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Microbiological diagnostics in oral diseases

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Abstract
Most infections of the oral cavity, including the major dental diseases caries and periodontitis, are opportunistic in nature. They are caused or maintained by microorganisms of the resident or transient flora normally present in low numbers and not pathogenic, but in certain circumstances develop infections. Mucosal infections have some degree of specificity [e.g. *Candida* spp., *Staphylococcus aureus*, and enterics] and a microbiological test can be interpreted accurately for clinical diagnosis and choice of treatment. Subepithelial or deep infections, however, include a number of species from the resident flora, mainly anaerobes whose role in the infections is difficult to interpret. However, microbiological tests and the presence of certain bacterial species could be used for treatment control, risk-evaluation and even for patient motivation in the prevention of these diseases. Microbiological diagnosis can be used in general practice for several purposes and in various situations that can be of great value for the dental patient.

Key Words: *Caries, diagnosis, endodontic infections, microbiological test, mucosal infection, oral microbiology, periodontitis*

Introduction

The major dental diseases, caries, marginal and apical periodontitis as well as many other oral lesions have a microbiological etiology or association with specific microorganisms. Microbiological tests can therefore be used in general dental practice to increase accuracy and quality in the diagnosis, treatment, and prevention of these diseases. This article describes the rationale behind the use of microbiological tests for diagnosis, choice of treatment, treatment control, and risk-evaluation, even patient motivation in various oral conditions suspected of having an infectious origin or microbiological association.

Infections and microbiological specificity

Microbiological tests deal with the detection and identification of target microorganisms; they quantify them and disclose their activity or at least what they are capable of doing. Most microorganisms that we deal with as dentists in relation to diseased conditions are part of the resident or transient flora and in most cases detection and identification are not enough to define their impact on the disease. One major question is whether we are dealing with a harmless colonization or microorganisms that go over the edge and cause infection. Generally, we live together with the normal microflora in a steady state known as microbial homeostasis [1]. This is illustrated particularly in dental plaque, where homeostasis takes place in a very complex microbial community, namely the biofilm, which provides the microorganisms with a number of advantages. One of the consequences of biofilm stability is the limited effect of antimicrobials [2]. This effect is present on the mucosa, especially the dorsum of the tongue, and in the infected root canal. The first stage in the change of the flora from a state of colonization to infection is therefore disruption of this stability and a subsequent change of the microbiological activity, quantity, and composition. This is the general character of an opportunistic infection [1]. Certain microorganisms thus increase in number and proportion as a result of increased activity and growth. Stomatitis induced by yeasts in relation to full dentures is classical; however, overgrowth of enterics

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in immunocompromised patients is another example. The overgrowths of lactobacilli in the active dentine lesion and of periodontopathogens in the periodontal pocket are examples of opportunistic infections due to an ecological shift in the dental plaque flora. This relation is summarized in the equation of infection (Figure 1) applying to most oral microorganisms of low virulence and that increase their numbers or, as a result of lowered general or local host defense, after a certain time start an infection process.

Another important step in the transition from colonization to infection is the invasion of microorganisms into the tissues, which occurs commonly in oral infections, e.g. spirochete invasion by motility in acute necrotizing ulcerative gingivitis (ANUG), invasion of the epithelial cell layer by *Candida* hyphae in fungal infections, ingrowth of bacterial cells in dentine tubuli in caries-active lesions and cell invasion of the pocket epithelium by periodontal pathogens in aggressive forms of periodontitis [3–5].

Dental treatment and prevention is characterized by being population-based and treatment protocols are usually standardized and non-specific, especially in relation to their microbiology origin. This approach has been successfully applied at least in relation to caries and periodontal disease, since it has been suggested that more or less the whole population is at risk. However, despite the cause, severe caries cases, advanced periodontitis cases, a number of quiet apical periodontitis cases, and an increasing number of oral mucosal infections are still a significant problem for many patients despite regular dental visits. A more individual case-related approach in treatment and prevention, aimed at identifying microbial risk factors or risk markers for the dental diseases, is needed.

Consequently, both detection and quantifying are necessary if microorganisms associated with the lesions are to be identified. Oral infections are of two kinds, mucosal infections and subepithelial infections.

$$\frac{\text{Virulence} \times \text{Times} \times \text{Number}}{\text{General and Local Host Defence}} \longrightarrow \text{Infection}$$

Virulence:
- Infectiosity - Capacity to colonize and establish
- Invasivity - Capacity to invade
- Pathogenicity - Capacity to survive and grow within the tissues

Figure 1. Infection equation.

