



UNIVERSIDADE DA BEIRA INTERIOR

Ciências da Saúde

Epidemiology of Dental Caries.

From prevention to the relationship with *Helicobacter pylori*
infection.

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Tese para obtenção do Grau de Doutor em

Biomedicina

(3º ciclo de estudos)

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Dedictory

To my wife Inês

and my daughter Alice

“Prevention is better than cure”.

Desiderius Erasmus

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List of papers

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- I. Veiga N, Pereira C, Ferreira P, Correia I. Prevalence of dental caries and fissure sealants in a portuguese sample of adolescents. PLoS ONE. 2015; 10(3):e0121299. doi:10.1371/journal.pone.0121299. eCollection 2015.
- II. Veiga N, Pereira C, Resende C, Amaral O, Ferreira M, Nelas P, Chaves C, Duarte J, Cirnes L, Machado JL, Ferreira P, Correia IJ. Oral and gastric Helicobacter pylori: effects and associations. PLoS ONE. 2015. *In press*.
- III. Pereira C, Veiga N, Resende C, Amaral O, Ferreira M, Nelas P, Chaves C, Duarte J, Cirnes L, Machado JL, Ferreira P, Correia IJ. Prevalence and determinants of oral microflora among Portuguese adolescents. Biomed Res Int. 2015. (Paper submitted for publication).
- IV. Veiga N, Ferreira P, Correia T, Correia MJ, Pereira C, Correia IJ. Characterization of the antibacterial effect resulting from the association between silver diamine fluoride and a resin-based fissure sealant. J Dent Res. 2015. (Paper submitted for publication).
- V. Veiga N, Pereira C, Amaral O, Ferreira P, Correia IJ. Oral Health Education: community and individual levels of intervention. Oral Health Dent Manag. 2015; 14(2):129-35. doi:10.4172/2247-2452.1000787.

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- II. Veiga N, Pereira C, Amaral O, Chaves C, Nelas P, Ferreira M, Ferreira P, Correia IJ. Prevalence of dental caries and fissure sealants in a portuguese sample of adolescents. *Aten Primaria.* 2014;46(Espec Cong 1):15.
- III. Veiga N, Amaral O, Pereira C, Chaves C, Nelas P, Ferreira M, Ferreira P, Correia IJ. Prevalence of oral hygiene habits and dental appointments among a portuguese sample of adolescents. *Euro J Public Health.* 2014;24(Suppl 2):42.

Resumo alargado

Actualmente, as doenças orais, como a cárie dentária e as doenças periodontais são as mais prevalentes a nível mundial. A Organização Mundial de Saúde (OMS) considera estas patologias como importantes problemas de saúde pública. É crucial focar a saúde oral de crianças e adolescentes, pois as doenças orais podem causar problemas nesta população, nomeadamente dificuldades de mastigação, ingestão de alimentos, aprendizagem e concentração, sono e redução da qualidade de vida.

A saúde oral tem vindo a ser uma área médica em constante desenvolvimento, tanto em Portugal como no resto do mundo. Assim, torna-se fundamental conhecer as várias vertentes de investigação que podem ser realizadas nesta área específica da saúde pública. A complementaridade entre a investigação epidemiológica e laboratorial é uma realidade actual e essencial para investigar e avaliar importantes problemas de saúde pública relacionados com a saúde oral e até mesmo melhorar biomateriais dentários que, no futuro, podem vir a tornar-se produtos importantes na prevenção de doenças orais.

Tendo em conta as várias vertentes de investigação em saúde oral que podem ser desenvolvidas, o presente plano de trabalhos de doutoramento consistiu em: 1) avaliar a prevalência de cárie dentária e o padrão de distribuição dos selantes de fissuras; 2) avaliar se a cavidade oral pode ser considerada um potencial reservatório de *Helicobacter pylori* (*H. pylori*) e determinar se existe uma associação entre a infecção por *H. pylori*, as variáveis sócio-demográficas e a prevalência de cárie dentária; 3) investigar se a prevalência de *Streptococcus mutans* (*S. mutans*), *Lactobacillus* e *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) salivar tem influência sobre o risco de desenvolvimento de cárie dentária e ainda determinar se existe alguma relação com os aspectos sócio-demográficos; 4) avaliar a eficácia de incorporação de um agente antibacteriano (fluoreto diamino de prata) no selante de fissuras resinoso com o intuito de prevenir o aparecimento da cárie dentária, através da inibição do crescimento de *S. mutans*; 5) descrever as diferentes estratégias que permitam a realização de programas adequados para a promoção da saúde oral, tanto a nível individual, como comunitário.

O estudo epidemiológico envolveu um estudo observacional transversal com uma amostra de conveniência, não probabilística de 447 adolescentes com idades entre os 12 e os 18 anos, que frequentavam uma escola pública em Sátão, Portugal. A recolha de dados foi efectuada através de um questionário que foi respondido pelos participantes na sala de aula seguido por uma observação intra-oral, recolha de saliva e, finalmente, a detecção de infecção por *H. pylori* utilizando o *Urease Breath Test* (UBT) teste respiratório da urease. A identificação das estirpes bacterianas na saliva foi efectuada através do recurso à técnica *Polymerase Chain Reaction* (PCR). Simultaneamente, iniciou-se o estudo *in vitro* através da incorporação do diamino fluoreto de prata no selante de fissuras resinoso e procedeu-se à análise dos halos inibitórios, usando como modelo a estirpe bacteriana *S. mutans*.

Os resultados obtidos revelaram um nível moderado de prevalência da cárie dentária, com um maior número de dentes restaurados em comparação com o número de dentes cariados e perdidos devido à cárie dentária. Esta situação indica que a maioria dos adolescentes têm vindo a ter, durante as suas vidas,

consultas médico-dentárias para a prevenção e tratamento da cárie dentária. Tal constatação permite verificar que nos últimos anos tem ocorrido uma melhoria dos níveis de saúde oral registados em Portugal, já que as crianças e os adolescentes têm tido um acesso mais fácil e regular à consulta do médico dentista.

A prevalência de *H. pylori* determinada na saliva dos adolescentes que participaram no estudo foi muito reduzida. Uma das possíveis explicações pode estar associada com o facto de os adolescentes não terem qualquer patologia gástrica, nem apresentarem sintomas compatíveis com a infecção por *H. pylori*, o que contrasta com alguns estudos referente a amostras de adultos, em que foi estabelecida uma correlação entre a patologia gástrica e a infecção por *H. pylori*. As diferenças observadas na prevalência do *H. pylori* na cavidade oral também podem ser justificadas pelas diversas metodologias / técnicas aplicadas nos diferentes estudos para detectar a presença desta bactéria. No presente estudo, a detecção de *H. pylori* salivar foi realizada por meio da identificação do gene específico característico do *H. pylori* denominado *vacuolating cytotoxin A (vacA)*, contrariamente ao descrito em estudos anteriores em que a presença da estirpe bacteriana foi determinada através da presença de ureia na cavidade oral.

A pesquisa desenvolvida permitiu também avaliar a influência da microflora oral na saúde oral dos adolescentes. A enorme diversidade da microflora existente na cavidade oral requer uma melhor compreensão do ecossistema oral, o que poderá fornecer novas ideias sobre a etiologia da cárie dentária e sugerir novos alvos terapêuticos para tratamento da doença. Por isso, e, como foi comprovado na presente pesquisa, não houve associação direta e independente entre estirpes bacterianas e a cárie dentária. Contudo, a presença do *A. actinomycetemcomitans* encontra-se associada a aspectos sócio-demográficos, nomeadamente a idade e a área de residência do adolescente. Estes resultados podem ser considerados importantes, pois permitirão detectar mais precocemente adolescentes portadores de uma estirpe bacteriana com potencial para desencadear uma doença periodontal que pode surgir na idade adulta.

Por outro lado, o desenvolvimento de biomateriais que possam ser usados em medicina dentária é também essencial para evitar o início e desenvolvimento de doenças orais. Tendo em conta esta ideia, a abordagem epidemiológica da investigação foi complementada com uma componente laboratorial, com o objetivo de demonstrar, numa fase inicial, a possibilidade de potenciar o efeito antibacteriano de um biomaterial usado como selante de fissuras existentes nos dentes. Neste estudo, o diamino fluoreto de prata foi usado, tendo em conta o seu potencial para a diminuição significativa de *S. mutans*. Este composto foi incorporado no selante de fissuras com o objectivo de potenciar o efeito antibacteriano do selante de fissura, e subseqüentemente reduzir o risco de desenvolver cárie dentária, na presença de infiltração ou perda parcial de um selante de fissura.

Na última parte do estudo realizado, foi constatada a necessidade de implementar programas de promoção de saúde oral orientados para a comunidade, a fim de melhorar os conhecimentos e os comportamentos relacionados com a saúde oral de crianças, adolescentes, pais, professores e profissionais de saúde. A prevenção primária deve ser tida em conta de modo a reduzir o risco de desenvolvimento de doenças orais, principalmente através da melhoria significativa dos comportamentos de saúde oral das populações.

Em suma, os estudos descritos nesta tese de doutoramento dão à comunidade científica a possibilidade de analisar: a epidemiologia das doenças orais e métodos de prevenção primária; a microflora oral e os fatores ambientais que podem influenciar a saúde oral; a influência da presença da estirpe bacteriana *H. pylori* na cavidade oral e a sua relação com o desenvolvimento de cáries; conhecer novos biomateriais que podem ser utilizados na medicina dentária; e as estratégias de educação em saúde oral para melhorar os comportamentos e diminuir a incidência das doenças orais.

Palavras-chave

Cárie dentária, fluoreto diamino de prata, *H. pylori*, microflora oral, saúde oral, selantes de fissuras.

Abstract

Oral diseases, such as dental caries and periodontal diseases, are the most prevalent worldwide nowadays. The World Health Organization (WHO) considers these pathologies important public health issues. It is fundamental to focus on the oral health, since oral diseases can cause problems related to difficulties in chewing, food intake, learning and concentration, sleep and reduced quality of life during childhood and adolescence.

Oral health is a medical field in constant development, both in Portugal and in the rest of the world. Therefore, it is important to know the various aspects of research that can be performed in this specific public health domain. The complementarity between the epidemiological and laboratory research is a current reality and it is essential to investigate and assess important public health problems related to oral health and even to allow the improvement of biomaterials aiming at the prevention of oral diseases.

The developed doctoral work plan consisted in: 1) assessing the prevalence of dental caries and the distribution pattern of fissure sealants; 2) evaluating the presence of *Helicobacter pylori* (*H. pylori*) in the oral cavity to determine if there is any association between *H. pylori* infection, socio-demographic variables and the prevalence of dental caries; 3) investigating the prevalence of salivary *Streptococcus mutans* (*S. mutans*), *Lactobacillus* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) and their influence on the risk of dental caries development and also their association with socio-demographic factors; 4) evaluating the effectiveness of incorporating an antibacterial agent (silver diamine fluoride) in a resin-based fissure sealant to inhibit the growth of *S. mutans*; 5) describing different strategies that can foster the development of appropriate oral health promotion programs, at a community and individual levels.

For the epidemiological approach an observational cross-sectional study was conducted with a non-probabilistic convenience sample of 447 adolescents, aged 12 to 18 years old, who attended a public school in Sátão, Portugal. Data collection was accomplished through a questionnaire that was answered by the adolescents in the classroom followed by an intra-oral observation, saliva collection and, finally, *H. pylori* infection detection using the Urease Breath Test (UBT). The bacterial strains in the saliva were identified using the Polymerase Chain Reaction technique (PCR). Simultaneously, an *in vitro* study was performed to check if the incorporation of silver diamine fluoride in a resin-based fissure sealant had an inhibitory effect on a bacterial strain *S. mutans*.

This study demonstrated that there was a moderate level of prevalence of dental caries, with a higher number of filled teeth in comparison with the number of decayed and missing teeth due to dental caries. This indicates that most adolescents are having dental appointments during their lives, at least for the treatment of dental caries. These results demonstrate the improvement of oral health registered in Portugal, since children and adolescents have easier and a more regular access to dental appointments.

The prevalence of *H. pylori* present in saliva was very low in the sample of adolescents analyzed. One possible explanation may be correlated with the absence of gastric pathology. In contrast, other studies

where adult samples were used demonstrated a clear diagnosis of gastric pathology associated with *H. pylori* infection. The observed differences in the prevalence of oral *H. pylori* can also be justified by the different methodologies / techniques applied in the previous studies to detect this bacteria. In the present study salivary detection of *H. pylori* was performed by identifying the specific gene of *H. pylori*, vacuolating cytotoxin gene A (*vacA*), whereas in previous studies the presence of the bacterial strain was determined based on the urea content which is also produced by other bacterial strains in the oral cavity.

Furthermore, the influence of oral microflora in the oral health of adolescents was also studied. The high diversity of oral microflora requires a fully characterization in order to understand the oral ecosystem. Such knowledge is crucial to know the etiology of dental caries and suggest new therapeutic targets to treat the disease. In this study no direct association was found between bacterial strains and dental caries. However, the presence of *A. actinomycetemcomitans* was noticed to be associated with socio-demographic factors, such as the age and residence area of adolescents. These results can be considered important because they can allow the detection, at an earlier stage, of a potential bacterial strain that originates a periodontal disease that will arise in adulthood.

The requirement to improve biomaterials applied in dental medicine is also essential to prevent the beginning and development of oral diseases. With this idea in mind, we developed a laboratorial approach, with the main goal of demonstrating at an early stage, the possibility of enhancing the antibacterial effect of a biomaterial used in the primary prevention, in this case, a resin-based fissure sealant. In this study, considering the proven clinical application of silver diamine fluoride (SDF) in the significant inhibition of *S. mutans*, its incorporation into the fissure sealant proved to be a possible association that may enhance the antibacterial effect of the same fissure sealant, and avoid the development of dental caries in the presence of infiltration or partial loss of a fissure sealant.

The last part of the study presented in this thesis shows the need to implement community-oriented oral health promotion programs, which must be considered and developed in order to improve the knowledge and behaviors related to oral health of children, adolescents, parents, teachers and health professionals. Primary prevention should be taken into account to reduce the risk of oral disease development, primarily by significantly improving population's oral health behaviors.

The research described in this PhD thesis gives the scientific community the opportunity to analyze various issues, such as: epidemiology of oral diseases and primary prevention methods; characterization of the oral microflora and associated environmental factors that can influence oral health; influence of *H. pylori* bacterial strain on oral health; new applications that can be assigned to biomaterials used in dental medicine with a view to improving clinical outcomes in the future; and description of oral health educational strategies to improve health behaviors and reduce the incidence of oral diseases.

Keywords

Dental caries, fissure sealants, *H. pylori*, oral health, oral microflora, silver diamine fluoride.

Thesis Overview

Bearing in mind the importance of oral health in modern society in recent years, the thesis entitled **“Epidemiology of Dental Caries. From prevention to the relationship with *Helicobacter pylori* infection”** presents various studies that address different issues about oral health and primary prevention methods. The work comprises simultaneously an epidemiological and a laboratorial component aiming at improving oral health.

The thesis comprises eight main chapters that are described in brief terms below.

The first chapter identifies and describes the main objectives established for the development of the PhD work plan. The second chapter presents a literature review, through which the state of the art related to the determinants of oral health, the importance of the cariostatic effect of diamine silver fluoride, and the current knowledge about *H. pylori* in oral health is presented.

Chapters 3-7 present the results obtained during the development of this PhD research work plan, and that were summarized in original research papers:

Chapter 3 – Prevalence of dental caries and fissure sealants in a Portuguese sample of adolescents.

Chapter 4 – Oral and gastric *Helicobacter pylori*: effects and associations.

Chapter 5 – Prevalence and determinants of oral microflora among Portuguese adolescents.

Chapter 6 – Characterization of the antibacterial effect resulting from the association between silver diamine fluoride and a resin-based fissure sealant.

Chapter 7 – Oral Health Education: community and individual levels of intervention.

Chapter 8 brings the concluding remarks about the work developed during this PhD and future trends are presented.

List of Acronyms

<i>A. actinomycetemcomitans</i>	<i>Aggregatibacter actinomycetemcomitans</i>
AFRO	African Region
AMRO	North America, Central America and South America Region
CFU	Colony-forming units
CI	Confidence Interval
DGS	Direcção-Geral de Saúde
DMAE-CB	Metacriloxiletil ethyl dimethyl ammonium chloride monomer
DMFT	Decayed, missing and filled permanent teeth
dmft	Decayed, missing and filled deciduous teeth
EMRO	East Mediterranean Region
EURO	European Region
<i>H. pylori</i>	<i>Helicobacter pylori</i>
OR	<i>Odds ratio</i>
Ppm	Parts per million
PCR	Polymerase Chain Reaction
SDF	Silver diamine fluoride
SEARO	Southeast Asia Region
SEM	Scanning Electron Microscopy
<i>S. mutans</i>	<i>Streptococcus mutans</i>
UBT	Urease Breath Test
WHO	World Health Organization
WPRO	Western Pacific Region

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Chapter 1

Global aims

1. Global Aims

The global aims of this research are the following:

1. To assess the prevalence of dental caries, the decayed, missing and filled permanent teeth index (DMFT index) and the distribution pattern of pit and fissure sealants on permanent teeth and also its association with socio-demographic factors.
2. To evaluate if the oral cavity may be considered a potential *reservoir* of *H. pylori* and to determine the association between *H. pylori* infection with socio-demographic variables and the prevalence of dental caries.
3. To investigate the prevalence of salivary *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and correlate their presence with the risk of dental caries development and also their association with socio-demographic aspects.
4. To assess the effectiveness of incorporating an antibacterial agent – silver diamine fluoride - into a resin-based fissure sealant to prevent the onset of dental caries by inhibiting the growth of *S. mutans in vitro*.
5. To describe the different strategies that can be used to accomplish collective programs that promote oral health at individual and community levels.

Chapter 2

Introduction

2. Introduction

2.1. Oral Health

Oral health is defined by the World Health Organization (WHO) “*as being free of chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, and other diseases and disorders that affect the mouth and oral cavity*” (1).

Oral health has become an important component of general health. Nowadays, oral diseases such as dental caries and periodontal diseases are still the most prevalent diseases worldwide, and WHO considers these pathologies as one of the most important public health issues (1, 2).

The WHO described that:

- 60–90% of school children and nearly 100% of adults have dental caries.
- Dental caries can be prevented if a constant low level of fluoride is maintained in the oral cavity.
- Severe periodontal disease, which may result in tooth loss, is found in 15–20% of middle-aged (35-44 years) adults.
- Globally, about 30% of people aged 65–74 have no natural teeth.
- Oral diseases in children and adults are higher among poor and disadvantaged population groups.
- Risk factors for oral diseases include an unhealthy diet, tobacco use, abusive alcohol use, poor oral hygiene and other social determinants (2, 3).

In the 1980s in a political debate a former surgeon of the United States of America named Everett Koop recognized the link between oral health and the general health. According to him, “*You are not healthy without good oral health*”. Later, David Satcher, a general-surgeon wrote the report entitled “*Oral Health in America: a Portrait of a General Surgeon*”. This report explains the role of oral health in general health, emphasizing that oral health is a mirror for general health, since the oral cavity is the main entrance for infectious organisms and disease development (4).

The state of oral health is assessed mainly by the Locker conceptual model which was established in 1988. This model is based on the WHO classification and on the concepts of impairment, disability and handicap. This model reveals that oral health problems can lead to several possible consequences, such as:

- functional limitation;
- pain/discomfort;
- physical, psychological and social disability.

Basically, the Locker model is an attempt to understand oral diseases and their consequences at the physical, psychological and social levels (5). In terms of public health, it is crucial to understand that since 1989 available data reveal that about 51 million school hours are lost annually because of diseases

related to dental problems. Several studies also reveal positive correlations between the state of oral health and school performance (1, 4, 6).

