy periodontium (Alaluusua and Asikainen, 1988; Gmür and Guggenheim, 1994). A. *actinomycetemcomitans* serotype b seems to be more frequent in subjects with periodontal disese, whereas serotype c is associated with periodontal health (Asikainen *et al.*, 1991b).

Data from studies on the effect of treatment on levels of A. *actinomycetemcomitans* are particularly relevant. When subjects with juvenile periodontitis were treated successfully, A. *actinomycetemcomitans* was suppressed below detectable levels, while unsuccessful treatment was associated with failure to suppress the species (Christersson *et al.*, 1985; Mandell *et al.*, 1986). Mechanical treatment alone does not reliably eliminate A. *actinomycetemcomitans*. Several studies have reported that A. *actinomycetemcomitans* still remained after debridement in a high proportion of sites initially infected with the bacteria (Christersson *et al.*, 1985; Renvert *et al.*, 1990). Some authors have shown that elimination can be achieved only by combining mechanical treatment with the use of antibiotics (Slots and Rosling, 1983; Pavicic *et al.*, 1994).

Porphyromonas gingivalis

Most studies indicate that a high P. *gingivalis* count is uncommon in health but is often found in destructive forms of periodontitis (Slots and Listgarten, 1988; van Winkelhoff *et al.*, 1988a; Moore *et al.*, 1991). The species was also shown to be elevated in active lesions compared with inactive sites (Dzink *et al.*, 1988; Walker and Gordon, 1990). P. *gingivalis* produces a variety of proteolytic enzymes, which may be important virulence factors. Data on the effect of therapy are of interest. In most

cases, P. *gingivalis* decreased below detectable levels after mechanical debridement (Gusberti *et al.*, 1988; Renvert *et al.*, 1990). P. *gingivalis* was shown to remain elevated in sites that responded poorly to therapy, as was the case for A. *actinomycetemcomitans* (van Winkelhoff *et al.*, 1988b; Choi *et al.*, 1990). However, these observations were not substantiated by others (Magnusson *et al.*, 1991). High proportions of P. *gingivalis* were found in sites that showed breakdown after therapy (Choi *et al.*, 1990), suggesting that the species might play an important role in the pathogenesis of disease recurrence.

Bacteroides forsythus

Initially, B. *forsythus* was not frequently reported among the predominent cultivable organisms from periodontal lesions. This was probably due to the fact that the organism is difficult to grow and has specific cultural requirements (Wyss, 1989). However, detection of B. *forsythus* in plaque samples was shown to be possible by immunofluorescence techniques with polyclonal or monoclonal antibodies (Lai *et al.*, 1987; Gmür, 1988) or by DNA probes (Moncla *et al.*, 1991). These studies reported that the species was found in higher proportions in gingivitis and adult periodontitis than in healthy subjects. The species is more often detected in destructive periodontitis by immunological methods or DNA probes than by culture (Gmür, 1988; Moncla *et al.*, 1991). In addition, B. *forsythus* was found in increased numbers in sites with periodontal breakdown (Lai *et al.*, 1987) or in refractory subjects (Listgarten *et al.*, 1993). These observations were confirmed by culture data showing that B. *forsythus*, together with other species, was isolated in higher levels and with increased frequency in active lesions than in quiescent sites of subjects with refractory periodontitis (Dzink *et al.*, 1988). However, the species has also been frequently detected in supragingival plaque of healthy subjects or in maintenance patients (Gmür and Guggenheim, 1994).

Treponema species

The presence of spirochetes in acute forms of gingival inflammation has been known for decades. These organisms can be readily observed by direct microscopy. They are usually not detected in healthy sites but are common in gingivitis and reach high levels in periodontitis sites. Spirochetes average from 30 to 50% of the microscopic counts in subgingival plaque samples recovered from periodontal pockets (Listgarten and Helldén, 1978). Many studies have shown that the proportion of spirochetes decreased after mechanical debridement without antimicrobial agents (Mousquès *et al.*, 1980; Magnusson *et al.*, 1984). However, studies on maintenance patients failed to predict future deterioration based on the presence of spirochete counts (Listgarten *et al.*, 1984). These organisms are fragile and difficult to isolate. T. *denticola* is one of the four human oral species which has been characterized. The species was detected in diseased sites and is not commonly found in healthy sites. Decrease in T. *denticola* was observed following successful treatment but not in non-responding sites (Simonson *et al.*, 1992).