Mucosal infections

Mucosal surface infections are opportunistic in the classical sense. The surface is colonized by bacteria from the resident flora, predominantly the streptococci *Neisseria* and *Haemophilus* species, and, if disturbed, microorganisms from the transient flora or the surroundings colonize, establish, and grow pathologically, thereby causing various types of lesions or discomfort. The most common forms of mucosal lesions are presented in Table I as well as the microbial findings (Table II). The occurrence *Candida* in denture stomatitis is well known for most dentists. Less well considered are stomatitis due to bacterial infections such as *S. aureus* and enterics [6–8]. Glossitis ["burning tongue"], in particular, is common in the immunocompromised and hospitalized patient; however, this kind of lesion can also be observed in apparently healthy individuals. Apart from the tongue, which is the most common site of infection, the cheek and palatum are sites for local mucosal infections. White lesions (hyperkeratosis) are commonly associated with *Candida*, which prevents the lesion from spontaneous healing. The red lesions (erythematous) are confined to atrophy and it is not possible clinically to distinguish between *Candida* and bacterial infections. Cheilitis (usually angular), too, are often secondarily infected either by *S. aureus* or *Candida*, and specific treatment is needed for healing [9].

Generally, the mucosal lesions have a high degree of specificity and consequently the treatment has to be selective and specific. Experience tells us that too many have been misdiagnosed and mistreated for mucosal lesions that could have been avoided by a fairly simple microbiological test and diagnosis.

Subepithelial infections

The subepithelial infections are presented in Table III. The list includes caries and periodontitis as well as a number of other deep infections. They all have in common that members of the oral flora enter normally sterile tissues for various reasons, start to multiply, and cause damage. Since the bacteria are normally of low virulence, they cooperate in a polymicrobial infection; specificity is low and anaerobes usually predominate. Table IV gives a number

Table I. Oral mucosal lesions associated with microorganisms and infection

Stomatitis
Glossitis, "burning tongue"
Gingivitis, mucositis
General or local oral mucosal lesions
Hyperkeratosis
Erytematous lesions
Angular cheilitis

Table II. Common microbiological findings in oral mucosal lesions

Candida
Staph aureus
Hemolytic streptococci
Enterococci
Pseudomonas
Enterics [G- rods]
E. coli
Enterobacter
Klebsiella
Proteus

of the more frequently occurring bacterial species in subepithelial infections. However, the list is far from complete and in fact almost any species in the oral cavity can be found in these infections. On the other hand, we cannot overlook the fact that some of the species are much more frequent and possess characteristics (virulence factors) that render them putative pathogenic.

Endodontic infections

Endodontic infections lead to acute or chronic apical periodontitis and are probably the most typical subepithelial infections in the oral cavity. The bacteria enter normally sterile tissues (pulp) or compartments (root canal). The infection is largely unspecific, polymicrobial, and predominantly anaerobic [10]. The presence of bacteria is directly related to the inflammatory response in the periapical tissues. No bacteria – no reaction, and therefore the treatment goal is clear, i.e. elimination. Most bacteria are sensitive to the standard treatment procedures applied. Bacteria left behind in the root canal when it is permanently root-filled is a strong risk factor for future failure in terms of persistent apical periodontitis [11]. Despite the obvious advantage of using microbiological tests as treatment control before root-filling, it is rarely done, probably because the failures are not dramatic enough. The developing apical periodontitis lesions are usually slow, chronic processes with little symptoms. In addition, the standardized mechanical and chemical procedures select bacterial species that are most resistant to the treatment strategy. The remaining flora is predominantly Gram-positive and facultative and with bacterial species low in virulence and hampered by the permanent root filling [12]. Furthermore, it is technically difficult to carry out the endodontic procedures aseptically and to reach (either mechanically or chemically) the bacteria hiding in the root-canal system, especially in the apical region. Consequently, microbiological sampling for treatment control unfortunately coincides with a significant number of false-positive and false-negative tests. Therefore, microbiological tests in endodontic infections are routinely used primarily in teeth with symptoms or with a complex clinic by so-called "adopters" [13]. However, the need for microbiological tests in endodontic treatment is potentially unlimited, along with increasing demands of quality of the endodontic treatment, for choice of treatment strategy and for risk evaluation and prognosis.

Table III. Subepthelial infections appearing in the oral cavity

Caries
Periodontitis
Periimplantitis
Endodontic infections
Apical periodontitis
Odontogenic abscesses
Periodontal
Periapical
Periocoronitis
Alveolitis[dry sockets]
Osteomyelitis
Non-odontogenic infections
Fractures and trauma

Table IV. Common bacterial findings in subepithelial infections

Streptococci
S. anginosus
S. intermedius
Peptostreptococci
Micromonas micros
P. anaerobius
Veillonella parvula
Actinomyces israelii
Eubacterium
Prevotella
P. intermedia/nigrescens
P. oralis/buccae and more
Porphyromonas
P. gingivalis
P. endodontalis
Fusobacterium nucleatum
Tannerella forsythia
Bacteroides
B. ureolyticus
Campylobacter rectus
Actinobacillus actinomycetemcomitans
Eikenella corrodens
Treponema (spirochetes)
T. denticola