Despite developments in medicine, current lifestyles and habits (eating of sugars and fats, smoking and alcohol consumption) lead to serious oral health problems. Consequently, these problems can disrupt the well being of a person (5, 7).

Various studies have shown that oral health is an important determinant to define the quality of life, namely among children and adolescents, and that oral health behaviors developed during childhood directly influence the risk of oral disease development during adulthood (8, 9).

However, oral diseases can be prevented and easily controlled if simple primary prevention methods that include infrequent sugar consumption, toothbrushing effectively and regularly at least twice a day, daily use of dental floss and having a dental appointment regularly to prevent and detect oral diseases in an early stage are adopted (2, 10). The application of topical fluoride supplements and fissure sealants are also considered as being important primary prevention methods in oral health (11, 12).

The impact of oral health is highly related with the quality of life (13). A study developed by Crocombe *et al.* demonstrates that the improvement in the frequency of dental appointments among low socioeconomic status groups would have the greatest effect on improving oral health and reducing oral health impacts (14).

Oral health must be focused on the prevention and treatment, since the burden of oral diseases and other chronic diseases can decrease simultaneously if common risk factors (1, 2, 15) are addressed. Consider, among others, the following measures:

- To reduce sugar intake and maintain a balanced nutritional intake to prevent dental caries and premature loss of teeth;
- To consume fruit and vegetable to protect against oral cancer;
- To stop tobacco use and reduce alcohol consumption to reduce the risk of oral cancer, periodontal disease and tooth loss;
- To ensure proper oral hygiene;
- To resort to protective equipment to reduce the risk of facial injuries in unsafe physical environments.

2.1.1. Definition of Dental Caries

Dental caries consists in a post-eruptive bacterial infectious disease characterized by a progressive demineralization process that affects the mineralized dental tissues. It is considered the most prevalent oral disease and the main cause of tooth loss among the population (16-18).

It is recognized as the most common oral disease worldwide and it is also the responsible for dental pain and tooth loss in populations throughout the world (2, 19).

This disease is also considered as being the most prevalent chronic disease in developed societies, being influenced by numerous genetic, cultural and social factors, which may explain, in part, large variations in the prevalence and incidence worldwide (2).

The absence of oral treatments invariably leads to an increase in lesion size progressing towards the dental pulp, resulting in a progressive development of pulpal inflammation accompanied by pain symptomatology and possible infection. Dental caries consists in a oral disease that is the main responsible for most of the consumption of biomaterials and human resources used in dental medicine, therefore dental caries have serious economic repercussions related to the high costs of their treatment and also with professional and school absenteeism (20-22).

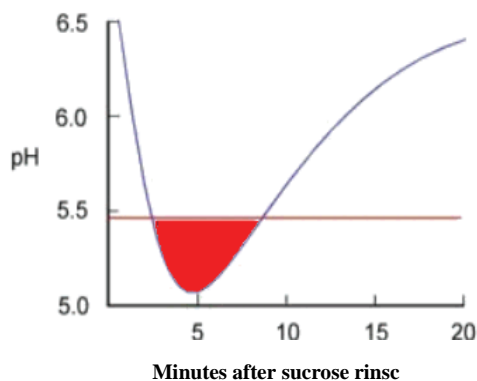
Dental caries is a result of a very complex interplay of multiple determinants and what has been experienced is a highly fortunate decrease in the rate which carie lesions develop and progress in children's dentition. However, much of the currently available evidence indicates that dental caries, unless carefully controlled, will continue to develop and progress throughout life. Thus, what has been considered an eradication of the disease should merely be considered a postponement of the time in life where the disease manifests itself with symptoms from lesions penetrating so deep into the dental hard tissue that cavities are formed (19).

Action mechanisms that lead to dental caries development

Dental caries are clinically characterized by a large polymorphism and a very complex etiology. Dental caries begins when there is a favorable interaction between multiple ethiological factors that create an imbalance in the oral cavity which allows the development of the disease (18).

A carious lesion initiates with the production of organic acids by the microorganisms of the oral cavity, namely *S. mutans* and *Lactobacillus*, that metabolize the extracellular carbohydrates of the individual's diet (18, 23). The presence of the organic acids produced will decrease the pH in the interface between the tooth surface and the bacterial plaque, allowing the development of the demineralization process on the tooth enamel (16). In the mouth, these changes over time are known as Stephan responses or Stephan curves (24). The pH of dental plaque under resting conditions (when no food or drink has been consumed), is fairly constant. The response after exposure of dental plaque to a fermentable carbohydrate is that pH decreases rapidly, reaching a minimum in approximately 5 to 20 minutes. This is followed by a gradual recovery to its starting value, usually over 30 to 60 minutes, although this can be longer in some individuals. When the oral cavity has a pH below 5,5 (considered the critical pH), the saturation of the dental tissues initiates causing desmineralization. If this process is frequent and constant a initial lesion will initiate and it may become the precursor of a dental carie (19, 20).

Figure 2.1: Illustration of the Stephan curve. Demonstration of the decrease of pH according to the time after sucrose rinse. The red zone corresponds to the critical pH (below 5.5) that takes place in the oral cavity and causes desmineralization of tooth enamel.



Prevention methods have as a main goal to decrease the time of exposure of tooth tissues to the low values of pH and, therefore, it is strictly necessary the frequent removal of bacterial plaque, avoiding an increase contact with tooth surfaces (25).

The buffering action of saliva must also be considered. Saliva is supersaturated with various ions that act as a buffer. In the physiological pH range (6.5-7.4) for saliva, a high saliva buffer capacity, mostly due to HCO_3^- concentration (nearly 10mg% are present in the oral cavity), is protective against dental caries, possibly reducing the rate of tooth demineralization caused by a lower pH value (19). Therefore, saliva has an important role in the prevention of dental caries, maintaining low levels of acidity in the oral cavity.

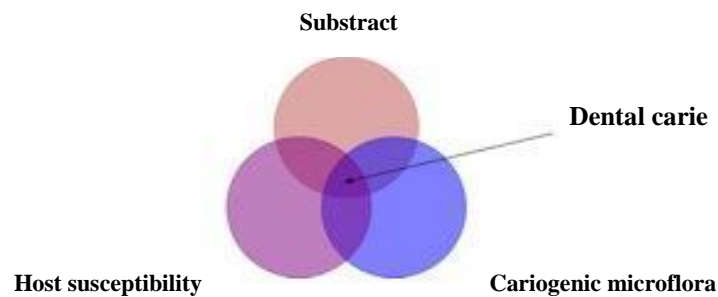
2.1.2. Etiological Factors of Dental Caries

Primary etiological factors

The researcher Paul Keyes developed a diagram that describes the multifactorial etiology of dental caries. In this diagram, we can observe that there are three main etiological factors that are essential for the initiation and development of the disease:

- Susceptible host;
- Cariogenic oral microflora;
- Substrat that depends on the host's diet, which is then metabolized by the microorganisms that constitutes that bacterial plaque (18).

Figure 2.2: Diagram proposed by Keyes about the multifactorial character of dental caries (Adapted from Lima *et al.*, 2007).



Host susceptibility

Refers to the characteristics that the individual's dental tissues may influence on the susceptibility to acid dissolution developed by the microorganisms in contact with the substrat (16). The presence of dental caries in dental morphology is influenced by the type of oral microflora, saliva composition and quantification in the oral cavity, by food intake such as high consumption of sugary foods and the application of fluorides or a combination of these and other individual and genetic factors.

We must also take into account the susceptibility of the individual, which is directly related to socioeconomical and cultural factors and that translates into specific individual behaviors, namely related with oral health (18).

Dental plaque and oral microflora

The oral cavity is inhabited by hundreds of bacterial species that play vital roles in maintaining oral health or in shifting to a diseased state such as dental caries and periodontal disease (26, 27).

The term biofilm is used to describe communities of microorganisms attached to a surface and can be formed very easily if the formation process is not interrupted, mainly by regular toothbrushing. Such organisms are spatially organized into a three-dimensional structure enclosed in a matrix of extracellular material derived from both the cells themselves and the environment. Dental plaque is considered as a microbial biofilm and its development can be divided into several stages:

1. Pellicle formation;
2. Attachment of single bacterial cells (0-4 hours);
3. Growth of attached bacteria leading to the formation of distinct microcolonies (4-24 hours);
4. Microbial succession and co-aggregation leading to increased species diversity concomitant with continued growth of microcolonies (1-14 days);
5. Climax community/mature plaque (2 weeks or more).

It should be noticed that plaque formation is a highly dynamic process, and that attachment, growth, removal and reattachment of bacterium may occur at the same time (19).

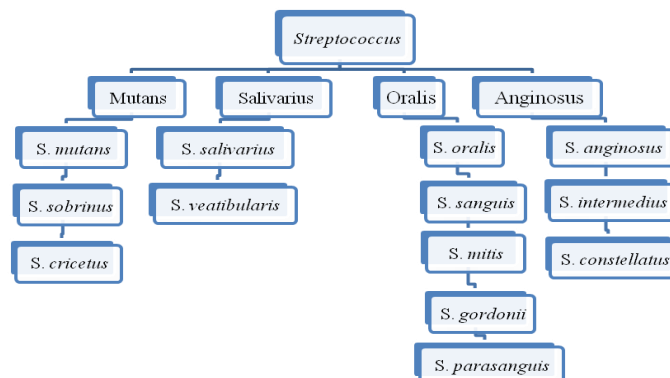
Dental caries, as a infectious disease, with bacterial etiology, correlates directly with bacterial strains that co-exist in the oral cavity, like *S. mutans* and *Lactobacillus* (19). The cariogenic properties of *S. mutans* and *Lactobacillus* are widely recognised and, as significant oral pathological agents, the former group is linked to enamel lesion formation while the latter is associated with cavity progression (28).

Recent studies have shown that *S. mutans* can also be a potential risk factor for other diseases such as the aggravation of ulcerative colitis (29).

A. actinomycetemcomitans is one of the most completely studied periodontal bacterial strains. It stays in the periodontal pocket of the oral cavity and damages tooth supporting tissues being considered as the major cause of periodontitis which is a bacterial infection of tooth-supporting tissues which may lead to tooth loss (30, 31).

A study developed by Hart *et al.* identifies the top 10 bacterial species or groups according to the score that have been definitely and/or that could be possibly implicated in caries onset and progression which included *S. mutans* and *Lactobacillus* (32).

Figure 2.3: Diagram presenting the principal strains of *Streptococcus* present in the oral cavity.



Aas *et al.* confirmed in their study that there is a distinctive predominant bacterial flora of the healthy oral cavity that is highly diverse and can be site and subject-specific. It is important to fully define the human microflora of the healthy oral cavity before we can understand the role of bacteria in oral disease (33).

It has concurrently been clearly established that social, economic, cultural, ethnic, and environmental factors also play an important role in the progression of dental caries and also influences the individual oral microflora highly related with oral health behaviors (34).

The bacteria present in the oral cavity are: other *Staphylococcus spp.* strains, which forms part of the oral normal microbial population; *Actinomyces spp.* which exists in large quantity in the dental plaque; the *Eubacterium spp.* found in periodontitis and abscesses; *Neisseria spp.* that are early colonizers of the oral cavity; the *Veillonella spp.* which is isolated from all surfaces of the oral cavity and there is a large number present in the tongue and dental plaque; *Haemophilus spp.* that is often present in saliva, dental plaque and epithelial surface; the *Eikenella spp.* which is most often present in the subgingival plaque,

and their number increases in gingivitis; the *Capnocytophaga spp.* predominantly present in periodontal disease; the *Aggregatibacter spp.* that lives isolated from periodontal pockets and is implicated in juvenile periodontitis; *Porphyromonas spp.*, *Prevotella spp.* and *Fusobacterium spp.*, that are three bacterial strains isolated from subgingival plaque and implicated in the etiology of periodontitis in adults (35).

Cariogenic diet

The level of cariogenic diet is determined by the presence of carbohydrates or refined sugars, especially sucrose, which serves as a substrate for microorganisms of the oral cavity (36). The oral microflora synthesizes extracellular polysaccharides that play a key role in dental plaque formation and also in the production of organic acids that promote and facilitate enamel demineralization and, thereby, the development of dental caries (18, 23, 37). Thus, for the genesis of the disease there is required in addition to cariogenic diet microorganisms to metabolize the substrate, and a host susceptible to the damaging effects of metabolism (38).

Today the world faces two kinds of malnutrition, one associated with hunger or nutritional deficiency and the other with dietary excess. Urbanization and economic development result in rapid changes in diets and lifestyles, which may be reflected by a higher risk of dental caries development. A study developed in Scotland confirms a lower prevalence of dental caries in the rural areas, mainly justified by the fact that adolescents may practice a better and healthier diet when compared with adolescents living in urban areas (39). Market globalization has a significant and worldwide impact on dietary excess leading to chronic diseases such as obesity, diabetes, cardiovascular diseases, cancer, osteoporosis and oral diseases. Diet and nutrition affects oral health in many ways. Nutrition, for example, influences cranio-facial development, oral cancer and oral infectious diseases. Dental diseases related to diet include dental caries, developmental defects of enamel, dental erosion and periodontal disease.

The nutrition transition is a relevant example on how common risks influence public health, including oral health. The public health community involved with oral health should gain an understanding of the health effects of these complex developments in order to prevent or control oral diseases (15).

Secondary etiological factors

Time

The time factor has an important role in the manifestation of clinical signs of the development of carie lesions (16). This factor was added by Newbrun to the primary etiological factors identified by Keyes, since these need to be present for a certain period of time, so that the progressive demineralization of enamel may develop (20, 40).

Fluorides

Research has shown that fluoride is most effective in dental caries prevention when a low level of fluoride is constantly maintained in the oral cavity. The goal of community-based public health programmes, therefore, should be to implement the most appropriate means of maintaining a constant low level of

fluoride in the oral cavity (36, 41). Fluorides can be obtained from fluoridated drinking-water, salt, milk, mouthrinse or toothpaste as well as professionally applied fluorides, or from combinations of fluoridated toothpaste with either of the other two fluoride sources (41). Fluoride is being widely used on a global scale, with much benefit. Millions of people worldwide use fluoridated toothpaste. Recent local studies have shown that affordable fluoridated toothpaste is effective in caries prevention and should be made available for use by health authorities in developing countries. The WHO Global Oral Health Programme is currently undertaking further demonstration projects in Africa, Asia and Europe in order to assess the relevance of affordable fluoridated toothpaste, milk fluoridation and salt fluoridation (1, 15).

There is clear evidence that long-term exposure to an optimal level of fluoride results in diminishing levels of caries in both child and adult populations (16). However, populations in many developing countries do not have access to fluorides for prevention of dental caries for practical or economic reasons (16).

There are some undesirable side-effects with excessive fluoride intake. Experience has shown that it may not be possible to achieve effective fluoride-based caries prevention without some degree of dental fluorosis, regardless of which methods are chosen to maintain a low level of fluoride in the mouth. The public health administrators must seek to maximize caries reduction while minimizing dental fluorosis (42).

Saliva

The mixed fluid in the mouth in contact with the teeth and oral mucosa, referred to as whole saliva, is derived predominantly from three paired major salivary glands: the parotid, submandibular and sublingual glands, but also from the minor salivary glands in the oral mucosa (16, 19). Normally, the daily production of saliva ranges between 0.5 and 1.0 liter and is composed of more than 99% water and less than 1% solids, mostly proteins and electrolytes. The multiple functions of saliva relate to both its fluid characteristics and specific components (20). Examples of the former are:

- Rinsing effect;
- Solubilization of food taste-substances;
- Bolus formation;
- Food and bacterial clearance;
- Dilution of detritus;
- Lubrification of oral soft tissues;
- Chewing capacity;
- Swallowing;
- Speech.

Other important functions of saliva consists in the protection of the teeth by neutralization of acids by buffering actions, the saliva maintains supersaturated calcium phosphate concentration with regard to hydroxyapatite, and also by participating in enamel pellicle formation. Furthermore, saliva components participate in mucosal coating and antimicrobial defense as well as digestive actions. Thus, saliva plays a

major role in oral health and changes affecting salivary function, it may also compromise hard and soft oral tissues structure and functions (19).

The oral cavity is constantly exposed to many different kinds of substances, some of which influence the caries process to a great extent. An important function of saliva is therefore the dilution and elimination of substances introduced into the oral cavity, through a physiological process usually referred to as salivary clearance or oral clearance (19, 20). In patients with reduced quantity of saliva the mechanistic and cleaning properties of this fluid in the mouth are impaired. With regard to prolonged oral clearance, a low oral sugar clearance inevitably increases the risk of caries development. Concerning this relation, the unstimulated flow rate has been found to be diagnostically more important than the stimulated one (19).

Oral hygiene

There is a strong correlation between oral hygiene and the prevalence of dental caries (43). Good oral hygiene habits help to prevent the development of caries by reducing the build-up of dental plaque (24).

The composition of the dental plaque varies not only from individual to individual, but also upon the location of the oral cavity and tooth surface. Control of bacterial plaque through proper hygiene, performed by each individual and complemented with the intervention of a dental professional are key preventive primary measures for the improvement of oral health and disease prevention, including dental caries (16).

2.1.3. Epidemiology of Dental Caries

Although there has been notable national and international epidemiological research for some years, the study of dental epidemiology is a relatively new field in dentistry that has been stimulated by its increasing concern in public health. Epidemiology is fundamental to understanding the clinical and the public health importance of the disease, as well as providing insights for devising and assessing methods of caries control (16, 44).

To understand the disease process and how caries affect the different groups of society, one needs to know the distribution of the disease in various communities. The average levels of disease seen in the inherently atypical groups of self-selected and/or referred patients attending dental clinics frequently give a false picture compared with the rest of the population. The mean levels and distribution of disease seen in representative samples of the total population will usually be different. The existence of this inherent and explainable difference is a key issue that dental students, dentists and public health planners must understand (19).

Prevalences Worldwide

Globally, it is estimated that 60-90% of young people (children and adolescents) and 100% of the adult population experience dental caries. Dental caries and periodontal diseases are common in industrialized countries and has been referred its increase in developing countries (2).

Analyzing the global caries burden for 12-year-old children in the six regions defined by the WHO, the highest values of the decayed, missing and filled permanent teeth index (DMFT index) were found in the

regions of North America, Central America and South America (AMRO) with 2.35, Europe (EURO) presented 1.95, followed by the Southeast Asia (SEARO) registering 1.87, regions of the East Mediterranean (EMRO) with 1.63, Western Pacific (WPRO) with 1.39 and Africa (AFRO) with an average score of 1.19. Globally it is estimated that the DMFT at 12 years of age is 1.67 (45). This value achieved in 2011 reflects an increase in the overall DMFT, considering that in 2004 it was 1.6 (2, 46). The most significant decrease in the DMFT index was registered in Europe, which registered an average score in 2004 of 2.57, and of 1.95 in 2011. In the East Mediterranean, Africa and Southeast Asia there was an increase in the DMFT index from 2004 to 2011, which proves the increase in the prevalence of dental caries among the developing countries, contrasting with a decrease verified in the developed regions like Europe and North America. However, these last two WHO regions still register the highest DMFT index scores in the world (45).

Table 2.1: DMFT reported for regions defined by WHO among 12-year-old children (45,46).

WHO Regions	DMFT	
	2004	2011
AFRO	1.15	1.19
AMRO	2.76	2.35
EMRO	1.58	1.63
EURO	2.57	1.95
SEARO	1.12	1.87
WPRO	1.48	1.39
Global	1.61	1.67

Despite the fact that various studies have demonstrated in the last years an increase in the prevalence of dental caries among children and adolescents in developed countries, caries levels have a tendency to increase with age and remain a problematic public health issue in adults (47, 48).

Various studies demonstrate the existence of problematic situations in certain countries when analyzing the prevalence of oral diseases and the lack of correct oral health behaviors (4, 39, 49, 50).