There has been a growing interest in other bacterial species such as *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Eikenella corrodens*, and unclassified *Treponema* species. These species have been found in high numbers in periodontal lesions; they are also detected with high frequency but in low numbers in healthy sites. Their exact role in destructive periodontal disease or in sites refractory to periodontal therapy remains to be evaluated.

The search for the etiological agents of periodontal diseases is still progressing. The specificity of bacteria associated with the diseases is still controversial. Current knowledge clearly indicates that the microbiota associated with healthy periodontal sites is different from that of diseased sites (Haffajee and Socransky, 1994). These differences in microbial composition or numbers may be used for diagnostic purposes. However, a few key questions need to be addressed. The first question is related to the mere presence of suspected pathogens. Most periodontal species found in diseased sites are frequently isolated but in low numbers in healthy subjects. Thus, colonization by these microorganisms is not synonymous with disease but is compatible with health. Microbial tests should therefore not be used without a careful evaluation of the patient's history and clinical status. Second, not all isolates of a given bacterial species are equally virulent. For example, leukotoxin expression may vary among strains of A. *actinomycetemcomitans*; various strains of P. *gingivalis* may differ in their production of proteolytic enzymes. Some of these differences may be partly due to environmental factors but seem to be also genetically regulated (Kuramitsu *et al.*, 1995; Spitznagel *et al.*, 1995). This might explain why species suspected to be pathogens can be found in healthy sites or subjects. If this is true, diagnostic tests should be targeted not at the species but at the virulent strains of the species. Last, the role of the host response in the equation must be considered. Periodontal disease, as an infectious process, requires virulent bacteria as well as a genetically susceptible host (Socransky and Haffajee, 1992; Hassell and Harris, 1995). Periodontal destruction and disease progression are the result of an imbalance between pathogenic species and the host defense mechanisms. Thus, microbiology represents only one element of this multifactorial disease.

(5.2) Detection of Bacterial Species in Periodontal Samples

Sampling methods and strategies for site selection have been evaluated. The most commonly used sampling techniques are dental curettes and paper points. Several

studies have shown that curettes yield higher colony-forming units than paper point samples (Kiel and Lang, 1983; Moore *et al.*, 1985), but in one study (Renvert *et al.*, 1992), higher counts of micro-organisms were obtained with paper points. The paper point technique recovers mostly non-adherent subgingival plaque. One study suggested that a stratified distribution of bacterial cells will not be adequately sampled by absorbent paper points (Baker *et al.*, 1991). Nevertheless, reproducibility of paper point sampling has been shown to be acceptable. Duplicate samples obtained one week apart (Renvert *et al.*, 1992) or during the same visit (Dahlén *et al.*, 1990) demonstrated little variation in the recovery of A. *actinomycetemcomitans* and P. *gingivalis*. A critical step is the microbial sampling, because it appears to be the most important source of variance in the recovery of subgingival bacterial markers (Wikström *et al.*, 1991). In this context, the presence of bacteria within gingival tissues may be of importance. Clusters of micro-organisms have been shown in biopsies from diseased sites and identified as members of the periodontal flora, suggesting bacterial invasion (Saglie, 1991). The exact significance of these observations is not known. However, if bacterial invasion proves to be related to disease progression, the sampling methods that are common today will be inadequate.