Periodontitis

In Sweden, advanced or severe periodontitis is present in about 7% of 50-year-old adults [15], but it is difficult to identify these patients clinically, and when this is possible it is often too late. It is likely that in the more aggressive forms of periodontitis the bacteria play a more active role compared to the chronic forms and that we are dealing with a true infection according to the definition. Clinically, the progression of periodontitis is episodic, e.g. the attachment loss occurs at certain sites, and in certain individuals under a limited period of time. Consequently, there is a need for identifying the

individuals and sites that are at risk of such a periodontal breakdown. However, microbiological tests are commonly performed after the breakdown and in quieter periods, and it is not known whether the bacteria in the sample represent the clinical "burst" of activity. In the late 1970s, *Actinobacillus actinomycetemcomitans* in juveniles first represented a more specific bacterial association with periodontitis. Still, there are subtypes of *A. actinomycetemcomitans* that are closely related to aggressive forms of periodontitis and that seem to behave like a classic infection [14]. This apparent cause relation between *A. actinomycetemcomitans* and periodontitis justifies the strategy of using antibiotics as an adjunct to surgery in these *A. actinomycetemcomitans* positive patients [16,17]. This treatment strategy in periodontitis patients is based on the detection of *A. actinomycetemcomitans* in the microbiological test sample.

Aggressive forms of periodontitis in adults are more difficult to identify, and many attempts including microbiology have been performed [18]. One report on individual cases shows that patients who were not responding adequately while receiving thorough periodontal treatment including both mechanical and surgical procedures could be identified by microbiological testing and presence of *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, and a change of treatment strategy including various antibiotics was applied successfully [19]. A similar treatment strategy for periimplantitis cases has shown a 5-year success rate of 75% [20]. A predominance of putative periodontal pathogens, single or in certain combinations [complex], is a sign of disturbed microbiological homeostasis and a marker of "diseased" flora [16,21]; the most significant markers (*A. actinomycetemcomitans, P. gingivalis, Tannerella forsythia, Treponema denticola* and *P. intermedia*) are included in Table V. In an attempt to identify other bacterial species relevant for this diseased flora, *Filifactor alocis*, *Prevotella tannerae*, and *Porphyromonas endodontalis* should be included in the list [22]. All these bacterial species can easily and rapidly be detected and quantified using various molecular biology based methods, e.g. checkerboard DNA hybridization or real time PCR [23–27].

Table V. Reasons for a microbiological test

Diagnosis
Choice of therapy
Treatment control
Risk evaluation
Motivation

Caries

It has long been known that caries is associated with certain acidogenic and aciduric microorganisms [28]. Lactobacilli are favored by having a lower pH in the oral cavity and establish themselves preferentially in the carious lesion, where the pH can be lower than 5.0 [3]. Mutansstreptococci, on the other hand, is favored by a high intake of fermentable sugars, especially sucrose. Mutansstreptococci is one of a limited number of bacterial species that actually ferment sucrose, and is regarded as the only one that subsequently produces water-insoluble polyglucans [29]. It is therefore favored in the sucrose exposed dental plaque and is associated with the early caries development ["white spot lesions"]. However, their role in the cause relation is not necessarily specific, and in a recent review Beighton [30] suggests that the etiology is complex and polymicrobial in nature. Furthermore, caries development seems to be age-related, site-related (fissure, smooth surface, and root surface), and related to host susceptibility (fluorides). So, even if there is a significant correlation between mutansstreptococci in the saliva and DMFT values in children 5–8 years of age [31], this correlation is weak if caries activity is considered. Kingsman et al. [32] showed that over the course of 17 months it was not possible to distinguish between those getting three more lesions from those developing none based on high numbers of salivary mutansstreptococci or lactobacilli. On the other hand, both bacterial parameters were significant negative predictors, e.g. no mutansstreptococci and no lactobacilli correlated to low risk for developing new lesions during the next 17 months. Ravald et al. [33] have shown that both bacterial parameters are useful predictors [$>75\%$] for future root-surface caries. Consequently, microbiological tests can be beneficial for both treatment control and for risk evaluation and prognosis in caries-susceptible patients [34,35].

Patient motivation, too, is a benefit from microbiological diagnosis that should not be overlooked (Table 5). We are in prevention, and the treatment strategy for both periodontitis and caries is dependent on patient cooperation and self-performed hygiene measures, and anything that can make things easier for the patient to follow the instructions are important. Bacteria and bacterial levels are easy to understand and the patient can contribute to the change of levels.

In conclusion, microbiological diagnosis can be used for several purposes and in various situations which can be of great value for the dental patient.

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