In a study developed in Thailand, it was found that 96.3% of children under 6 years of age had dental caries and the DMFT and dmft index in children with 12 years was 8.1 and 2.4, respectively. In the same study it was found that 53% of children under the age of 12 reported toothache in the last 12 months. Of these, only 66% had a dental appointment in the last year. A large number of children who participated in this study consumed daily sugary foods and this has been correlated with the onset of dental caries (49).

Traebert *et al.* conducted a study in the municipality of Santa Catarina, Brazil, and found that the prevalence of caries in the primary dentition in children aged 6 years was 60.9% in public schools, enrolling more disadvantaged children (dmft index 2.98) and 34.9% in private schools (dmft index 1.32) (50).

In another study performed to compare the prevalence of dental caries among children in specific regions of Estonia and Denmark, there were significant differences. Thus, 83.8% of children with 7 years of age had caries in Estonia while in Denmark this prevalence was only 62.1 % in the same age group. Conclusions of this study highlights the unsatisfactory level of oral hygiene of children in Estonia, as described in other studies, and its relation to excessive intake of foods and sugary drinks (51).

Szoke and Petersen found that in Hungary the prevalence of caries of 73% and 84.5% in children with six and twelve, respectively. It is a high prevalence reflecting the need to implement measures to promote oral health at community level (52).

Sweden is a country that has the lowest prevalence of dental caries worldwide, having been recorded in the last decades a significant decrease in prevalence to values under 12%, especially among children and adolescents (53). However, in this country, there is still a prevalence of caries of 10% among adolescents, although cases are derived predominantly from immigrant families according to the authors (54). A swedish study developed by Hasselkvist *et al.* established an association between certain unhealthy lifestyle habits and poorer oral health, mainly related with the consumption of soft drinks (48).

Prevalences in Portugal

In recent decades, the prevalence of dental caries in Portugal has decreased significantly, especially among children and adolescents. This decrease in the prevalence of dental caries began to widen with the introduction of the national program of oral health promotion in Portugal. Such programs is based on a global intervention strategy that focus on health promotion throughout the life cycle, family context and the specific environments that children attend, including school (55, 56). It has been found among Portuguese children a significant improvement in oral health behaviors, including increased frequency of brushing, use of fluoride toothpaste and an increase in the number of queries to the dentist, in order to perform preventive treatments. Not withstanding these positive developments, dental caries still present a high prevalence, and also constitute a public health problem (55).

The first national survey in 1983-84, in partnership with WHO, the *Direcção-Geral de Saúde* of Portugal (DGS-Portugal) and the Faculty of Dental Medicine of the University of Lisbon showed that for children with 6 years old the decayed, missing and filled deciduous teeth (dmft) and DMFT indexes were 5.2 and 0.5, respectively, while the 12 year old children had a DMFT index for permanent teeth of 3.8 (57).

Almeida *et al.* estimated that the prevalence of caries in children with 6 years of age was 46.9% in children and 12 years was 52.9%. The dmft index was 2.1 for children aged 6 years and DMFT index was 1.5 in children aged 12 years. According to this study, Portuguese children have prevalence similar to that found in countries with more advanced oral health programs such as the United Kingdom and Denmark (57).

Through the national study of the prevalence of dental caries in the educated population, conducted by the DGS-Portugal in 2000, the DMFT at age 12 was 2.95 reaching the goal advocated for the WHO European region in 2000. However, in this study, the number of children under 6 years without any dental carie only reached 33% (58).

It was therefore concluded that a decrease in the prevalence of dental caries in Portugal occurred in recent years compared with the data collected in the first national survey in 1983-84.

In 2008, a national study of the prevalence of oral diseases carried out by the DGS-Portugal in partnership with the WHO aimed to assess the prevalence of dental caries, dental fluorosis and periodontal at 6, 12 and 15 diseases and analyze raises some their determinants (55). This study highlights the fact that at 6 years of age, 51% of Portuguese children did not have any dental carie. This demonstrates a significant increase in 20 years of oral health programs aimed primarily at children and adolescents, considering that the percentage of children free of dental caries at age 6, increased from 10% in 1986 to 51% in 2008. The national study also highlights the DMFT index at 6, 12 and 15 years that was, 0.07, 1.48 and 3.04, respectively. Using as reference values for the WHO scores of DMFT at age 12, Portugal ranks among the countries with low caries prevalence, reaching in 2008 the recommendations for the European region predicted for 2020. From 2000 to 2006 it was found an increase of 30% of young people with treated teeth. These gains in oral health resulted, in large part, of the process of contractual agreements with the private sector for the provision of medical and dental healthcare to children and young people (55). Comparing the current oral health indicators in Portugal with those of 20 years ago, the portuguese population has been experiencing a significant improvement in oral health (55).

Rodrigues and Reis developed a study which stated that dental caries is the most common chronic diseases during childhood. The prevalence of dental caries in Portugal among 6 year-old children is 49%, increasing to 72% at 15 years of age. This study demonstrates that Portugal needs to continue the development and improvement of oral health promotion programs directed to children and adolescents for doing primary prevention of oral diseases (59).

2.1.4. Prevention and Non-Invasive Treatments of Dental Caries

Primary prevention involves the adoption of measures to prevent disease or ill health. An example would be immunizations, which attempt to prevent people from developing disease in the first place. This is in contrast to secondary prevention, which involves strategies to diagnose and treat an existing disease in its early stages before it results in significant morbidity. An example of this would be a mammogram, which is aimed at finding breast cancer in its earliest stage. Tertiary prevention involves treatment of established disease, by restoring function and also reducing disease-related complications (24, 44).

Primary prevention in dentistry essentially covers the following methods: brushing, use of dental floss, regular dental appointments, fluoride administration, application of fissure sealants; balanced and non-cariogenic diet. The application of these methods are dependent of the knowledge about primary prevention and oral health and sociodemographic and cultural factors of the community (24, 25).

Sociodemographic aspects

The increased risk of oral disease is associated with low socioeconomic status, low educational level and cultural factors that often determine a greater difficulty in access to information and health (38, 60-62).

Overtime, and despite the continuing high prevalence of dental caries worldwide, the prevalence of the disease has declined from high to low and it is moderate for the majority of the developed countries (20, 63). There is a persistence of oral problems in many communities, particularly among the less privileged with worse socioeconomic status, both in developed and in developing countries (21, 61).

In some industrialized countries, the decrease of the prevalence of dental caries and other oral diseases is explained largely by the interventions achieved through oral health promotion programs (2, 15). This pattern of evolution of the prevalence of dental caries is directly related to the many preventive measures implemented in recent years, as the fluoridation of public water supply, administration of fluoride mouthwashes and fluoride toothpastes and the improvement in standards of oral hygiene. In addition to these factors, some authors described the improvements in the provision of oral health services as responsible for the decline of dental caries (64).

As an example, the large decrease in the prevalence of dental caries in the Scandinavian countries (Sweden and Finland), Australia and New Zealand, was achieved through the implementation of oral health promotion programs aimed at the prevention of dental caries (2, 53).

However, in many developing countries whose populations do not have access to oral healthcare the health system implemented give very insufficient attention to the need for primary prevention towards oral diseases (6, 60). In some of these countries there has been an increase in the prevalence of dental caries justified by the direct influence of the existing socioeconomic conditions in these populations. The exceptions were noticed for the countries where programs to promote oral health and disease prevention have been implemented (23, 65).

The impact of oral diseases both at individual and community level, in terms of pain and suffering, disability and reduced quality of life is quite considerable. This impact is also higher in developed countries (3, 6).

A cross-sectional study developed by Peres *et al.*, where 695 adolescents were involved, showed that the higher the prevalence of dental caries the higher the dissatisfaction levels related with chewing function described by the adolescent. This situation occurred mostly in public schools, and was not so often among private school students (42).

One of the main problems in the unequal general and oral health situation is related with socioeconomic status and social inequalities. The damage to health combines the deprivation of goods and services necessary to minimize physical, psychological and social problems that is a major characteristic of poverty (65). The socioeconomically disadvantaged cannot afford healthcare services and have less access and interest in information, having a higher predisposition in developing health risk behaviors (13). They have a worse nutritional condition, have less access to early diagnosis, fewer therapeutic resources and poorer prognosis due to a higher risk of disease development (44).

Brushing

Brushing is the best form of mechanical removal of dental plaque and food debris and is seen to be the primary method of oral hygiene, when complemented with the use of a fluoride toothpaste (25). The use of fluoride toothpaste with brushing allows an increasing abrasive action of the bristles of the brush and

the release of chemicals in the prevention and control of dental plaque. Several authors conclude that the recommended frequency of daily brushing should be at least twice a day and the hygiene should be applied also on other oral structures such as the tongue, gums and palate (16, 25, 53).

In the last national prevalence study of oral diseases, it was demonstrated that nearly 50% of children aged 6 years and 67 % and 69% of teens with 12 and 15 years, respectively, toothbrush at least twice a day, with a fluoride toothpaste. Brushing their teeth at bedtime was made, always or almost always, by only 50% of children and adolescents that participated in the national survey (55).

Dental Floss

The use of dental floss daily to prevent the development of caries in the interproximal surfaces of the teeth and periodontal diseases is highly recommended (16, 24).

The most difficult areas to remove dental plaque are the interproximal surfaces that lie at the level of interdental spaces (66, 67). Dental floss is a key addition to oral hygiene, allowing the cleaning of interdental spaces with difficult access during brushing (66).

The efficient use of dental floss allows the:

- Removal of plaque and food debris that adheres to the tooth surface restorations, orthodontic appliances, fixed prostheses, soft tissue located in the interproximal spaces and implants;
- Assistance in identifying the presence of subgingival calculus, interproximal carious lesions or unadapted dental restorations;
- Reduce gum bleeding (66, 67).

However, flossing is not widely used by the population, especially by adolescents. There is a clear need to educate the communities for daily use of dental floss and emphasize its importance as a complement in oral hygiene (66).

In the last national study of the prevalence of oral diseases, the daily use of dental floss was reported by 14 % and 10 % of young people with 12 and 15, respectively (55).

Dental appointments

Regular dental appointments are critical and must occur at least once every six months, because it allows early detection of oral diseases, provides specific guidance on certain oral hygiene procedures and apply a set of other primary preventive measures such as topical application of fluoride and fissure sealants (1, 14).

Regarding the periodicity of routine appointments there are different opinions about what would be more appropriate. However, the frequency of the dental appointments should take into account the needs of each population (16).

The self-perception of the need for regular dental healthcare, level of education and socioeconomic status, the high cost of medical and dental appointments and the phobia are often associated with these preventive measures (23, 53, 68, 69).

One of the main reasons for going to the dentist is the emergence of pain (21). A dental pain (or toothache) among children and adolescents has been classified as a major public health problem and is associated with poor oral healthcare, including unregular attendance to the dentist (70, 71).

Children and adolescents with better socioeconomic conditions, those who live in urban areas and whose parents perform medical and dental appointments regularly, are those who visit the dentist more frequently, favoring medical and dental care prevention (2, 70).

Accessibility to medical and dental care, at 12 and 15 years, was reported by more than 85 % of young people in the last national prevalence study of oral diseases performed in 2008. In this study, the main reasons for a dental appointment were routine appointment or check-up and for dental treatments, not in an emergency situation (55).

Fluoride applications

Maintaining adequate levels of fluoride in the oral cavity is achieved not only through the application of fluoride by brushing with a fluoride toothpaste, but also by the periodical use of fluoride mouthwashes and fluoride gel application during a dental appointment (24, 25).

Schulte *et al.* performed a study in Germany and France that revealed the importance of the application of fluoride for promoting the reduction of dental caries in children with 12 years of age (72).

Maintaining adequate levels of fluoride in the oral cavity is an important determinant of oral health and contributes to the prevention of dental caries. The preventive and therapeutic action of fluoride is achieved predominantly for its topical action, both in children and in adults, through three different mechanisms:

- Inhibition of demineralization process;
- Potentiation of remineralization process;
- Inhibition of dental plaque formation (41, 73).

Between 1987 and 2005, the guidelines set for oral health programs developed in Portugal advocated the intake of fluoride by children, in the form of oral drops (6 months to 2 years) and tablets (from 2 to 16 years) whose dosage could reach 1mg/day, depending on the age and contents of this element in public water supplies. This administration could be done in schools. Furthermore, in primary schools, the strategy was complemented by a rinse with a solution of sodium fluoride 0.2%, every two weeks (41).

Nutrition education and practice of oral hygiene by brushing with a fluoride toothpaste twice a day, has proven a collective means of preventing dental caries, with great effectiveness and low cost, and should be considered as a strategy of community oral health programs. Toothpastes that are in the market nowadays, should have a concentration of fluoride ranging between 500 and 1500 parts per million (ppm) (41).

Before the widespread use of fluoridated toothpaste, use of fluoride mouthwash was considered of great importance, especially in terms of use at a community level due to its low cost, high efficiency and simplicity of use with the great advantage that it can be used outside the clinical context. A prescription of mouthwash solutions should be made by the dentist according to the individual risk of each patient. The

daily rinses usually have a sodium fluoride concentration of 0.05% and weekly or biweekly using a concentration of 0.2%. It is recommended that children with more than 6 years of age make a mouthwash, fortnightly, with a solution of sodium fluoride 0.2% (41).

Currently it is considered that the topical application of fluorides have a benefic effect directly on the tooth surface, whereas the systemic action of fluorides is much less important (24, 41).

Fissure sealant application

Fissure sealants are resinous materials professionally applied on the occlusal surfaces of the posterior teeth. Several epidemiological studies show an association between the morphology of the occlusal surface and the risk of developing dental caries (25). Dental anatomy is characterized by the presence of a complex set of pits and fissures with variable depths. The tooth morphology is a major factor associated with the increased susceptibility of dental caries development due to the greater difficulty of cleaning these areas and greater bacterial and plaque retentiveness (25). Thus, the sealants are indicated when there are pits and fissures very deep and narrow creating a physical barrier to the build up of plaque in these specific anatomical regions of the tooth preventing the formation of dental caries (20). The placement of fissure sealants in these areas highly susceptible to the development of dental caries is considered a primary preventive measure to reduce the risk of the disease and preventing the need to perform more dental restorations. Therefore, the application of sealants is covered by community oral health programs and is considered an effective and economical method for primary prevention of dental caries (16).

In the national study of the prevalence of oral diseases in 2008 made in Portugal 38% of teens at age 12 presented one or more teeth with fissure sealants (74).

A study developed in Greece with 12 and 15 year old teens was able to demonstrate that fissure sealants had a positive effect on the decrease of dental caries development (75). In general, we can establish that fissure sealants is an effective way to prevent caries in permanent teeth in childhood and adolescence (12).

Decrease of cariogenic foods and beverages

The use of cariogenic foods with a high content of carbohydrates, is an important risk factor for the development of dental caries and premature loss of teeth has been increasing, mainly in industrialized countries (2, 38, 48, 76). It is important to regulate the consumption of some foods, such as fruits and vegetables, which also contribute to the prevention of cancer diseases, namely in the oral cavity (2, 38).

Several studies also indicate that there is an association between obesity and oral diseases (77-79). However, the relationship between obesity and dental caries is not clear. The presence of a common risk factor, which is the high intake of sugary foods, appears to be a justification to explain the relationship between obesity and dental caries (77, 80).

There is evidence that the nutritional status influences the formation and development of the teeth increased risk for the occurrence of caries in individuals who experience episodes of mild to moderate malnutrition in the first year of life (62). Therefore, the more economically advantaged groups would

have greater opportunity to have a better nutritional/immune status and consequently lower risk of caries and dental pain (21, 62). On the other hand, the socioeconomically wealthier groups consume more foods with high sugar contents, which do not cease to be an influential factor in oral health, considering that they also practice better oral health habits. A study developed by Zaborskis *et al.* showed that children and adolescents, from higher socioeconomic levels, have worse care with their diet, which translates to higher consumption of cariogenic foods (81).

2.2. The Importance of the Cariostatic Effect of Diamine Silver Fluoride

Nowadays, nanotechnology has become a key research field that has brought beneficial applications to medicine and healthcare. One of the most advanced methods applied in healthcare consists in the development of controlled release systems of silver particles, which increases the therapeutical antibacterial effect of silver ion particles (82, 83). Silver is a natural antibacterial agent and has important medical properties, namely the fact of being a biocompatible material, non-toxic for the human cells, long-term bactericide effect and stability in high temperatures and low volatility (83). Silver ions (Ag^+) are expected to have antibacterial effects whereas metallic silver (Ag) is relatively inert. However, metallic silver can interact with moisture in the oral environment and subsequently release silver ions. Silver ions have been suggested to have the following three main antibacterial effects: destruction of cell wall structure; denaturation of cytoplasmic enzyme and inhibition of microbic DNA replication. Silver compounds have been used due to their properties for centuries in various areas and in dentistry for more than a century (84).

Today, silver is again seen as an antimicrobial agent due to its wide spectrum, low toxicity and lack of cross-spectrum bacterial resistance and has also been used in various situations like water purification, wound treatments, bone prosthesis, surgical orthopedic reconstruction, development of heart functioning devices and even has been incorporated in textile aimed for hospital use made in order to decrease the risk of hospital infections (84-86).

In dental medicine, silver compounds have been used as early as the 1840s, when silver nitrate was used for reducing the incidence of caries in the primary dentition and later in the permanent molars (87, 88). In the 1960s, silver was advocated to combine with fluoride as an anticariogenic agent presumably for a combined beneficial effect (82).

Other studies demonstrated the possible association of silver ion particles with filled resins applied in dental medicine which increases the antibacterial effect and assures a better protection against the development of recidivating dental caries (89).

We can also find a biomaterial that associates the advantages of sodium fluoride and silver nitrate: silver diamine fluoride (SDF). This is a biomaterial, with proven cariostatic properties, that can stop the development of dental caries in the hard tissue of the tooth with a mechanism that consists in the self-stimulation of the calcified and sclerotic dentin formed by the silver ion (90, 91).

Other studies have shown the synergism established between the fluoride and the silver that increases the enamel resistance against the organic acids produced in the oral cavity and that is responsible for the

dental tissue demineralization, which gives this biomaterial the possibility to become an important product that can be applied in primary prevention (82, 92). SDF has also been shown to inhibit bacterial growth, mainly cariogenic strains of *S. mutans* (84, 93).

Due to these specific properties of SDF, in resource-limited situations, applications of SDF may become a beneficial procedure to stop dental caries progression, mainly among low socioeconomic status communities (84, 90).

2.3. *Helicobacter pylori* and Oral Health

From a historical perspective, in 1875, German scientists found a helical-shaped bacterium on the inner wall of the human stomach. The bacterium did not grow in culture and the results were forgotten. In 1892 the Italian researcher Giulio Bizzozero describes the existence of a helical-shaped bacterium living in the acidic environment of the stomach of dogs. Years later, Professor Walery Jaworski of the Jagiellonian University in Krakow, Poland, investigates sediments of gastric washings obtained from humans. Smooth helical-shaped bacteria was also found in the sediments and were designated as *Vibrio*. This researcher was the first to suggest a possible role of this organism in the pathogenesis of gastric diseases. In 1979 the bacterium is rediscovered by Robin Warren, who describes the presence of this microorganism in biopsies of patients suffering from gastritis (94). In 1981, two Australian researchers – Robin Warren and Barry Marshall – initiate further investigations and detected signs of inflammation when bacteria was present in the gastric mucosa. The discovery of the *H. pylori* was accomplished (95). *H. pylori* is a gram-negative, microaerophilic, rod-shaped bacterium that colonizes the human stomach. It resides beneath the gastric mucous layer, adjacent to the gastric epithelial cells, and although it is not invasive, it causes inflammation of the gastric mucosa. When the organism is infected with this microorganism it is now recognized as a serious, transmissible infectious disease, highly associated with the development of atrophic gastritis, chronic gastritis, duodenal ulcers, gastric mucosa-associated lymphoma, and gastric cancer (96).