Another issue concerns the number of sites to be sampled *per* patient and the strategies for site selection. Studies on topographic distribution of black-pigmenting bacteria have shown that these micro-organisms are not evenly distributed within the mouth but cluster in certain areas of the dentition (Mombelli *et al.*, 1991a). Most authors agree that sites with the deepest probing depth and that bleed when probed are most likely to harbor suspected pathogens (Mombelli *et al.*, 1991b; Savitt *et al.*, 1991). Others have suggested that multiple sites should be sampled for the reliable detection of species such as A. *actinomycetemcomitans* and P. *gingivalis* (Christersson *et al.*, 1992; Loos *et al.*, 1992). One study demonstrated that, in 70% of cases, when A. *actinomycetemcomitans* was recovered from subgingival sites, it was also found in stimulated saliva samples (Asikainen *et al.*, 1991a). Analysis of saliva samples may be useful to identify a carrier state.

As discussed earlier, different technologies have been used for detecting and quantifying bacteria in samples from periodontal pockets. Several commercial tests and services are now available in the United States and in Europe. These tests involve different strategies (Dewhirst and Paster, 1991). Some tests use ^{32}P-labeled DNA probes. This requires that the sample be processed in a specialized laboratory approved for the use of radioactive material. Plaque samples are collected by the clinician and sent to the reference laboratory. Quantitation is performed by comparison of the signals generated by the samples with signals obtained with standards, *e.g.*, known quantities of DNA from target micro-organisms (French *et al.*, 1986; Savitt *et al.*, 1988; Dix *et al.*, 1990; Moncla *et al.*, 1990). An automated system based on this technology uses enzymatically tagged DNA probes. This procedure can be performed in the dental office, provided that appropriate equipment is available (Listgarten, 1992). However, *quantitation* of the reaction is not possible in the system that is designed for use in the dental practice.

Another technology that has been applied to periodontal samples and commercialized is the latex agglutination test (Zambon *et al.*, 1986). In this technique, the antibody-bound latex is mixed with the plaque sample suspension and agitated. A positive test is visualized by the clumping of the mixture, which occurs when the target micro-organisms form complexes with the species-specific antibodies. The major problem is that these tests have a low sensitivity and provide only qualitative results (Zambon *et al.*, 1986).

The detection of bacteria based on their enzyme profiles has also been applied to certain subgingival species. For example, P. *gingivalis*, T. *denticola*, and B. *forsythus* possess a trypsin-like enzyme(s) that can be detected in the benzoyl-arginine naphthylamide (BANA) test. Detection of these micro-organisms by BANA, compared with fluorescent immunoassay for P. *gingivalis* and T. *denticola*, has shown good levels of agreement between the two methods (Loesche *et al.* 1992a). However, false-positive reactions may occur because some of the enzyme activity detected by the test may be derived from the host. Another drawback is that the test is not specific, since all three bacterial species can cleave the same substrate, and, thus, the test cannot distinguish among the three species.

Microscopic examination of plaque samples has been used to detect certain indicator micro-organisms such as motile rods and spirochetes. These species are often associated with periodontal destruction but they are also found at high levels in gingivitis. Phase-contrast or dark-field microscopy is useful to determine distribution of different bacterial morphotypes but does not give information about bacterial species (Listgarten, 1992).

(5.3) The Value of Microbial Tests in the Management of Periodontal Diseases

In theory, microbial diagnosis should improve our ability to identify subjects at risk for developing disease or to monitor disease progression, and it should enhance periodontal diagnosis and assist in treatment decisions. Data from studies on risk assessment suggest that elevated levels of certain species including A. *actinomycetemcomitans*, P. *gingivalis*, P. *intermedia*, and B. *forsythus* are associated with an increased risk for new attachment loss (Haffajee *et al.*, 1991; Beck *et al.*, 1992; Haffajee and Socransky, 1994). In these studies, bacteria were identi-

fied by culture methods, immunofluorescence, or colony-lift procedures combined with DNA probes. Thus, the value of the diagnostic tests discussed above for risk evaluation has not yet been determined.