Nowadays, it is estimated that approximately 50% of the world's population is infected. Developed countries typically have a lower prevalence of *H. pylori* infection at all ages, but this difference is especially noticeable among younger people (95, 97, 98).

The fecal-oral tract is believed to be a common route of transmission of *H. pylori* so low levels of sanitation and socioeconomic status are associated with an increased prevalence of *H. pylori* infection (95, 98, 99).

Almeida *et al.* claim that in Portugal, "*the decreasing prevalence of H. pylori infection has been reported worldwide, mainly due to the improvement social and economic conditions.*" However, the same author declares that some endoscopic and histological findings were associated significantly with the presence of infection by *H. pylori*. These results emphasize the relevance of *H. pylori* infection in symptomatic Portuguese pediatric population and the need to adopt strategies that are cost-effective (100).

Some authors have suggested that *H. pylori* may belong to the normal oral microflora of the human oral cavity, maintaining a commensal relation with the host (101, 102), although, due to their limited presence,

their identification is difficult (103, 104). Studies have demonstrated that *H. pylori* presence in the oral cavity has a clear association with gastro-esophageal infection (105). Therefore, the failure to eliminate *H. pylori* in the oral cavity may lead to a gastrointestinal re-infection (105). Others have suggested that *H. pylori* is not consistently present in dental plaque and, when present, may be the result of occasional gastroesophageal reflux (96).

A prospective epidemiologic study reported a positive association between tooth loss and the risk of noncardia gastric cancer. However, specific oral health conditions that may be responsible for this association remain unknown (106).

The *H. pylori* and their presence in the oral cavity have been widely studied in order to establish a possible relation between its presence and the appearance of a particular disease. Their presence in the oral cavity by itself doesn't mean they are pathogenic; but studies also have showed the presence of *H. pylori* in periodontium and their potential role in causing the periodontal infection (107).

It has been noted that the presence of *H. pylori* in the oral cavity may affect the outcome of eradication therapy. This condition is associated with the recurrence of gastric infection. The optimum secretion of saliva promotes oral health consequently influencing *H. pylori* eradication. Vahedi *et al.* investigated the relation between salivary secretion and the efficacy of *H. pylori* eradication from the stomach. The research developed demonstrated that the efficacy of *H. pylori* eradication from the stomach might be reduced by lower salivary secretion (105).

Numerous factors may influence the effectiveness of anti-*H. pylori* therapy. Since oral cavities that can be considered a localized "sanctuary" for *H. pylori* survival in the mouth despite their eradication from the stomach, may promote early reinfection of the stomach reducing the efficacy of eradication therapy. In cases of low salivary secretion, the oral cavity becomes a favorable environment for the growth of bacterium including *H. pylori* (105).

Momtaz *et al.* considered that regard to high similarity in genotype of *H. pylori* isolated from saliva, stomach and stool, their study supports the idea that the fecal-oral tract is the main route of *H. pylori* transmission and that the oral cavity may serve as a *reservoir* for *H. pylori*, however, remarkable genotype diversity among stomach, saliva and stool samples showed that more than one *H. pylori* genotype may exist in a same patient (96). Furthermore, the question remains, what are the characteristics of the *H. pylori* detected in the oral cavity. It is possible, that *H. pylori* simply belong to the normal flora of the oral cavity, maintaining a commensal relationship with the host?

Two virulence factors that are expressed by the alleles of the cytotoxin genes, *cagA* and *vacA*, have been identified in the presence of *H. pylori*. Momtaz *et al.*, after comparing *cagA* and *vacA* genotypes of *H. pylori* between stomach and saliva in a same patient, verified genotypic diversity between stomach and saliva in the same patient in 38.8% of the cases, suggesting that more than one different *H. pylori* strain may exist in stomach and saliva of the same patient (96).

An important issue that still needs to be well studied and determined is the influence of oral *H. pylori* on dental caries development and oral health status. Liu *et al.* verified a correlation between the presence of *H. pylori* and the occurrence of dental caries. The authors analyzed a total of 841 subjects, 574 (68.25%) were infected with *H. pylori*, and 516 (61.36%) were diagnosed with dental caries. Among the 574

subjects with *H. pylori*, the prevalence of dental caries was 73.52%, while the prevalence among the 267 cases without *H. pylori* was 35.21%. Therefore, Liu *et al.* conclude that *H. pylori* infection in the oral cavity is associated with dental caries and poor dental hygiene (108).

Liu *et al.* also investigated whether individuals with oral *H. pylori* show more possibility of gastric infection and also examined the relationship between gastric *H. pylori* infection and the presence of the bacteria in the oral cavity. The researchers verified that the prevalence of gastric infection was significantly higher among the patients with positive tests for *H. pylori* in their dental plaque than in the patients with no *H. pylori* in their dental plaque ($p < 0.05$). Based on this data, they conclude that the oral cavity may be a potential *reservoir* for *H. pylori*, and the prevalence of oral *H. pylori* was quite similar of gastric *H. pylori*, in the studied population. Moreover, a close relationship may exist between *H. pylori* in the oral cavity and the bacteria found in the stomach or gastric infection, and dyspeptic patients with gastric infection are more likely to harbor *H. pylori* in their oral cavity (109).

In a study developed by Pytko-Polonczyk *et al.*, the authors conclude that, among the Polish population including dyspeptic and duodenal peptic ulcer patients, the mouth is a permanent *reservoir* of *H. pylori* and that the success in its eradication from the stomach by systemic therapy fails due to the presence of *H. pylori* in the oral cavity that might be a potential source of gastric reinfection in these patients (97). Therefore, and having into account what the actual state of the art presents, we consider that it is essential to analyze among portuguese adolescents if the oral cavity can be considered a *reservoir* for *H. pylori*.

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Chapter 3

**Prevalence of dental caries and fissure sealants
in a portuguese sample of adolescents**

Paper I

Prevalence of dental caries and fissure sealants in a portuguese sample of adolescents.

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3.1. Abstract

Introduction: The aims of this study were to assess the prevalence of dental caries and the DMFT index, as well as the distribution pattern of pit and fissure sealants on permanent teeth in a Portuguese sample of adolescents, and to assess whether the existing usage of sealants and socio-demographic factors are correlated to caries prevalence on the examined sample.

Materials and Methods: A cross-sectional study was designed with a sample of 447 adolescents aged 12 to 18 years old, attending a public school in Sátão, Portugal. A self-administered questionnaire with questions about oral health behaviours and socio-economic status was answered by adolescents in the classroom. Clinical examination of oral health status and assessment of fissure sealants were accomplished by only one trained member of the research team.

Results: We obtained a DMFT index of 3.32 (2.92), which indicates a moderate level of prevalence of dental caries. When considering a DMFT=0, we found significant statistical differences between the parents' level of education ($\leq 4^{\text{th}}$ grade=26.3 vs 5^{th} - 12^{th} grade=18.8 vs $>12^{\text{th}}$ grade=43.3, $p=0.001$), gender (male=27.3 vs female=19.6, $p=0.04$), age (≤ 15 years=27.1 vs >15 years=18.5, $p=0.02$), presence of fissure sealants (yes=30.6 vs no=13.5, $p=0.001$) and experience of dental pain (no=25.4 vs yes=16.8, $p=0.02$). When analyzing the prevalence of fissure sealants, we verified that 58.8% of adolescents had at least one fissure sealant applied. Significant statistical differences were found when analyzing the presence of fissure sealants related with parents' educational level ($>9^{\text{th}}$ grade, OR=1.56 CI95%=1.05-2.54), gender (female, OR=1.86 CI95%=1.19-2.98), experience of dental pain (yes, OR=0.62 CI95%=0.39-0.97) and presence of dental caries (yes, OR=0.35 CI95%=0.19-0.65).

Conclusions: The moderate level of caries prevalence reveals the need of improvement of primary prevention interventions among Portuguese adolescents. The establishment of a more targeted preventive program with better and more effective oral health education is essential, having into account socio-demographic aspects.

Keywords: Adolescents, dental caries, fissure sealants, oral health.

3.2. Introduction

In the last decades the World Health Organization (WHO) has dedicated special attention to oral health and the prevalence and causes of oral diseases, namely dental caries and periodontal diseases, and, most recently, oral cancer (1).

The risk of development of oral diseases, namely dental caries and periodontal diseases, is strongly related with lifestyle habits. Health-promoting lifestyles include infrequent sugar consumption, toothbrushing effectively and regularly at least twice a day, daily use of dental floss and visiting a dentist regularly to prevent and detect oral diseases in an early stage. Most researchers also believe that oral health knowledge and parents' attitudes mostly determine and promote children's oral health behaviour (1-3).

One of the most important oral health indicators used in epidemiological studies is the decayed, missing and filled permanent teeth index (DMFT index) developed by Klein and Palmer in 1937 (4, 5).

After obtaining the DMFT index, it is possible to determine the level of severity of dental caries in the study sample: very low (DMFT index between 0.1 and 1.1); low (DMFT index between 1.2 and 2.6); moderate (DMFT index between 2.7 and 4.4); high (DMFT index between 4.5 and 6.5) and very high (DMFT index higher than 6.5) (1).

In Portugal, dental healthcare is mainly provided by the private sector. In the past years, several oral health programs have been developed in order to finance the dental treatments of the poorer socioeconomic status to compensate the very low number of dental appointments in the public sector (health centers and hospitals). The oral health programs are mainly addressed to children and adolescents, focusing mainly on primary prevention with the application of fluoride products and fissure sealants in the dental appointment (6, 7).

Pits and fissures on occlusal surfaces of permanent teeth are particularly susceptible to the development of dental caries. This susceptibility to dental caries is related with the individual morphology of the tooth's pits and fissures, which can be prosperous shelters for microorganisms and make the hygiene procedures of these areas more difficult, allowing greater plaque aggregation (8). Consequently, with a view to preventing dental caries, fissure sealant application is recommended if pits and fissures are very deep and narrow, thus creating a physical barrier for the plaque's accumulation in these specific anatomical areas of the tooth (9). This primary prevention measure is highly recommended to prevent dental caries formation, but also to control and arrest the development of incipient dental caries (10, 11). Fissure sealants are used mostly as a primary preventive measure in dental medicine (12).

Fissure sealants are considered to be an effective and economical method for dental caries primary prevention, so it has been integrated in oral health community programs (10, 13-15). However, a study developed by Martin *et al.* (2013) concludes that dentists may be underusing fissure sealants, even when knowing that a fissure sealant is effective and has positive effects on the prevention of oral diseases (16).

The aim of this study was to assess the prevalence of dental caries and the DMFT index, as well as the distribution pattern of pit and fissure sealants on permanent teeth (molars and pre-molars) in a Portuguese sample of adolescents, and to assess whether the existing usage of sealants and sociodemographic factors are correlated to caries prevalence on the examined sample.

3.3. Materials and Methods

A non-probabilistic convenience sampling of 447 adolescents aged between 12 and 18 years old, attending a public school in Sátão, Portugal, was enrolled in this epidemiological observational cross-sectional study that was carried out from September to December of 2012. The studied sample represents the entire school group of the area, and 88.6% of its pupils were enrolled in the study. Questionnaires without information about gender and age were excluded from the study as well as the adolescents whose parents did not sign the informed consent before data collection.

A self-administered questionnaire with questions about sociodemographic variables, social and daily habits and oral health behaviours was filled out by adolescents in the classroom. Sociodemographic variables such as gender (male/female), age, school grade at the moment of the study, residence area (living in a village near the town of Sátão was considered rural area and living in the town of Sátão was considered urban area), parents' educational level (choosing the higher educational level between father and mother) and father's professional situation (employed/unemployed) were determined. Oral health behaviours were assessed by questioning adolescents about their ideas on their own oral health, the frequency of toothbrushing per day, time spent toothbrushing (in minutes), period of the day that each adolescent toothbrushed, daily use of dental floss, having a dental appointment in the last 12 months and the reason of the last dental appointment, if he or she had experienced at least one episode of dental pain during their lives, if adolescents were afraid of going to the dentist, if they knew what was a fissure sealant and if their dentist had ever applied fissure sealants during a dental appointment and finally if they frequently consumed sugary beverages or soft drinks.

Clinical examination of oral health status was carried out according to the WHO criteria to determine the prevalence of dental caries and the DMFT index (17). Teeth were clinically examined with dental instruments by using visual-tactile method with the use of a dental mirror and a probe for cavitation detection only (approved by the WHO for caries diagnosis) and took place in the classroom under standardized conditions recommended by the WHO (17). Cotton rolls and gauze were available to remove moisture and plaque when necessary. Data collection of the adolescent's oral health status was registered by only one trained calibrated dentist/researcher.

The recorded variables of the clinical examination were caries experience, using the DMFT as oral health indicator, which consists in the sum of teeth decayed, teeth missing due to dental caries and teeth filled for each analyzed adolescent. The presence of fissure sealants was also assessed by clinical examination and, if present, each tooth was classified considering the following classification:

- 1: Completely intact fissure sealant;
- 2: Infiltrated fissure sealant without dental caries;
- 3: Infiltrated fissure sealant with dental caries;
- 4: Partial fissure sealant, without dental caries;
- 5: Partial fissure sealant, with dental caries.

Data analysis was carried out using the *Statistical Package for Social Sciences* (SPSS 18.0 version). Prevalence was expressed in proportions and crude odds ratio (OR) with 95% confidence intervals (CI) was used to measure the strength of association between variables. Proportions were compared by the Chi-square test and continuous variables by the Kruskal-Wallis and Mann-Whitney tests. The significance level established the inferential statistics was 5% ($p < 0.05$). A multivariate analysis - logistic regression - was applied for analysis of the association between variables.

This research involving human data has been performed in accordance with the Declaration of Helsinki and was submitted and approved by the Ethics Committee of the Research Centre for Education, Technology and Health Studies of the Polytechnic Institute of Viseu, Portugal (CI&DETS).

The information collected by the questionnaires was provided voluntarily and confidentially. The anonymity of the information collected was guaranteed by telling adolescents not to sign their names or write down any other form of identification in any part of the questionnaire. Data collection taken into account only considered the answers given by adolescents whose parents signed an informed consent that explained the objectives of the study. After collection, questionnaires were numbered, stored and processed by computer. The results do not make reference to adolescents' names or contain any information that may identify any of the participants.

3.4. Results

The final sample was composed by 447 adolescents, 38.3% male and 61.7% female, all between the age of 12 and 18 years old, from a public school of Sátão, Portugal. When analyzing the parents' educational level, we can verify that 4.3% have parents that only attended school up to the 4th grade of the primary school, 53.5% attended school from the 5th to the 12th grade and 15.0% had access to a higher education degree after finishing the 12th grade. The analysis of the distribution of the sample by residence area indicates that the majority live in rural areas (65.3% vs 34.7%) (Table 3.1).

Table 3.1: Socio-demographic characterization of the studied sample of portuguese adolescents.

	Male		Female		Total	
	N	%	N	%	N	%
	171	38.3	276	61.7	447	100.0
Age						
12	31	18.2	27	9.8	58	13.0
13	29	17.0	38	13.8	67	15.0
14	18	10.5	34	12.3	52	11.6
15	28	16.4	27	9.8	55	12.3
16	16	9.4	34	12.3	50	11.2
17	16	9.4	29	10.5	45	10.1
18	33	12.3	87	24.3	120	26.6
Grade						
7	34	19.9	26	9.4	59	13.4
8	26	15.2	38	13.8	64	14.3
9	19	11.1	33	12.0	52	11.6
10	30	17.5	38	13.8	68	15.2
11	19	11.1	32	11.6	51	11.4
12	44	25.2	109	39.4	153	34.1
Parents' educational level						
≤ 4 grade	3	2.0	16	6.9	19	4.3
5-12 grade	105	61.4	134	48.6	239	53.5
> 12 grade	30	17.5	37	13.4	67	15.0
Without information	33	19.1	89	31.1	122	27.2
Residential area						
Rural	100	58.5	192	69.6	292	65.3
Urban	71	41.5	84	30.4	155	34.7

The DMFT index obtained in this study was 3.32 (2.92). We obtained a mean of 0.99 (1.72) for the decayed component of the index, 0.10 (0.40) for the missing component and 2.23 (2.37) for the filled component.

Out of the total sample, we verified by intra-oral examination that 61.0% of the adolescents did not present any dental caries at the moment of the observation, 92.6% did not lose any tooth due to dental caries and 66.5% had at least one filled tooth due to dental caries. Only 22.6% presented a DMFT=0, which indicates the prevalence of adolescents who never had dental caries.

When analysing the DMFT by participant, we verified that 22.6% present a DMFT=0, 34.2% DMFT between 1 and 3 and 43.2% higher than 4.

This study showed that females had a higher DMFT than males. But a lower prevalence of dental caries at the moment of data collection as 59.9% indicated that female adolescents were free of dental caries, while for the male fraction of the sample this value was 40.1%.

When associating the presence of dental caries according to the sample and sociodemographic variables we can verify that the prevalence of dental caries was lower among adolescents whose parents had a higher level of education ($>9^{\text{th}}$ grade, OR=0.42 CI95%=0.25-0.72) and among those who live in an urban area (urban, OR=0.69 CI95%=0.41-1.15), and higher among adolescents whose father was unemployed (unemployed, OR=1.16 CI95%=0.51-2.65), female gender (female, OR=1.54 CI95%=0.97-2.45) and older (>15 years, OR=1.87 CI95%=1.06-3.29). When considering a DMFT=0, we found significant statistical differences between parents' level of education ($\leq 4^{\text{th}}$ grade=26.3 vs 5^{th} - 12^{th} grade=18.8 vs $>12^{\text{th}}$ grade=43.3, $p=0.001$), gender (male=27.3 vs female=19.6, $p=0.04$) and age (≤ 15 years=27.1 vs >15 years=18.5, $p=0.02$) (Table 3.2).

Table 3.2: Prevalence of DMFT (per individual) and association with sociodemographic variables.

	DMFT=0		DMFT=1 to 3		DMFT ≥ 4		<i>p</i>
	N	%	N	%	N	%	
Parents' educational level							
$\leq 4^{\text{th}}$ grade	5	26.3	3	15.8	11	57.9	0.001
5-12 grade	45	18.8	89	37.2	105	43.9	
>12 grade	29	43.3	21	31.3	17	25.4	
Total	79	24.3	113	34.8	133	40.9	
Father's professional situation							
Employed	70	23.6	102	34.5	124	41.9	0.5
Unemployed	8	21.1	17	44.7	13	34.2	
Total	78	23.3	119	35.6	137	41.1	
Gender							
Male	44	27.3	52	32.3	65	40.4	0.04
Female	51	19.6	92	35.4	117	45.0	
Total	95	22.6	144	34.2	182	43.2	
Age							
≤ 15 years	62	27.1	88	38.4	79	34.5	0.02
> 15 years	32	18.5	51	29.5	90	52.0	
Total	94	23.4	139	34.6	169	42.0	
Residential area							
Rural	56	19.9	97	34.5	128	45.6	0.1
Urban	39	27.9	47	33.6	54	38.6	
Total	95	22.6	144	34.2	182	43.2	

When associating the presence of dental caries and oral health behaviours, dental pain and fissure sealant application, it is possible to verify that the prevalence of dental caries was lower among adolescents who toothbrushed more frequently (\geq twice a day, OR=0.89 CI95%=0.46-1.71) and those to whom fissure sealants had been applied (yes, OR=0.35 CI95%=0.19-0.65). The prevalence of dental caries was higher among adolescents who had experienced at least one episode of toothache during their lives (yes, OR=1.68 CI95%=0.98-2.84). When considering a DMFT=0, we found significant statistical differences

between adolescents with and without fissure sealants applied to their teeth during intraoral examination (yes=30.6 vs no=13.5, $p=0.001$) and when associated with the variable experience of dental pain (no=25.4 vs yes=16.8, $p=0.02$) (Table 3.3).

Table 3.3: Prevalence of DMFT (per individual) and association with oral health behaviours, experience of dental pain and fissure sealant application.

	DMFT=0		DMFT=1 to 3		DMFT ≥4		<i>p</i>
	N	%	N	%	N	%	
Toothbrushing							
< 2 times/day	11	28.9	9	23.7	18	47.4	0.3
≥ 2 times/day	83	22.1	133	35.4	160	42.6	
Total	94	22.7	142	34.3	178	43.0	
Daily dental floss							
No	87	21.9	135	34.0	175	44.1	0.3
Yes	8	36.4	8	36.4	6	27.3	
Total	95	22.7	143	34.1	181	43.2	
Dental appointment (last 12 months)							
No	38	27.3	39	28.1	62	44.6	0.1
Yes	57	20.5	102	36.7	119	42.8	
Total	95	22.8	141	33.8	181	43.4	
Fear of dental appointment							
No	70	24.1	105	36.2	115	39.7	0.06
Yes	8	16.3	14	28.6	27	55.1	
Total	78	23.0	119	35.1	142	41.9	
Experience of dental pain							
No	72	25.4	99	34.9	113	39.8	0.02
Yes	23	16.8	45	32.8	69	50.4	
Total	95	22.6	144	34.2	182	43.2	
Fissure sealants							
No	17	13.5	37	29.4	72	57.1	0.001
Yes	55	30.6	69	38.3	56	31.1	
Total	72	23.5	106	34.6	128	41.9	

When questioning adolescents if they knew what a fissure sealant was, only 28.9% referred knowing what fissure sealants were and only 15.5% knew if their dentist had applied any on them in a past dental appointment.

When analyzing the prevalence of fissure sealants, we verified that 58.8% of adolescents had at least one fissure sealant applied. As shown in Table 3.4, from our total sample, we observed 830 teeth sealed, in which 63.3% were completely intact, 11.3% infiltrated but still without dental caries, 1.6% infiltrated but

with the presence of dental caries, 23.5% with a partial fissure sealant but without dental caries and only 0.3% with a partial fissure sealant with dental caries present on the tooth surface.

Table 3.4: Assessment of the integrity of fissure sealants.

	N	%
Completely intact fissure sealant	526	63.3
Infiltrated fissure sealant without dental caries	94	11.3
Infiltrated fissure sealant with dental caries	13	1.6
Partial fissure sealant, without dental caries	195	23.5
Partial fissure sealant, with dental caries	3	0.3
Total	830	100.0

Through the analysis of the distribution of fissure sealants in different teeth groups we could verify that from the 830 identified fissure sealants, 20.0% were applied on the upper molars, 27.7% on the upper premolars, 27.6% on the lower molars and 24.7% on the lower premolars. These results lead us to conclude that there is an equal distribution of fissure sealants among teeth (Table 3.5).

Table 3.5: Assessment of the distribution of fissure sealants by teeth groups.

	N	%
1 st and 2 nd upper molars	166	20.0
1 st and 2 nd upper premolars	230	27.7
1 st and 2 nd lower molars	229	27.6
1 st and 2 nd lower premolars	205	24.7
Total	830	100.0

When associating the presence of fissure sealants with sociodemographic variables, experience of dental pain and presence of dental caries, we can verify that the prevalence of fissure sealants was higher among adolescents whose parents had a higher level of education (>9th grade, OR=1.56 CI95%=1.05-2.54), females (female, OR=1.86 CI95%=1.19-2.98), older adolescents (>15 years, OR=1.44 CI95%=0.82-2.53) and adolescents living in an urban area (urban, OR=1.44 CI95%=0.84-2.49). The prevalence of fissure sealants was lower among adolescents whose father was unemployed when the data collection took place (unemployed, OR=0.94 CI95%=0.43-2.09), that had experienced dental pain (yes, OR=0.35 CI95%=0.19-0.65) and with dental caries (yes, OR=0.35 CI95%=0.19-0.65). Significant statistical differences were found when analyzing the presence of fissure sealants related with parents' educational level, gender, experience of dental pain and presence of dental caries.

After adjustment by non-conditional logistic regression for sociodemographic variables, oral health behaviors (toothbrushing, daily use of dental floss and dental appointments) and experience of dental pain, the findings of the present study demonstrate that dental caries is associated with fissure sealant application (OR=0.09 95%CI=0.03-0.3).

3.5. Discussion

In 2008, the General Health Directory of Portugal presented the last nationwide study of the prevalence of oral diseases. In that study, the DMFT index among adolescents aged 12 and 13 years was 1.48 and 3.04 respectively. The prevalence of dental caries increased with age and was also higher among the poorer socioeconomic classes (7).

In this study we obtained a moderate level of prevalence of dental caries, with a higher number of filled teeth in comparison with the decayed and missing component of the DMFT index applied. This situation indicates that most of the adolescents have had, during their lives, dental appointments for dental caries treatment. These results demonstrate the improvement in oral healthcare registered in Portugal in the last years in which children and adolescents have easier access to dental appointments. However, the oral health indicators show the need of continuous improvement mainly towards primary prevention, specifically better oral hygiene habits (6, 7).

This can be obtained if oral health education initiatives are introduced in school programs, so that students and their families will be able to understand the importance of adequate oral hygiene habits, a balanced diet with less sugary food intake, beverage consumption and regular dental appointments. Another study developed by Barata *et al.* (2013) in another city of the central region of Portugal, obtained a DMFT index of 4.05 in adolescents with ages between 12 and 19. These results indicate the need of improvement in oral health behaviours among adolescents of this specific region of the country (18). In Spain, a survey developed by Almerich Silla *et al.* (2006) indicates a caries prevalence of 55.9% among 15-16 year olds, but a DMFT index of 1.84. In this case, we can verify a higher caries prevalence, but a lower DMFT index when compared with the results obtained in this study (19).

The prevalence of dental caries was highly associated with sociodemographic factors such as parents' educational level. This association has been identified in several studies in which a lower socioeconomic status is not only associated with a higher risk of oral disease development, but also with worse oral health behaviours (20-22).

Dental pain was also highly associated with higher levels of DMFT, which is also identified in various studies, and demonstrates that many adolescents have a dental appointment only in an emergency situation and maintain untreated dental caries (23).

This study also showed that females had a higher DMFT than males, but a lower prevalence of dental caries at the moment of data collection. This demonstrates the higher care that female adolescents have on their oral health when compared with males. The higher rate of oral health care among females can also be justified by the existence of a higher prevalence of fissure sealant application among the females in comparison with males (24, 25). The fear of dental appointment was associated with lower application of fissure sealants, which can be explained by the lower frequency of routine check-up dental appointments. Studies show that adolescents and children that do not regularly visit a dentist do have a higher risk of dental caries development, failing, thus, the application of primary preventive measures that could prevent the appearance and development of dental caries and other oral diseases (26, 27).

The application of fissure sealants is associated with the higher risk of oral disease development and higher levels of DMFT. This can be explained by the fact that adolescents do not have proper access to

adequate primary prevention methods. The correlation between dental caries and fissure sealants may be explained by the fact that adolescents with more dental caries and less fissure sealants are those that do not have regular dental appointments and do not benefit from the Portuguese oral health program that also includes the application of fissure sealants in permanent molars and premolars as a primary prevention method (28). A study developed by Oulis *et al.* (2011) revealed that only 8.0% of Greek adolescents had at least one sealed molar, and the presence of fissure sealants was associated with a lower risk of dental caries prevalence (29).

The application of fissure sealants is also associated in this study with parents' educational level which is the main variable that permits the establishment of a significant statistical association between the application of primary prevention methods and socioeconomic status. Therefore, economic variables appear to be one of the reasons for the absence of fissure sealants among adolescents (16, 30, 31).

When analysing the application of fissure sealants, we verify that less than one third of the adolescents know what a fissure sealant is. Several adolescents refer not knowing what a fissure sealant is, but do have fissure sealants applied to their own teeth. This can be justified by the fact that some adolescents do not understand what is being applied to their teeth during a dental appointment. Moreover, dentists do not always explain the treatment that is being done and the importance of the application of fissure sealants accurately. This reveals a low knowledge level of the definition and importance of primary prevention among adolescents and their families (32). It is important to refer that 37.7% of the observed fissure sealants were not completely intact and were at risk of developing dental caries. Studies conclude that an infiltrated or a partial fissure sealant, when not detected early, can permit the development of dental caries under the reminescent fissure sealant (33, 34). This may occur because the majority of adolescents do not visit a dentist at least twice a year in order to reassess applied fissure sealants (35).

Studies conducted by Griffin *et al.* (2010) analyzed dental caries risk in teeth with partially or totally lost sealants when compared to those that have never been sealed. The authors concluded that sealed teeth (either with completely or partially lost sealants) showed no greater risk of developing dental caries, when compared to those which have never been sealed. These results were conflicting and suggest a heightened concern because partially lost sealants may retain food debris and increase the risk of dental caries development (36).

When considering fissure sealants, the earlier the application, the more effective they are. Therefore, in children fissure sealants are recommended to be applied soon after tooth eruption, mainly at the level of the first permanent molars (8, 37). Studies have shown that fissure sealants applied both in clinics and in schools, are highly effective in preventing dental caries, reducing caries in pits and fissures up to 60% for 2 to 5 years after its implementation (38). A reassessment of fissure sealants should be held annually, not exceeding 12 months between dental appointments, for children and adolescents at high risk of developing dental caries. However, it is appropriate to reassess and reapply the fissure sealant within 6 months, in the particular cases of patients with high risk of developing dental caries and with insufficient oral health behaviors (39).

In this specific population, the application of fissure sealants is an important complement of primary prevention because in Portugal there is no water fluoridation and the application of fluoride gel and

varnish is not a common primary prevention treatment applied in dental medicine practice nowadays. This situation is verified in both urban and rural areas. However, previous studies have proven the use of fluoride toothpaste during daily oral hygiene by children and adolescents (6, 7).

In order to increase the knowledge of oral health behaviors and the application of primary prevention methods, more studies should be developed in Portugal nationwide to increase sample size and to better understand the reality of the Portuguese population. It is important to understand the determinants and distribution of oral diseases, but it is also important to identify the population with worse oral health behaviors in order to aim oral health education programmes towards the most problematic communities.

The application of primary preventive methods, namely fissure sealant application, in the dental appointment, complemented with oral health education, will certainly decrease the financial impact of oral treatments in the population and will guarantee a decrease in the risk of oral disease development during adulthood.

3.6. Conclusions

The moderate level of caries prevalence devised in this study reveals the need of improvement as far primary prevention interventions among Portuguese adolescents are concerned. Oral health has improved in the last years in Portugal, especially among children and adults. However, the establishment of a more targeted preventive program with better and more effective oral health education is essential, bearing in mind sociodemographic aspects, with a special focus on adolescents and families with a lower socioeconomic status. The application of fissure sealants should be complemented with oral health education, in order for children, adolescents and their families to assimilate adequate oral hygiene habits and understand the need of regular dental appointments for primary prevention and early diagnosis of oral diseases.

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3.9. Competing interests

The authors have declared that no competing interests exist.

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Chapter 4

Oral and gastric *Helicobacter Pylori*: effects and associations

Paper II

Oral and gastric *Helicobacter Pylori*: effects and associations.

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4.1. Abstract

Introduction: This study consisted in the comparison of the prevalence of *Helicobacter pylori* (*H. pylori*) present in the stomach and in saliva of a sample of Portuguese adolescents and the assessment of the association between *H. pylori* infection with socio-demographic variables and prevalence of dental caries.

Materials and Methods: A cross-sectional study was designed including a sample of 447 adolescents aged 12 to 18 years old, attending a public school in Sátão, Portugal. A questionnaire about socio-demographic variables and oral health behaviors was applied. Gastric *H. pylori* infection was determined using the urease breath test (UBT). Saliva collection was obtained and DNA was extracted by Polymerase Chain Reaction (PCR) in order to detect the presence of oral *H. pylori*.

Results: The prevalence of gastric *H. pylori* detected by UBT was 35.9%. Within the adolescents with a gastric UBT positive, only 1.9% were positive for oral *H. pylori*. The presence of gastric *H. pylori* was found to be associated with age (>15years, *Odds ratio* (OR)=1.64 95%CI=1.08-2.52), residence area (urban, OR=1.48 95%CI=1.03-2.29) and parents' professional situation (unemployed, OR=1.22 95%CI=1.02-1.23). Among those with detected dental caries during the intra-oral observation, 37.4% were positive for gastric *H. pylori* and 40.2% negative for the same bacterial strain (p=0.3).

Conclusions: The oral cavity cannot be considered a *reservoir* for infection of *H. pylori*. Gastric *H. pylori* infection was found to be associated with socio-demographic variables such as age, residence area and socioeconomic status.

Keywords: Dental caries, *H. pylori*, infection, Polymerase Chain Reaction, Urease Breath Test.

4.2. Introduction

An important bacterium species that may have an important pathobiological role, especially in the gastric mucosa, is *Helicobacter pylori* (*H. pylori*) – a gram-negative, microaerophilic, rod-shaped bacterium that colonizes the human stomach (1,2). It resides beneath the gastric mucous layer, adjacent to the gastric epithelial cells, and, although it is not invasive, it can give rise to chronic inflammation of the gastric mucosa (3). Infection with this organism is now recognized as a serious, transmissible infectious disease, highly associated with the development of chronic and atrophic gastritis, duodenal ulcers, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric carcinoma (4). The recognition of the pathological aspects of *H. pylori* infection in the upper gastro-intestinal tract, was firstly originated from a series of studies performed by Marshal and Warren in 1982 (3).

Various studies demonstrate that approximately 50% of the world's population may be infected, with developed countries showing a lower prevalence of *H. pylori* infection at all ages when compared with developing countries. Notably, the difference in the infection pattern is especially noticeable among younger people. The fecal-oral tract is believed to be a common route of transmission of *H. pylori*, so demographic areas with reduced levels of sanitation and low socioeconomic status are associated with an increased prevalence of *H. pylori* infection (5,6).

A study developed in 2012 by Almeida *et al.* in Portugal, revealed a prevalence of 40.6% of *H. pylori* infection among portuguese schoolchildren between 11 and 18 years old, but with a tendency to decrease in the following years (7). Relevantly, the worldwide infection by *H. pylori* has been decreasing due to better sanitary and socioeconomic conditions, and the same should happen in Portugal.

It has long been speculated that dental plaque might harbour *H. pylori*, and, by that reason, it can be a source of re-infection of the gastric mucosa. Additionally, the presence of the bacteria in the oral cavity was suggested to be associated with a higher risk of dental caries development (8-10). Some studies have also shown that the presence of *H. pylori* in the periodontium may be one of the main causes of periodontal infection (11-14). Other studies demonstrate the association between inadequate oral hygiene habits and the presence of oral *H. pylori* in the dental plaque (13-16). Further, some studies indicate that the presence of *H. pylori* in the oral cavity can be associated with gastro-esophageal infection, suggesting the mouth as the first extra-gastric *reservoir* for *H. pylori* (12,17). Therefore, the failure to eliminate *H. pylori* present in the oral cavity can lead to gastrointestinal re-infection (17).

Moreover, other authors suggest that *H. pylori* may belong to the normal oral flora of the human oral cavity, maintaining a commensal relation with the host, but present in very low numbers such that reliable identification is difficult (18,19). The difficulty in establishing a connection between the role of *H. pylori* in the oral cavity and gastric infection remains controversial, since the detection rate of the bacterium in the mouth is very diverse, ranging between 0% and 100% (4).

The two main aims of our work were: (i) to explore the hypothesis if the oral cavity may be considered a potential *reservoir* for *H. pylori* – to assess this we performed the detection of gastric *H. pylori* through Urease Breath Test (UBT) followed by the detection of oral *H. pylori* among those with positive UBT by

Polymerase Chain Reaction (PCR) in a sample of Portuguese adolescents; (ii) to determine the association between *H. pylori* infection and socio-demographic variables, and the prevalence of dental caries.

4.3. Materials and Methods

Material collection

A non-probabilistic convenience sample of 447 adolescents aged between 12 and 18 years old, attending a public school in Sátão, Portugal, was enrolled in this study. All samples were obtained from September to December of 2012. Questionnaires without information about gender and age were excluded of the study as well as the adolescents whose parents did not sign the informed consent before data collection.

A self-administered questionnaire focusing socio-demographic variables, social and daily habits and oral health behaviors was filled out by all participants in this study. Questions about socio-demographic variables such as gender (male/female), age, school grade at the moment of the study, residence area (urban/rural), parents' educational level (choosing the higher educational level between father and mother), parents' professional situation (employed/unemployed) and the number of rooms and people living in the house were used to determine the crowding index.

This research has been performed in accordance with the Declaration of Helsinki and was submitted and approved by the Ethics Committee of the Health School and Research Centre for Education, Technology and Health Studies of the Polytechnic Institute of Viseu, Portugal (CI&DETS). The information collected by the questionnaires was provided voluntarily and confidentially, guaranteeing, anonymity of the information collected by telling the adolescents not to sign their names or write down any other form of identification in any part of the questionnaire. Data collection was only made on adolescents from whom we obtained written informed consent from the next of kin, caretakers, or guardians on behalf of the minors that were enrolled in the present study. After collection, the questionnaires were numbered, stored and processed by computer. The results do not refer to nominal adolescents or contain any information that may identify any of the participants.

Clinical sample characterization

Clinical examination of oral health status was carried out according to the World Health Organization (WHO) criteria (20). The teeth were clinically examined with dental instruments using visual-tactile methods with the use of a dental mirror and a probe (approved by the WHO for caries diagnosis) and took place in the classroom under standardized conditions recommended by the WHO. Cotton rolls and gauze were available to remove moisture and plaque when necessary. There was only one observer that registered the results of each clinical observation during the study. The recorded variables of the clinical examination were caries experience, using the decayed, missing and filled permanent tooth index (DMFT) as an oral health indicator, which consists in the sum of teeth decayed, teeth missing due to dental caries and teeth filled for each analyzed adolescent. Each tooth would be classified with only one

of the following codes: 0 - sound crown or root, showing no evidence of either treated or untreated caries; 1 - indicates a tooth with caries; 2 - filled teeth, with additional decay; 3 - filled tooth with no decay; 4 - tooth that is missing as a result of caries; 5 - a permanent tooth missing for any other reason than decay; 6 - teeth on which sealants have been placed; 7 - indicate that the tooth is part of a fixed bridge; 8 - this code is used for a space with an unerupted permanent tooth where no primary tooth is present; 9 - erupted teeth that cannot be examined; T - indicates trauma in the presence of a fractured crown.

***H. pylori* detection**

From the total sample of 447 adolescents, 437 were screened for gastric *H. pylori* infection using the UBT that consists in the exhalation of carbon dioxide in samples before and after swallowing urea labeled with non-radioactive carbon-13. The samples were then analyzed and each result would be classified as positive or negative for *H. pylori* infection.

To detect *H. pylori* on oral cavity, saliva was collected by the passive drool method into a polypropylene tube until reaching 2 milliliters of saliva in each tube per adolescent. Next, DNA was extracted using the MagNA Pure LC DNA Isolation Kit (Bacteria, Fungi) (Roche), quantified with Nanodrop (Thermo Lifesciences), and bacterial DNA was amplified using the Multiplex PCR kit (Qiagen) with primers that recognize all *H. pylori* strains: VacA_Fw: ATGGAAATACAACAAACACAC and VacA_Rv: CTGCTTGAATGCGCCAAAC(21). PCR products were observed, after electrophoresis in a 2% agarose gel stained with RedSafe (Intron), in a UV chamber (Bio-Rad). DNA bands with the expected molecular weight were excised from the agarose gel, purified with ULTRAPrep Agarose Gel Extraction kits (AHN), and sequenced with BigDye Terminator Sequencing Kit (Applied Biosystems). The obtained DNA sequences were compared with a control (*H. pylori* positive DNA sample diluted in saliva at different concentrations) and with NCBI database (<http://www.ncbi.nlm.nih.gov/>).