Microbial diagnostic tests have also been suggested as adjuncts in treatment selection and planning. Although the concept is appealing, it does not apply to adult periodontitis. Indeed, in adult periodontitis, treatment by conventional methods including mechanical debridement with or without surgery was shown to arrest progression of disease in most cases (Lindhe, 1989). In addition, it has to be acknowledged that treatment modalities are essentially the same and that the choice among therapies is limited: Local mechanical debridement is performed in all cases, regardless of the type of diagnosis. One key issue related to treatment is the choice of antibiotics. It has been suggested that selection of an appropriate antibiotic in subjects with severe or aggressive disease should be based on proper microbiological analysis (van Winkelhoff *et al.*, 1993). In such cases, culturing is a prerequisite for determining the *in vitro* antimicrobial susceptibility of a given pathogen. Unfortunately, most of today's available data related to this issue are case report studies. Clearly, the goal of linking microbial diagnosis to specific therapies has not been achieved. Validation of the concept will have to be accomplished through controlled clinical trials where the true utility of bacterial monitoring would be matched against the therapeutic outcome.

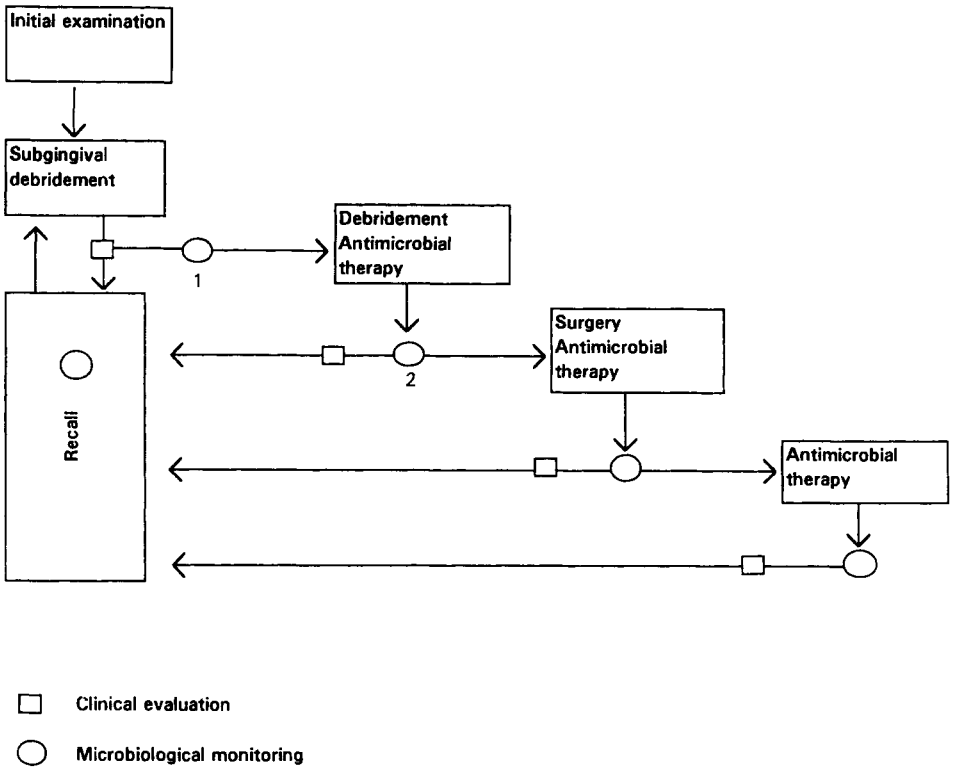

Figure 2. Flow chart of periodontal treatment for refractory periodontitis including microbial monitoring.

Thus, at the moment, microbial testing is of little value in the routine management of adult periodontitis. It results in unnecessary costs and sometimes misuse of antibiotic therapy. The most appropriate applications of microbial testing are in the early and rapidly progressing forms of periodontitis, juvenile periodontitis, and refractory periodontitis. Refractory periodontitis is a disease in sites or patients who continue to experience a high rate of attachment and tooth loss despite intensive therapy. This implies that mechanical treatment has been adequately performed and the clinical history well-documented. When indicated, bacterial monitoring after an active phase of therapy will help to assess the efficacy of treatment and determine whether the bacterial load has been controlled. Subgingival scaling has major effects on the subgingival environment and the microbiota. It lowers the total number of micro-organisms and produces shifts in the ecology of subgingival plaque (Socransky and Haffajee, 1993). Several studies have shown that A. *actinomycetemcomitans* or spirochetes may still be detected after treatment (Mousquès *et al.*, 1980; Slots and Rosling, 1983; Mandell *et al.*, 1986). Some results indicate that failure to lower certain suspected pathogens is associated with poor response to therapy (Socransky and Haffajee, 1993). It appears likely that the success of therapy is related to the magnitude of the quantitative and/or qualitative changes of the microbial flora. The adequacy of treatment should be based on the absence of detectable pathogens instead of on a tactile or visual basis. The end-point of therapy would then be determined by results of the microbial analysis.