Statistical analysis

Data analysis was carried out using the *Statistical Package for Social Sciences* (SPSS 18.0 version). Prevalence was expressed in proportions and crude odds ratio (OR) with 95% confidence intervals (CI) were used to measure the strength of association between variables. Proportions were compared by the Chi-square test. The significance level established the inferential statistics was 5% ($p < 0.05$).

4.4. Results

The sample used in this study was composed by 447 adolescents, 38.3% male gender and 61.7% female gender, with ages between 12 and 18 years old, from a public school of Sátão, Portugal. When analyzing the parents' educational level, we could verify that 4.3% of participants have parents that only frequented school for less than the 4th grade, 53.5% stayed in school from the 5th to the 12th grade and 15.0% went to a superior degree after finishing the 12th grade. Crowding index < 1.0 is presented among 71.4% of adolescents, while 14.1% are equal to 1 and only 4.5% > 1.0 , which indicates possible overcrowding at

home. Performing the analysis of the distribution of the sample by residential area, we could observe that the majority of participants live in rural areas (65.3% vs 34.7%) (Table 4.1).

Table 4.1: Socio-demographic characterization of the studied sample of portuguese adolescents.

	Male		Female		Total	
	N	%	N	%	N	%
	171	38.3	276	61.7	447	100.0
Age						
12	31	18.2	27	9.8	58	13.0
13	29	17.0	38	13.8	67	15.0
14	18	10.5	34	12.3	52	11.6
15	28	16.4	27	9.8	55	12.3
16	16	9.4	34	12.3	50	11.2
17	16	9.4	29	10.5	45	10.1
18	33	12.3	87	24.3	120	26.6
Grade						
7	34	19.9	26	9.4	59	13.4
8	26	15.2	38	13.8	64	14.3
9	19	11.1	33	12.0	52	11.6
10	30	17.5	38	13.8	68	15.2
11	19	11.1	32	11.6	51	11.4
12	44	25.2	109	39.4	153	34.1
Parents' educational level						
≤ 4 grade	3	2.0	16	6.9	19	4.3
5-12 grade	105	61.4	134	48.6	239	53.5
> 12 grade	30	17.5	37	13.4	67	15.0
Without information	33	19.1	89	31.1	122	27.2
Residential area						
Rural	100	58.5	192	69.6	292	65.3
Urban	71	41.5	84	30.4	155	34.7

Furthermore, the prevalence of gastric *H. pylori* detected by the UBT was 35.9% (157 positive individuals from a total of 437).

The presence of gastric *H. pylori* was associated with age, residence area and parents' professional situation, registering a higher prevalence among older adolescents (OR=1.64 95%CI=1.08-2.52), those who live in urban areas (OR=1.48 95%CI=1.03-2.29) and among the adolescents whose parents are unemployed at the moment of data collection (OR=1.22 95%CI=1.02-1.23) (Table 4.2).

Table 4.2: Association between the presence of gastric *H. pylori* (detected=positive test; non-detected=negative test) and socio-demographic variables.

	Positive test		Negative test		P
	N	%	N	%	
Gender					
Male	57	34.8	107	65.2	
Female	100	36.6	173	63.4	0.4
Age					
≤ 15 years	72	34.1	139	65.9	
> 15 years	67	45.9	79	54.1	0.02
Residential area					
Rural	79	35.4	144	64.6	
Urban	60	44.8	74	55.2	0.04
Parents' educational level					
≤9 th grade	90	38.5	144	61.5	
>9 th grade	53	36.1	94	63.9	0.4
Parents' professional situation					
Employed	89	36.6	154	63.4	
Unemployed	43	41.3	61	58.7	0.04

There is also no correlation between the presence of gastric *H. pylori* and the prevalence of dental caries. The results of the present study demonstrated that, among adolescents positive for gastric *H. pylori*, 62.6% were caries-free, while 59.8% of the adolescents negative for gastric *H. pylori* did not present any dental carie. Among those with detected dental caries during the intra-oral observation, 37.4% were positive for gastric *H. pylori* and 40.2% negative for the same bacteria in study (p=0.3).

In order to detect the presence of *H. pylori* in the oral cavity, we performed PCR of the DNA extracted from saliva of positive UBT participants. Interestingly, from the individuals with positive UBT, indicating gastric *H. pylori* infection, only 1.9% were positive for oral *H. pylori* (Figure 4.1). Additionally, and since we only detected the presence of a very-low percentage of oral positive-*H. pylori* from the DNA extracted from UBT positive cases, we performed a PCR experiment to validate the sensibility of our PCR results. Noteworthy, the primers that were used in this work are highly specific for *H. pylori*, since they recognize the *VacA* gene that is present in all strains of this bacteria (21). To do this, we diluted 50ng of DNA extracted from a cultured *H. pylori* (strain 7354) in two independent saliva samples that were previously shown to be negative for *H. pylori*. Relevantly, this approach has proven also to be useful to decipher if the non-amplification of previous PCR in this two negative cases was caused by the possible presence of chemical inhibitors in the saliva of those two individuals. Then, we performed a successive series of dilutions in saliva of those individuals, ranging from 1:10 to 1:100000, followed by the PCR with the *VacA* primers. As shown (Figure 4.2), it was possible to amplify the

specific *VacA*-amplicon from undiluted samples to the most diluted ones (in this case, a *H. pylori* DNA with a concentration of 0.5 µg). Thus, our results indicate that the employed PCR approach to identify *H. pylori* proven to be highly sensitive, and the existence of possible PCR inhibitors in saliva samples, if they exist, played an insignificant role in the amplification process. Finally, we confirmed the identity of the PCR product by nucleotide sequencing.

Figure 4.1: *H. pylori*- specific PCR for DNA extracted from oral cavity. In this figure, one can see the amplification of *VacA* in the three cases previously identified as oral cavity positives for *H. pylori* (Hp positive sample #1 to #3), in comparison with other three cases that are oral cavity *H. pylori* negatives (Hp negative sample #1 to #3). As a positive control (Hp positive control) PCR for DNA extracted from *H. pylori* (strain 7354) diluted in saliva was used. Blank – PCR negative control.

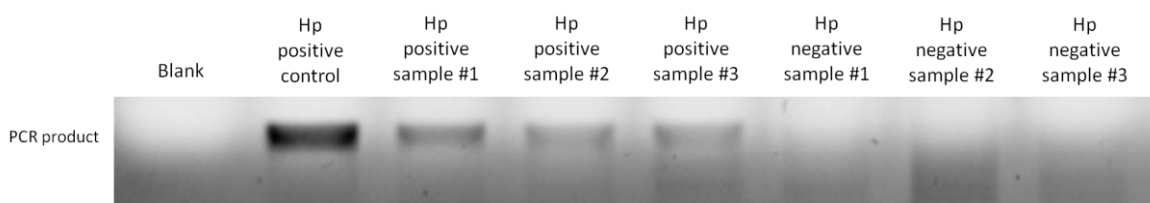
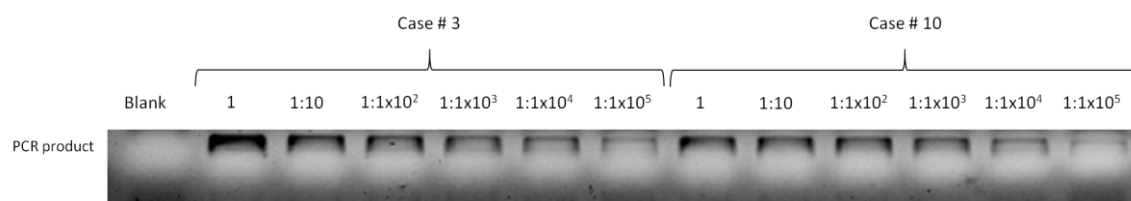


Figure 4.2: Highly sensitive PCR for detection of *H. pylori*. In order to evaluate the sensitivity of *VacA*-specific PCR, 50ng of DNA from *H. pylori* was successively diluted (1 to 1:100000) in saliva from two random cases (#3 and #10) that were shown previously to be negative for the presence of *H. pylori*. The PCR allowed the amplification of the expected product for all different dilutions.



4.5. Discussion

When analyzing the prevalence of gastric *H. pylori*, the portuguese adolescents included in the present study showed a higher prevalence of infection when compared with other studies, namely by Mana *et al.* (11.0%) in Belgium and Sousa *et al.* (24.9%) in Brazil, but on the other hand registered a lower prevalence when compared with studies developed by Constanza *et al.* (47.6%) in Mexico (22-24). The results of the present study demonstrated that the adolescents that live in urban areas and with worse socioeconomic status have a higher prevalence of gastric *H. pylori* infection, which may be related with poorer living conditions, lack of hygiene education and running water contamination (5,25). The prevalence of *H. pylori* was also higher among older adolescents, which is also verified in the study

developed by Miranda *et al.* and Kodaira *et al.* (26,27).

Our results reveal a very low prevalence of *H. pylori* in the oral/salivary niche, which leaves the question if the young age of the sample studied can justify such a low prevalence of *H. pylori* present in the oral cavity. One possible explanation may be the fact that the presence of the bacterium in the oral cavity might only happen after a certain period of time after the appearance in the stomach. The direct relation between oral/salivary *H. pylori* and age is not well established and must be studied in future research.

The prevalence of oral/salivary *H. pylori* verified in the present study was lower than the results obtained in studies developed by Fernando *et al.* (27.7%) and Wichelhaus *et al.* (82.0%) (28,29). The differences observed in the prevalence of oral *H. pylori* may be justified by the diverse methodologies/techniques employed in the different studies to detect the bacteria (30). Another possible justification relay on the fact that the adolescents involved in this study do not have gastric pathology diagnosed nor they have symptoms compatible with *H. pylori* infection, which contrasts with some of the previous studies that use adults as study targets, and that present a clear diagnosis of gastric pathology associated with *H. pylori* infection (4,10, 31-33). Also, in the present study, the detection of oral *H. pylori* was accomplished by the direct amplification of a portion of a specific gene of the bacteria: *VacA*. All the tests that identified the specific gene *VacA* were considered positive for the presence of *H. pylori* in the oral cavity. What we need to understand is that *H. pylori* is not the only microorganism that produces urea (13,14). Importantly, when identifying the presence of oral *H. pylori* through the amplification of the urease gene by PCR there is a high risk of detecting other microorganisms, such as *Streptococcus salivarius* (34). So, the amplification of the urease gene to identify *H. pylori* in the oral cavity may overvalue the true prevalence of this bacteria in that niche. Moreover, this fact can justify the differences of prevalence of *H. pylori* in the oral cavity when comparing the present results with those presented in other studies (10,13, 35, 36). Therefore, the low prevalence of *H. pylori* in the oral cavity of the present study is justified by the highly specific methodology applied characterized by the identification of the *VacA* specific for *H. pylori* and identified by PCR.

Medina *et al.*, referred that there may be differences in results when analyzing the presence of *H. pylori* in dental plaque and saliva (37). Another reason that could underlie the infrequent detection of *H. pylori* on DNA samples derived from saliva can reside in low amount of that bacteria in that particular niche, which will hamper the reliable identification, as stated previously (18,19). Nevertheless, and corroborating our results regarding clinic and pathological features, Burgers *et al.* verified that the occurrence of *H. pylori* in the oral cavity was not found to be correlated neither to any general or oral health parameters.[9], an observation that was also verified in our study. Kabir suggested that the detection rate in saliva was lower than that observed in feces, making saliva a less suitable specimen for the diagnosis of *H. pylori* infection (38).

The results obtained in the present study demonstrate a lack of significant correlation between the presence of oral and gastric *H. pylori*, since only three cases of 157 participants with UBT positive test for gastric *H. pylori* were also positive for oral/salivary *H. pylori*. Therefore, at least among adolescents

the oral cavity may not be considered a *reservoir* for *H. pylori* in individuals with gastric infection (6,39). Interestingly, some authors suggested that *H. pylori* is not consistently present in the oral cavity environment, but is only transiently present due to contaminated ingested food or because of the uprising of the bacteria due to gastroesophageal reflux (2,30).

An important issue that still needs to be well studied and determined is the influence of the putative oral *H. pylori* presence on dental caries development and oral health status. The results of this study also demonstrated a lack of association between *H. pylori* infection and the prevalence of dental caries. Namiot *et al.*, referred that the occurrence of *H. pylori* antigens in dental plaque of natural teeth is not associated with oral health status or dental plaque removal practices from both natural teeth and removable dentures (30). Berroteran *et al.*, reported a lack of correlation between *H. pylori* infection and dental hygiene, dental caries, periodontal disease or use of dentures (40). The diversity of results of various studies established the necessity of developing more studies in order to understand the role and consequences of the presence of *H. pylori* in the oral cavity, mainly among adolescents. A review article developed by Al-Sayed *et al.*, confirmed the need of more epidemiological studies in order to determine the real quantification of *H. Pylori* in the oral cavity and understand its importance in human health (41).

4.6. Conclusions

Considering the results of the present study, we can conclude that the prevalence of oral *H. pylori* is very low, even among those with a positive test for gastric *H. pylori*, which reveals that, in this specific sample composed by adolescents, the oral cavity cannot be considered a *reservoir* for infection and re-infection of *H. pylori*, and cannot be considered for diagnosis of gastric *H. pylori*. The present study reveals that there is no significant correlation between the occurrence of *H. pylori* infection in the stomach and the oral health of adolescents not verifying any association with the presence of dental caries. However, the presence of the gastric *H. pylori* infection may be correlated with socio-demographic variables such as age, residence area and socioeconomic status. Nevertheless, it is urgent to perform more studies in samples from different age-clusters, and not only in adolescents, in order to understand the possible biological role of *H. pylori* in the oral cavity.

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4.9. Competing interests

The authors have declared that no competing interests exist.

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Chapter 5

**Prevalence and determinants of oral microflora
among portuguese adolescents**

Paper III

Prevalence and determinants of oral microflora among portuguese adolescents

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5.1. Abstract

Introduction: The aim of the present study was to determine the prevalence and determinants of salivary *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and the associated risk of development of dental pathologies on a sample of portuguese adolescents.

Materials and Methods: A cross-sectional study was designed including a final sample of 447 adolescents aged between 12 and 18 years old, attending a public school in Sátão, Portugal. A self-administered questionnaire was filled out by the adolescents. Clinical examination of oral health status was carried out and saliva collection was accomplished by the passive drool method. The identification of the different types of bacterial strains was accomplished using the Polymerase Chain Reaction technique.

Results: The prevalence of *S. mutans* in the sample studied was 80.8%, *Lactobacillus* 99.5%, and *A. actinomycetemcomitans* only 15.2%. The presence of *S. mutans* was associated with gender (male=76.1% vs female=83.6%, $p=0.04$) and dental pain in the presence of severe dental caries (77.3% vs 87.8%, $p=0.006$). The infection with *A. actinomycetemcomitans* was associated with age (<15yrs=12.3% vs ≥ 15 yrs=20.3%, $p=0.03$) and residence area (rural=18.2% vs urban=11.0%, $p=0.04$), and may be related with a higher risk of periodontal disease development in adulthood.

Conclusions: *A. actinomycetemcomitans* infection was found to be associated with socio-demographic variables, suggesting that, if not clinically well identified and treated, may cause serious oral diseases during adulthood. It has been described that the oral microflora is one of the main etiological factors for dental caries and periodontal diseases development, but cannot be considered in an isolated manner.

Keywords: *A. actinomycetemcomitans*, *Lactobacillus*, oral health, oral microflora, Polymerase Chain Reaction, *S. mutans*.

5.2. Introduction

The oral cavity is inhabited by different bacterial species that play vital roles in maintaining oral health or in shifting to a diseased state such as dental caries and periodontal disease (1, 2). Dental caries consists in a post-eruptive bacterial infectious disease characterized by a progressive demineralization process that affects the mineralized dental tissues. It is considered the most prevalent oral disease and the main responsible for tooth loss among the population (3, 4). A carious lesion initiates with the production of organic acids by the microorganisms of the oral cavity that metabolize the extracellular carbohydrates of the individual's diet (5, 6). The presence of the organic acids produced will decrease the pH in the interface between the tooth surface and the bacterial plaque, which permits the development of the demineralization process on the tooth enamel (4). When the oral cavity has a pH below 5,5 (considered the critical pH), the saturation of the dental tissues initiates causing a initial lesion that will be the precursor of the dental carie (7). Prevention methods have the main goal of decreasing the time of exposure of the tooth tissues to the low values of pH. Therefore, it is strictly necessary the frequent removal of bacterial plaque to avoid the increased contact with tooth surfaces (8).

The researcher Paul Keyes developed a diagram that describes the multifactorial aetiology of dental caries. In this diagram, we can observe that there are three main etiological factors that are essential for the initiation and development of the disease: susceptible host; cariogenic oral microflora; substratum that depends on the host's diet, which is then metabolized by the microorganisms that constitutes that bacterial plaque (5).

Dental caries, as an infectious disease, correlates directly with bacterial strains that co-exist in the oral cavity, like *S. mutans* and *Lactobacillus* (9). The cariogenic properties of *S. mutans* and *Lactobacillus* are widely recognised and, as significant odontopathogens, the former group is linked to enamel lesion formation while the latter is associated with cavity progression (10). *A. actinomycetemcomitans* is one of the most well studied periodontal bacterial strains. It stays in the periodontal pocket of the oral cavity and damages tooth supporting tissues, being considered as the major cause of periodontitis (11). Kaplan and colleagues found that all *A. actinomycetemcomitans* strains possess strong virulence potential (12). A study developed by Hart *et al.*, indicated the main bacterial species or groups that could be implicated in caries onset and progression. Both *S. mutans* and *Lactobacillus* were reported in that study has been linked with caries onset and progression (13).

Aas *et al.*, reported a distinctive predominant bacterial flora of the healthy oral cavity that is highly diverse and subject specific. It is important to fully define the human microflora of the healthy oral cavity before we can understand the role of bacteria in oral disease (14). Furthermore, it has concurrently been clearly established that social, economic, cultural, ethnic, and environmental factors also play an important role in the formation of dental caries and also influences the individual oral microflora highly related with oral health behaviours (15).

The aim of the present study was, therefore, to investigate the prevalence of salivary *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and the influence on the risk of development of dental caries and the association with socio-demographic aspects among a sample of Portuguese adolescents.

5.3. Materials and Methods

Material collection

A sample of 447 adolescents aged between 12 and 18 years old, attending a public school in Sátão, Portugal, was enrolled in this study. All samples were obtained from September to December of 2012. Questionnaires without information about gender and age were excluded of the study as well as the adolescents whose parents did not sign the informed consent before data collection.

All participants in this study filled out a self-administered questionnaire focusing socio-demographic variables, social and daily habits and oral health behaviours. Questions about socio-demographic variables such as gender (male/female), age, school grade at the moment of the study, residence area (urban/rural), parents' educational level (choosing the higher educational level between father and mother), parents' professional situation (employed/unemployed) and the number of rooms and people living in the house were used to determine the crowding index.

This research has been performed in accordance with the Declaration of Helsinki and was submitted and approved by the Ethics Committee of the Health School and Research Centre for Education, Technology and Health Studies of the Polytechnic Institute of Viseu, Portugal (CI&DETS). The information collected by the questionnaires was provided voluntarily and confidentially, guaranteeing, anonymity of the information collected by telling the adolescents not to sign their names or write down any other form of identification in any part of the questionnaire. Data collection was made only for adolescents whose parents signed an informed consent that explained the objectives of the study. After collection, the questionnaires were numbered, stored and processed by computer. The results do not refer to nominal adolescents or contain any information that may identify any of the participants.