Fig. 2 represents a proposed treatment scheme for refractory periodontitis in which therapeutic decisions are based on microbiologic testing. Microbial analysis aims at determining the presence of a limited set of bacterial markers, as defined earlier. In the proposed scheme, after a clinical examination the patient receives oral hygiene instructions and is treated by subgingival scaling. In the event treatment is effective in arresting periodontal breakdown, the patient is placed on mainte-

nance. Regular prophylaxis is intended to prevent recurrence of disease. If the clinical situation fails to improve, microbiological testing is indicated to gain information on the residual flora (see #1, Fig. 2). If the results show that bacterial markers have not been adequately reduced, therapy is continued: Mechanical treatment is repeated, with the possible addition of local or systemic antimicrobial therapy. Use of antibiotics should always be accompanied by longitudinal microbial monitoring to evaluate the efficacy of treatment. Active treatment is discontinued when the results show bacterial markers below detectable levels (see #2, Fig. 2). This approach is justified by the fact that failure to reduce the target bacteria below detection limits is associated with unsuccessful treatment. Microbial diagnosis is also indicated in the recall phase. A test performed during these periodic visits will give information on the microbial status and on possible recurrence of the disease. It will then help to determine whether a conservative (mechanical) or chemotherapy-supported approach should be considered.

(6) *Conclusions*

In recent years, remarkable advances in technology have been made in the field of oral microbiology. On the one hand, new tools have been made available which have the potential to identify known bacteria as well as novel species and as-yet-uncultured organisms. These techniques should facilitate the search for etiologic agents, particularly of periodontal diseases, and help to clarify the taxonomy of oral bacteria based on nucleotide sequence data. On the other hand, attempts have been made to put existing knowledge on microbial etiology into practice. Methods to detect suspected pathogens in a reliable fashion are being made accessible to the clinician. The following conclusions can be drawn concerning caries:

(1) With few exceptions, salivary microbial tests are useless for determining future individual caries activity due to their low sensitivity and low positive prognostic value. There are far more cost-effective alternatives with a high sensitivity and positive predictive value.

(2) That active caries is highly correlated with high numbers of mutans streptococci and/or lactobacilli appears to be trivial and needs no further confirmation.

(3) Caries is a disease with a multifactorial etiology and not an infectious disease due singularly to mutans streptococci and/or lactobacilli. If fluoride prophylaxis is available, high numbers of these bacteria may be tolerated in the oral flora without causing injury to teeth. This is one of the main reasons for the low predictive values of salivary microbial tests.

With regard to periodontal diseases:

(1) Periodontal destruction is a result of an imbalance between host defense mechanisms and a limited set of bacterial species. However, not all clonal types of a suspected pathogenic species are identical in expressing virulence factors. Furthermore, the host defense capacity varies not only between patients but also between affected sites. Thus, for identical microbial conditions, health may either be maintained over prolonged periods of time, or, in other cases, rapid breakdown may occur.

(2) Although diagnostic tests have been commercialized and introduced into the clinic, current indications for such tests are limited. Microbial diagnosis should be considered in early-onset periodontitis or in subjects who respond poorly to conventional therapy. Microbial tests should then be applied to monitor the efficacy of mechanical treatment as well as antimicrobial chemotherapy and to determine the end-point of active treatment.

The technology developed in the past decade is opening up new horizons to the dental community and should enhance our ability to conduct clinical research and provide treatment. However, in this search one should be fully aware that microbiology represents only one element of a multifactorial equation.

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