Clinical sample characterization

Clinical examination of oral health status was carried out according to the World Health Organization (WHO) criteria (16). The teeth were clinically examined with dental instruments using visual-tactile method with the use of a dental mirror and a probe (approved by the WHO for caries diagnosis) and took place in the classroom under standardized conditions recommended by the WHO. Cotton rolls and gauze were available to remove moisture and plaque when necessary. There was only one observer that registered the results of each clinical observation during the study. The recorded variables of the clinical examination were caries experience, using the decayed, missing and filled permanent tooth index (DMFT) as oral health indicator, which consists in the sum of teeth decayed, teeth missing due to dental caries and teeth filled for each analysed adolescent. Each tooth would be classified with only one of the following codes: 0: sound crown or root, showing no evidence of either treated or untreated caries; 1: indicates a tooth with caries; 2: filled teeth, with additional decay; 3: filled tooth with no decay; 4: tooth that is missing as a result of caries; 5: a permanent tooth missing for any other reason than decay; 6: teeth on which sealants have been placed; 7: indicate that the tooth is part of a fixed bridge; 8: this code is used for a space with an unerupted permanent tooth where no primary tooth is present; 9: erupted teeth that cannot be examined; T: indicates trauma in the presence of a fractured crown.

Bacterial strain identification

Saliva collection was accomplished by the passive drool method into a polypropylene tube until reaching 2 milliliters of saliva in each tube per adolescent. Next, DNA was extracted using the MagNA Pure LC DNA Isolation Kit (Bacteria, Fungi) (Roche), quantified with Nanodrop (Thermo Lifesciences), and bacterial DNA was amplified using the Multiplex Polymerase Chain Reaction (PCR) kit (Qiagen) with primers specific for the bacterial strains analysed. To validate primers specificity, DNA bands with the expected molecular from 3 positive-cases were excised from the agarose gel, purified with ULTRAPrep® Agarose Gel Extraction kits (AHN), and sequenced with BigDye Terminator Sequencing Kit (Applied Biosystems). The obtained DNA sequences were compared with a control and with NCBI database (<http://www.ncbi.nlm.nih.gov/>). Primers employed in this study are as described: *Lactobacillus* (GGAATCTTCCACAATGGACG and CGCTTTACGCCCAATAAATCCGG); *S. mutans* (TCGCGAAAAAGATAAACAACA and GCCCCTTCACAGTTGGTTAG); *A. actinomycetemcomitans* (AAACCCATCTCTGAGTTCTTCTTC and ATGCCAACTTGACGTAAAT) (17-19).

Statistical analysis

Data analysis was carried out using SPSS for Windows (version 18.0). Prevalence was expressed in proportions and crude odds ratio (OR) with 95% confidence intervals (CI) were used to measure the strength of association between variables. Proportions were compared by the Chi-square test and continuous variables by the Kruskal-Wallis and Mann-Whitney tests. The significance level established the inferential statistics was 5% ($p < 0.05$).

5.4. Results

The sample used in this research was composed by 447 adolescents (38.3% were males and 61.7% females), all between the age of 12 and 18 years old, from a public school of Sátão, Portugal. When analysing the parents' educational level, we observed that 4.3% have parents that only frequented school to or less than 4th grade, 53.5% stayed in school from the 5th to the 12th grade and 15.0% went to a superior degree after finishing the 12th grade. Crowding index < 1.0 is presented among 71.4% of adolescents, while 14.1% are equal to 1 and only 4.5% > 1.0 , which indicates possible overcrowding at home. The analysis of the distribution of the sample by residence area revealed that the majority of adolescents live in rural areas (65.3% vs 34.7%) (Table 5.1).

Table 5.1: Socio-demographic characterization of the studied sample of portuguese adolescents.

	Male		Female		Total	
	N	%	N	%	N	%
	171	38.3	276	61.7	447	100.0
Age						
12	31	18.2	27	9.8	58	13.0
13	29	17.0	38	13.8	67	15.0
14	18	10.5	34	12.3	52	11.6
15	28	16.4	27	9.8	55	12.3
16	16	9.4	34	12.3	50	11.2
17	16	9.4	29	10.5	45	10.1
18	33	12.3	87	24.3	120	26.6
Grade						
7	34	19.9	26	9.4	59	13.4
8	26	15.2	38	13.8	64	14.3
9	19	11.1	33	12.0	52	11.6
10	30	17.5	38	13.8	68	15.2
11	19	11.1	32	11.6	51	11.4
12	44	25.2	109	39.4	153	34.1
Parents' educational level						
≤ 4 grade	3	2.0	16	6.9	19	4.3
5-12 grade	105	61.4	134	48.6	239	53.5
> 12 grade	30	17.5	37	13.4	67	15.0
Without information	33	19.1	89	31.1	122	27.2
Crowding index						
<1.0	110	64.3	209	75.7	319	71.4
1,0	25	14.6	38	13.8	63	14.1
>1.0	8	4.7	12	4.3	20	4.5
Without information	28	16.4	17	6.2	45	10.0
Residential area						
Rural	100	58.5	192	69.6	292	65.3
Urban	71	41.5	84	30.4	155	34.7

The prevalence of *S. mutans* in the cohort studied was 80.8%, *Lactobacillus* 99.5% and *A. actinomycetemcomitans* 15.2%. When analysing the combinatorial presence of two or more bacterial strains, we observed the following: *S. mutans* and *Lactobacillus*: 80.5%; *Lactobacillus* and *A. actinomycetemcomitans*: 15.0%; *S. mutans* and *A. actinomycetemcomitans*: 12.1%; *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans*: 12.1%.

Looking for the association between the bacterial species and gender, we observed a significant difference among gender (higher incidence in females) for *S. mutans* (male=76.1% vs female=83.6%, $p=0.04$) and the co-infection with both *S. mutans* and *Lactobacillus*. Focusing on socio-demographic variables, we found that infection with *A. actinomycetemcomitans* was significantly associated with both age (<15 years=12.3% vs ≥ 15 years=20.3, $p=0.03$) and residence area (rural=18.2% vs urban=11.0%, $p=0.04$). The same situation was observed in adolescents infected with *A. actinomycetemcomitans* and any of the other bacterial species mentioned above. Taken together, these results demonstrate that *A. actinomycetemcomitans* may be the main bacterial strain associated with socio-demographic variables independently of the presence of the other bacterial strains analysed in the present study. These results may also be associated with a higher prevalence of periodontal disease among older adolescents and those that present worse socioeconomic status that live in rural areas as verified in table 5.2.

Table 5.2: Association between bacterial strains *S. mutans* (SM), *Lactobacillus* (LA) and *A. actinomycetemcomitans* (AA) and socio-demographic variables.

Bacterial strain	Gender					Age					Residential area				
	Male		Female		<i>p</i>	<15 yrs		≥ 15 yrs		<i>p</i>	Rural		Urban		<i>p</i>
	N	%	N	%		N	%	N	%		N	%	N	%	
SM	121	76.1	219	83.6	0.04	161	79.3	107	77.5	0.4	169	79.0	78	99.0	0.5
LA	158	99.4	261	99.6	0.6	203	100.0	136	98.6	0.2	213	99.5	126	99.2	0.6
AA	19	11.9	45	17.2	0.09	25	12.3	28	20.3	0.03	39	18.2	14	11.0	0.04
SM+LA	121	76.1	218	83.2	0.05	161	79.3	107	77.5	0.4	169	79.0	99	78.0	0.5
LA+AA	18	11.3	45	17.2	0.07	25	12.3	27	19.6	0.05	39	18.2	13	10.2	0.03
SM+AA	14	8.8	37	14.1	0.07	21	10.3	20	14.5	0.2	32	15.0	9	7.1	0.02
SM+LA+AA	14	8.8	37	14.1	0.07	21	10.3	20	14.5	0.2	32	15.0	9	7.1	0.02

Our study revealed the lack of a significant correlation between worse oral health behaviours and the presence of the various bacterial species analysed. However, some bacterial strains were associated with dental pain, which occurs in the presence of severe dental caries. We observed that adolescents who suffered one or more episodes of dental pain due to dental caries had a higher rate of *S. mutans* infection (episode of dental pain= 87.8% vs no dental pain=77.3%, $p=0.006$) and both *S. mutans* and *Lactobacillus* as seen in table 5.3. The results demonstrate that dental pain might be associated mainly with *S. mutans*

infection than with *Lactobacillus* infection, considering that 99.5% of the sample used in this study showed infection with this bacterial strain.

Table 5.3: Association between bacterial strains *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and oral health behaviours and dental pain.

Bacterial strain	Oral hygiene			Dental appointment			Dental pain								
	≤1/day		≥2/day	No		Yes	No		Yes	<i>p</i>					
	N	%	N	%	N	%	N	%	N	%	N	%	<i>p</i>		
SM	56	80.0	205	78.2	0.5	117	81.8	219	79.9	0.4	218	77.3	122	87.8	0.006
LA	70	100.0	260	99.2	0.6	143	100.0	272	99.3	0.4	282	100.0	137	98.6	0.1
AA	9	12.9	44	16.8	0.3	22	15.4	42	15.3	0.5	46	16.3	18	12.9	0.2
SM+LA	56	80.0	205	78.2	0.5	117	81.8	218	79.6	0.3	217	77.0	122	87.8	0.005
LA+AA	9	12.9	43	16.4	0.3	22	15.4	41	15.0	0.5	46	16.3	17	12.2	0.2
SM+AA	9	12.9	32	12.2	0.5	18	12.6	33	12.0	0.5	36	12.8	15	10.8	0.3
SM+LA+AA	9	12.9	32	12.2	0.5	18	12.6	33	12.0	0.5	36	12.8	15	10.8	0.3

Despite the observed association between dental pain and the presence of *S. mutans* alone or in combination with *Lactobacillus*, no significant association was found between the presence of the bacterial species studied and the risk of dental caries development (Tables 5.4 to 5.6). In fact, we detected the presence of the three species studied not only adolescents that have active caries during the time of intra-oral observation but also in caries-free individuals.

Table 5.4: Association between bacterial strains *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and dental caries.

Bacterial strain	Carie-free		1 to 3 caries		≥ 4 caries		<i>p</i>
	N	%	N	%	N	%	
SM	36	14.9	16	13.7	7	18.9	0.7
LA	241	99.6	117	100.0	37	100.0	0.7
AA	36	14.9	16	13.7	7	18.9	0.7
SM + LA	194	80.2	98	83.8	31	83.8	0.7
LA + AA	36	14.9	16	13.7	7	18.9	0.7
SM + AA	29	12.0	14	12.0	5	13.5	0.9
SM + LA + AA	29	12.0	14	12.0	5	13.5	0.9

Table 5.5: Association between bacterial strains *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and number of teeth decayed, missing and filled due to dental caries per adolescent (DMFT).

Bacterial strain	DMFT = 0		DMFT = 1-3		DMFT ≥ 4		<i>p</i>
	N	%	N	%	N	%	
SM	71	78.9	110	80.9	143	84.1	0.5
LA	90	100.0	136	100.0	169	99.4	0.5
AA	11	12.2	17	12.5	31	18.2	0.3
SM + LA	71	78.9	109	80.1	143	84.1	0.5
LA + AA	11	12.2	17	12.5	31	18.2	0.3
SM + AA	10	11.1	11	8.1	27	15.9	0.1
SM + LA + AA	10	11.1	11	8.1	27	15.9	0.1

Table 5.6: Association between bacterial strains *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans* and DMFT and dental caries.

Bacterial strain	DMFT					Dental caries				
	0		≥ 4		<i>P</i>	0		≥ 3		<i>p</i>
	N	%	N	%		N	%	N	%	
SM	71	78.9	143	84.1	0.2	195	80.6	46	83.6	0.4
LA	90	100.0	169	99.4	0.7	241	99.6	55	100.0	0.8
AA	11	12.2	31	18.2	0.1	36	14.9	10	18.2	0.3
SM+LA	71	78.9	143	84.1	0.2	194	80.2	46	83.6	0.4
LA+AA	11	12.2	31	18.2	0.1	36	14.9	10	18.2	0.3
SM+AA	10	11.1	27	15.9	0.2	29	12.0	8	14.5	0.4
SM+LA+AA	10	11.1	27	15.9	0.2	29	12.0	8	14.5	0.4

5.5. Discussion

The results obtained in this work demonstrate that the salivary presence of *S. mutans*, *Lactobacillus*, and *A. actinomycetemcomitans* is not associated with development of dental pathologies in our cohort of adolescents. Dividing the sample in caries-free adolescents and caries-active adolescents, we observed that both groups have similar levels of infection by *S. mutans* and *Lactobacillus*. Nevertheless, our findings go against some studies that correlate the presence of *S. mutans* and *Lactobacillus* with a higher risk of caries formation (20, 21). Also Fysal *et al.*, observed that the number of *S. mutans* and *A. actinomycetemcomitans* determined by microscopic counts was twice as high in caries patients (22). On the other hand, and strengthening our results, Reyes *et al.* demonstrated that *Streptococcus* species were present in caries-active and also in caries-free individuals. In this study, the authors also observed that dental caries can occur in the apparent absence of the bacterium *Streptococcus* and can even be associated with healthy states (1). Parisotto *et al.* explored the association between caries development, colonization with caries-associated microflora, and immunity as children begin the transition to mixed dentition. In baseline level there was no significant differences in *S. mutans* and *Lactobacillus* between caries-free and caries-active groups (23). Beighton confirmed that dental caries may develop in the absence of these species and their presence does not necessarily indicate dental caries activity (24). Additionally, Wolff *et al.* demonstrated that there is no significant differences of oral bacterial strains between caries-free and caries subjects (25).

A question may arise from the present study: can an association of other bacterial strains with *S. mutans*, *Lactobacillus* and *A. actinomycetemcomitans*, potentiate and increase the risk of oral disease development? Looking at the oral health behaviours, no differences were observed between oral hygiene habits and the presence or absence of the three bacterial strains in study. Our results are different from

those obtained by Plonka *et al.* that determined that the presence of *S. mutans* and *Lactobacillus* would increase with children's age and was also associated with worse oral health behaviours (26). Levels of *S. mutans* and *Lactobacillus* were found to be strongly associated with socioeconomic status among Palestinian children in East Jerusalem. The relatively high prevalence of cariogenic bacteria suggests that oral care prevention and treatment demands special attention from the health care institutions and authorities (15).

In the present study, *A. actinomycetemcomitans* was associated with age and residence area. Adolescents from rural areas showed a higher prevalence of this bacteria in comparison with the ones who live in urban areas. Paolantonio *et al.* demonstrated that *A. actinomycetemcomitans* colonization in children and adolescents from central Italy is affected by socioeconomic and cultural factors, namely residence area (urban vs rural areas), and that these factors may also affect the periodontal condition of the subjects (27). The same situation is verified in the presence of *Lactobacillus* and *A. actinomycetemcomitans*, *S. mutans* and *A. actinomycetemcomitans* and in the presence of all three bacterial strains. This fact can be considered important, because the adolescents that present *A. actinomycetemcomitans* can have, in the near future, a higher risk of periodontal disease development (28, 29). Therefore, special attention should be given to adolescents living in rural areas, which present worse oral health behaviours and more difficulties in attending frequent dental appointments biannually.

We verified that adolescents who suffered one or more episodes of dental pain due to dental caries had a higher incidence of *S. mutans* alone or in combination with *Lactobacillus*. This may be justified by the fact of adolescents that suffer dental pain have worse dental caries lesions and more retentive sites on the tooth surface that may increase the levels of anchored *S. mutans*, as previously suggested by Thaweboon *et al.* (30). However, it is important to understand that this association between the prevalence of certain bacterial strains and the occurrence of dental pain does not occur in an isolated manner. Even knowing that *S. mutans* is one of the main bacterial strains responsible for dental caries development (31), we must have into account other aetiological factors. The consumption of sweet foods and oral hygiene habits are clearly described as being significantly associated with the severity of dental caries development (32).

5.6. Conclusions

The presence of oral microflora is clearly one of the main etiological factors for dental caries and periodontal diseases development, but cannot be considered in an isolated manner. For the development of oral disease, various other factors need to be present such as high and daily sugar intake, inadequate oral hygiene habits and even genetic susceptibility. *A. actinomycetemcomitans*, even among adolescents, can be considered associated with socio-demographic variables, and, if not clinically well identified and treated, may cause serious oral diseases during adulthood. Adolescents who suffered one or more episodes of dental pain due to dental caries had a higher incidence of *S. mutans*.

Probably the imbalances in the resident microflora may be the ultimate mechanism of oral disease development. Oral diseases can appear in the presence of changes of the oral bacterial communities' structure and that may be related with the shift from health to disease. The enormous diversity of oral

microbiota allowed for a better understanding of oral micro ecosystem, and these pathogenic populations present in the oral cavity provide new insights into the etiology of oral diseases and suggest new targets for interventions of the disease. The present study indicates that epidemiological surveys with the assessment of etiologic risk factors are crucial tools for oral health planners and clinicians in order to implement a risk-based preventive strategy.

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5.9. Competing interests

The authors have declared that no competing interests exist.

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Chapter 6

**Characterization of the antibacterial effect
resulting from the association between silver
diamine fluoride and a resin-based fissure
sealant**

Paper IV

Characterization of the antibacterial effect resulting from the association between silver diamine fluoride and a resin-based fissure sealant

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6.1. Abstract

Introduction: The present study consists in the incorporation of a cariostatic agent – silver diamine fluoride (SDF) – in a resin-based fissure sealant followed by the study of release kinetics by spectrophotometry analysis of the association between both biomaterials and assessment of the inhibitory effect on the growth of the reference bacterial strain *S. mutans* in an *in vitro* study.

Materials and Methods: An experimental *in vitro* study was designed consisting in the entrapment of SDF (*Cariestop*[®] 12% and 30%) into a commercially available fissure sealant (*Fissurit*[®]), by photopolymerization and photocrosslinking. The same sealant, without SDF was used as a negative control. The effect of the sealants on the growth of *S. mutans* was determined by the presence of bacterial inhibitory halos in the cultures at the end of the incubation period. In order to confirm the absence of bacteria in the surface of the materials, Scanning Electron Microscopy (SEM) characterization was performed. Also, to analyze the release profile of SDF along time spectrophotometry technique was applied.

Results: The obtained results indicate that the association of SDF to a resin-based fissure sealant may be able to increase the inhibition of *S. mutans* growth. Although, no SDF release was noticed during the *in vitro* release studies and no statistically significant difference was noticed in inhibitory halo sizes obtained for test and control group.

Conclusions: In this study, the entrapment of SDF in the resin-based fissure sealant did not potentiate the antibacterial effect of the fissure sealant or avoid the immediate development of dental caries. The development of more laboratorial research and, afterwards, long-term clinical data are necessary in order to verify if this association between these biomaterials is effective and can be considered for being used in oral health management. Also, other methodologies for associating cariostatic agents and sealant should be addressed.

Keywords: Biomaterial, fissure sealant, primary prevention, silver diamine fluoride, *S. mutans*.

6.2. Introduction

Pits and fissures on occlusal surfaces of permanent teeth are particularly susceptible to the development of dental caries, which is the most prevalent oral disease worldwide (1). This susceptibility is related with the physical size and individual morphology of pits and fissures of the tooth surface, which can be considered as being ideal “shelters” for microorganisms and make the oral hygiene procedures of these areas more difficult, allowing greater amount of dental plaque retention (2). This is considered as being an important risk factor because the accumulation of bacterial biofilms is responsible for oral disease development, such as dental caries and periodontitis (3). For the prevention of dental caries, fissure sealants application is recommended if pits and fissures are very deep and narrow, creating a physical barrier for the bacterial biofilm accumulation in these specific anatomical areas of the tooth (4, 5). Fissure sealants application in high susceptible tooth decay areas is one of the primary preventive measures to minimize the risk of dental caries development, reducing its incidence in pits and fissures, preventing to carry out more invasive dental fillings with silver amalgam or filling resins (1, 6, 7). A study developed by Hiiri *et al.*, evidences the superiority of pit and fissure sealants over fluoride varnish application in the prevention of occlusal dental caries (8).

A key problem of fissure sealants is the high risk of microleakage development sometime after their application. This situation may happen due to chewing forces applied during time and also because of polymerization shrinkage of resin-based fissure sealants that might facilitate the formation of gaps between the material and the tooth surface, providing space for bacterial invasion and proliferation (9). This microleakage may lead to dental plaque accumulation, which in contact with enamel, can turn into a carious lesion (10).

The reassessment of fissure sealants should be made each six months in order to avoid the development of dental caries in teeth that have microleakage on the fissure sealant applied. This reassessment is especially important in particular cases of patients with high risk of developing dental caries and insufficient oral health behaviors (11).

It would be important to have a fissure sealant which offers a genuinely antimicrobial and antibiofilm efficacy that would be a considerable clinical benefit. Such a material could reduce recurrent decay providing an antibacterial seal, protecting the enamel surface of the tooth (12).

Various attempts have been made in order to reduce plaque accumulation on the surface of restorative and preventive materials by the incorporation of bacterial agents (3). A study developed by Li *et al.* demonstrated that the incorporation of the antibacterial agent metacriloxiletil ethyl dimethyl ammonium chloride monomer (DMAE-CB) influences the antibacterial properties of fissure sealants after photopolymerization. This conclusion was confirmed through analysis of the antibacterial effect on the bacterial strain *S. mutans*, being verified an inhibitory effect on bacterial growth (9). Furthermore, it was found that the combination of an antibacterial agent in fissure sealants did not affect their chemical and physical properties and there is no higher risk of microleakage. Moreover, other studies have demonstrated the antibacterial beneficial combination between a bactericidal and / or bacteriostatic agent and an adhesive system, without changing their physical and chemical properties (13).

Another primary prevention method that has proven to be efficient consists in the application of fluoride on the enamel surface of the teeth. Fluoride is well known to reduce dental caries by reducing the solubility and enhancing remineralization of dental enamel by the incorporation of available fluoride into the tooth hard tissue structure during the presence of the organic acids (14). Studies showed that the use of fluoride on dental surfaces, when contacting with the fissure sealant or even incorporated into the biomaterial, may increase their antibacterial effect (8, 15-18). A study undertaken by Matalon *et al.* showed that rinsing with a mouthwash fluoride, for two weeks, allows a greater replacement of the sealant antibacterial properties (16). However, there is still some debate among researchers about whether a sealant containing fluoride actually prevents dental caries more effectively than does a sealant without fluoride (9).

So the association between an antimicrobial agent (with specific characteristics) with several biomaterials used in clinical practice of dental medicine, can be performed to increase the inhibitory effect on the growth of bacterial strains, in this case, of *S. mutans* (9, 13, 19, 20).

Nowadays, nanotechnology has become a key research field that has brought beneficial applications in medicine and healthcare. One of the most advanced methods applied in healthcare consists in the development of releasing controlled systems of silver particles that have an antibacterial activity (21, 22). Silver is a natural antibacterial agent and that has been used in medicine due to its properties, namely its biocompatibility for human cells, long-term bactericide effect and stability at high temperatures and low volatility (22).

Silver has been used in various purposes like water purification, wound treatments, bone prosthesis, surgical orthopedic reconstruction, development of heart functioning devices and has even been incorporated in hospital clothing in order to decrease the risk of nosocomial infections (23, 24).

Some studies demonstrated also the possible association of silver ion particles with filled resins applied in dental medicine which increases the antibacterial effect and assures a better protection against the development of recidivating dental caries (25).

Furthermore, a compound that associates the advantages of sodium fluoride and silver nitrate: silver diamine fluoride (SDF) is currently commercially available. This compound has proven to possess cariostatic properties that being able to stop the development of dental caries in the hard tissue of the tooth with a mechanism that consists in the self-stimulation of the calcified and sclerotic dentin formed by the silver ion (19, 26-28).

Other studies show that the synergism established between the fluoride and the silver increases the enamel resistance against the organic acids produced in the oral cavity that is responsible for the dental tissue demineralization, which gives this biomaterial the possibility of becoming an important primary prevention method for deciduous and permanent teeth in the future (21, 29). SDF has also been shown to inhibit bacterial growth, mainly cariogenic strains like *S. mutans* (30).

In the present study, SDF was incorporated by physical entrapment in a resin-based fissure sealant. The prepared system was afterwards analyzed by assessing its inhibitory effect on the growth of *S. mutans* strain.

6.3. Materials and Methods

The purpose of this work was to assess the efficiency of incorporating SDF in a selected resin-based fissure sealant. *S. mutans* was used as model bacteria, since it is considered to be the primary responsible for the initiation of dental caries as well as for the progression of an established lesion (3).

This study was developed in three different stages:

Stage 1: Samples with SDF were prepared. Two commercially available SDF solutions with different concentrations were used: 12% (Cariestop 12%) and 30% (Cariestop 30%). Samples were prepared by adding the same concentration of cariostatic to the sealant (10%), meaning that the only variable was the initial SDF solution concentration. Once prepared, the mixtures were photopolymerized under UV irradiation for 30s. Samples without incorporation of SDF were prepared directly from the sealant to be further used as a negative control. At the end of this phase, three groups of samples were obtained: resin fissure sealant (sample A); resin fissure sealant with SDF 12% solution (Sample B) and resin fissure sealant with SDF 30% solution (Sample C).

Stage 2: Assessment of the inhibitory effect on the bacterial strain *S. mutans* growth in the presence of the samples prepared during stage 1. The bacteria were cultured in Brain Heart Infusion broth, at 37°C, anaerobically, for 24 hours. *S. mutans* (1.0×10^8 colony-forming units (CFU/mL)) was inoculated in an agar plate with the different materials. After 24 hours, the inhibitory halos were analyzed and measured, as previously described in literature (31). Furthermore, in order to confirm the absence of bacteria at the surface of the materials, Scanning Electron Microscopy (SEM) analysis was also performed (32).

Stage 3: Analysis of the release profile of SDF from samples was performed by spectrophotometric technique. In a first approach, a scanning of the SDF solutions was performed in the range of 200 to 500 nm of the electromagnetic spectrum. The wavelength in which the maximum absorbance value was registered and established as the one to be used during samples analyses. Afterwards, 0.5g of each sample were individually immersed in distilled water and placed in an oven at 37°C. At predetermined times, the samples were removed from the oven and the absorbance of the incubation medium was measured at previously established wavelength.

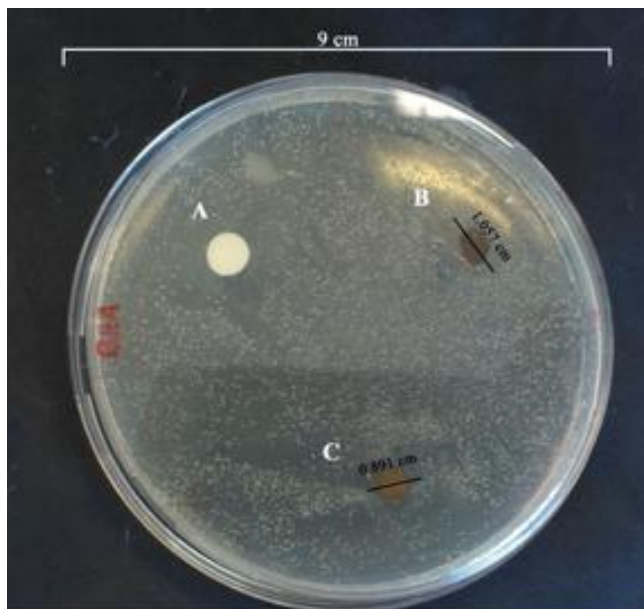
6.4. Results

Inhibition of bacterial growth

The obtained results demonstrate that the materials have inhibited the bacterial growth after 24 hours. The determination of the inhibitory halos surrounding the samples was assessed. In the sample containing the resin-based fissure sealant with 12 % SDF solution (sample B) the inhibitory halo had a diameter of 1.06 cm while the sample containing the resin-based fissure sealant with 30 % SDF solution (sample C) had a

0.89 cm inhibitory halo (Figure 6.1). Therefore, no statistically significant differences were found between the inhibitory halos obtained for samples B and C.

Figure 6.1: Evaluation of the inhibitory effect of SDF on *S. mutans*: A) Resin-based fissure sealant without SDF incorporated (control); B) Resin-based fissure sealant with 12 % SDF solution and C) Resin-based fissure sealant with 30 % SDF solution.



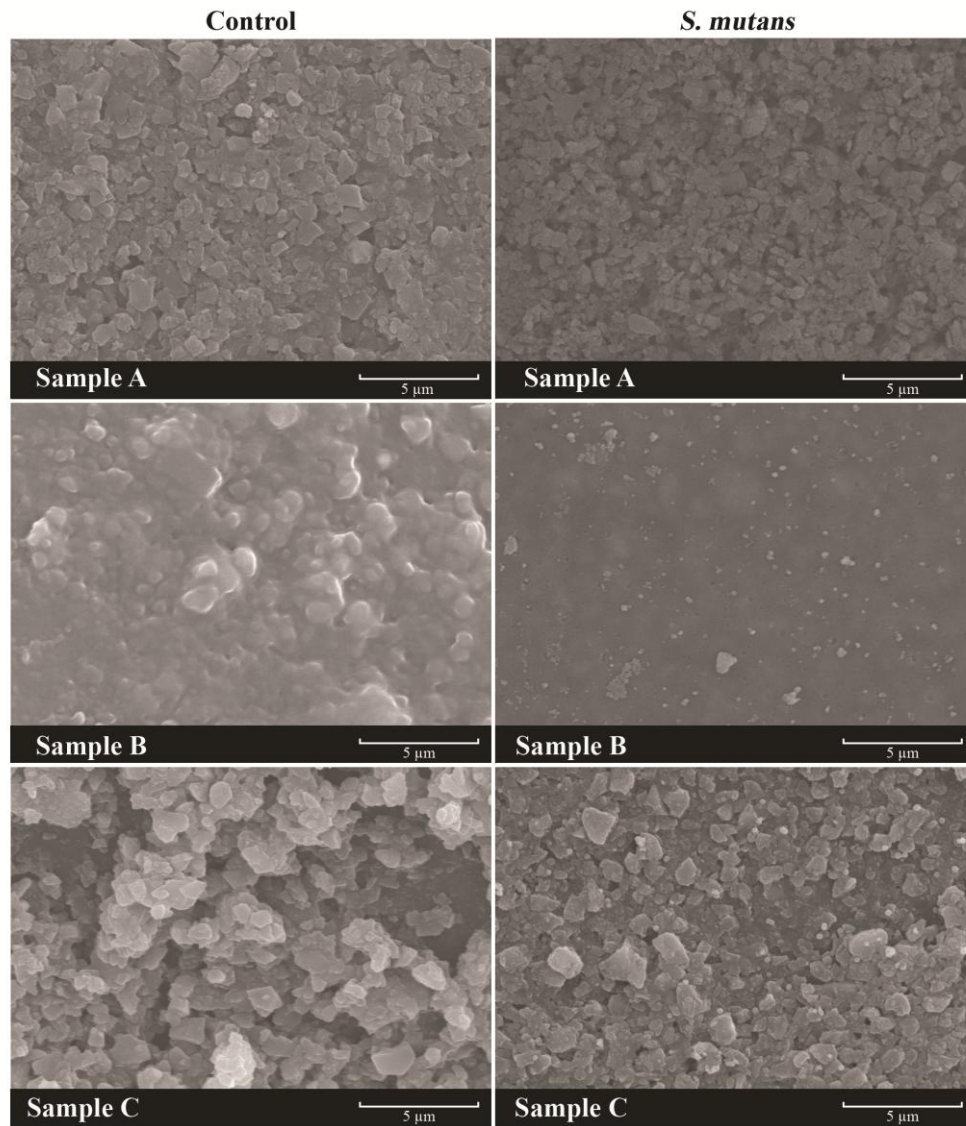
Moreover, SEM images of the surfaces of the materials were also acquired in order to fully characterize bacterial growth on the materials surface (Figure 6.2). As it can be seen, no bacterial growth was noticed on the surfaces of the materials. The authors found that the cured sealant containing SDF had an inhibitory effect on the growth of *S. mutans*. Although, the control sample (sample A) did not show any detectable growth of the tested bacteria. Further studies have to be done in order to check the antibacterial activity of the produced materials.

SDF release

Release of SDF from the samples produced during stage 1 was studied by using a spectrophotometric approach. For that purpose, a scan in the range between 200 and 500 nm of the electromagnetic spectrum was performed in order to determine the correct wavelength to be used during further analyses. The obtained values of absorbance indicated that maximum absorbance occurred at 300 nm.

As previously described, 0.5 g of each sample were incubated in distilled water at 37°C in order to evaluate the delivery of SDF from the polymeric matrix. The analyses were performed by measuring the incubation medium's absorbance at 300 nm. However, the obtained results indicated that no measurable amount of the drug was released. Such may be attributed to the highly rigid structure of the photopolymerized sealant which is crosslinked by UV radiation.

Figure 6.2: SEM images of the surfaces of the materials in the presence of *S. mutans* (right column) and in the absence of it (left column) after 24 hour for the resin fissure sealant (sample A); resin fissure sealant with SDF 12% solution (Sample B) and SDF with SDF 30% solution (Sample C).



6.5. Discussion

Providing dental materials with antibacterial properties has been attempted to prevent the formation and development of dental caries (9). New biomaterials with optimized antibacterial properties are crucial for their future application in communities where oral health behaviors are not well implemented, in order to avoid the increase of oral disease development.

Simultaneously to this study, an epidemiological approach was performed, with a sample of 447 adolescents, aged 12 to 18 years old, attending a public school in the portuguese town of Sátão. In this study we assessed the prevalence and integrity of fissure sealants after intra-oral observation of the adolescents in classroom. When analyzing the prevalence of fissure sealants, we verified that 58.8% of adolescents had at least one fissure sealant applied. From our total sample, we observed 830 teeth sealed, in which 63.3% were completely intact, 11.3% infiltrated but still without dental caries, 1.6% infiltrated but with the presence of dental caries, 23.5% with a partial fissure sealant but without dental caries and only 0.3% with a partial fissure sealant with dental caries present on the tooth surface. After the analysis of the epidemiological approach in adolescents, we can verify that fissure sealants, when infiltrated, increases the risk of dental caries development, losing all the characteristics of a primary preventive biomaterial, and, therefore, the need to investigate how biomaterials can be improved in order to prevent oral diseases considering that the oral cavity has such a complex micro-environment and the risk of dental caries development is high among the population.

It is essential to understand that the research described in this paper, reports preliminary results of an initial phase of biomaterial development that needs to be complemented in a near future. The results obtained in the present study do not demonstrate significant differences in the inhibitory effect of *S. mutans* growth between the control sample and the samples with SDF incorporated into the fissure sealant. Also, the SDF release profile from the fissure sealant could not be determined through the spectrophotometric method. However, forthcoming studies will allow the authors to optimize entrapment methodology as well as quantification techniques. As an example, HPLC may be used to quantify SDF release allowing detection of lower amounts of the drug.

Moreover, the antibacterial activity determination of the resin-based fissure sealant and SDF was made only with the bacterial strain *S. mutans*, since in literature it is described that *S. mutans* can be easily isolated from dental plaque samples and are considered as being the main bacterial strain involved in the initiation of dental caries (26, 33). Further bacterial strains can be tested in the near future to characterize antimicrobial effect on samples.

Clinical studies have shown that the topical application of SDF is effective in arresting enamel and dentin caries and inhibiting cariogenic biofilm formation (26). Therefore, the main goal of this study was to provide information concerning the potential clinical performance of the sealant with antibacterial activity, when SDF was added to its production (9, 13, 19, 20).

The methodology employed in the present study has some limitations with respect to the reproduction of the physiological and biological conditions of oral cavity. Due to these limitations, the results obtained during *in vitro* studies cannot be directly extrapolated to an *in vivo* situation because they may not reflect

the actual effect of the material when applied under clinical conditions. However, more laboratorial tests have to be done for a complete characterization of the materials performed.

Another point that has to be taken into account as a disadvantage of the use of SDF to arrest caries, is that the lesions will be stained black. It has been suggested that when carious dentin is treated with SDF, silver phosphate is formed and precipitated (30, 34).

However, previous studies have demonstrated that SDF application is effective for dental caries arrest, as an alternative for more complex restorative treatments and do not cause adverse effects (3, 28).

In addition, it was also described that the incorporation of the SDF in the crosslinked structure of the fissure sealant permits the presence of a high concentration of silver and fluoride ions, which can inhibit the growth of different cariogenic bacteria that are responsible for biofilm formation (34, 35). However, and also due to the highly complex crosslinked structure of the cured fissure sealant, the release of SDF is precluded.

Another issue that has to be taken into account in future studies are the systemic symptoms (argyria) that can occur after topical use of silver. In some cases, silver also produces a localized discoloration of various tissues (3).

6.6. Conclusions

In this study, the entrapment of SDF in the resin-based fissure sealant did not show to be an effective method to potentiate the antibacterial effect of the fissure sealant, and also avoid the development of dental caries. Further laboratorial research and, afterwards, long-term clinical data is necessary in order to verify if SDF impregnation in fissure sealants is effective and can be considered advantageous in the field of oral health management. Further research may allow the identification of other dental biomaterials that can prevent and reduce the risk of dental caries development and protect the tooth from bacterial infections.

6.7. Competing interests

The authors have declared that no competing interests exist.

6.8. References

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Chapter 7

**Oral health education: community and individual
levels of intervention**

Paper V



Oral health education: community and individual levels of intervention

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7.1. Abstract

Introduction: The objective of this study was to characterize the oral health behaviors among a sample of Portuguese adolescents and introduce different strategies that enable the accomplishment of collective programs appropriate for the promotion of oral health at individual and community levels.

Materials and Methods: A cross-sectional study was designed with a sample of 447 adolescents aged 12 to 18 years old, attending a public school in Sátão, Portugal. An interview was made questioning about socio-demographic factors and oral health behaviors to each adolescent. Considering the obtained results, a revision of the literature was made in order to define oral health promotion strategies to be applied among children and adolescents to improve oral health behaviors in a specific Portuguese community.

Results: The prevalence of toothbrushing (twice-a-day or more) was 90.6%. Five point eight percent of adolescents reported daily flossing. Sixty-seven percent had at least one dental appointment in the previous twelve months. Considering the results obtained, various oral health promotion strategies should be developed based on the following topics: oral health education for children and adolescents in schools and public institutions; oral health promotion for teachers and parents; technology application in oral health education; education and motivation for oral health behaviors given by health professionals.

Conclusions: Community programs should be considered and developed in order to improve knowledge and behaviors related to adolescents' oral health, giving special attention to the intervention of various health professionals, teachers and parents in the oral health education that should be transmitted to children and adolescents.

Keywords: Community, oral health education, oral health promotion, oral hygiene.

7.2. Introduction

The WHO defines health promotion as being a “*process of enabling people to increase control over, and to improve, their health. It moves beyond a focus on individual behavior towards a wide range of social and environmental interventions*”(1).

The promotion of oral health should include the creation of healthy public policies and supportive environments, development of personal skills and reorientation of the oral health services. This last definition is different from oral health education that is mostly aimed at improving oral health through the acquisition of knowledge, eventually leading to motivation and finally, to behavioral changes according to the health belief model (2,3).

The remarkable improvements in oral health in the last years reflect the strong scientific basis for prevention of oral diseases that has been developed and applied in the community, in clinical practice, and at home (4,5). However, and despite this approach, the majority of these programs fail to achieve their aims due to the lack of attention mostly resulting from the inadequate and insufficient relationship patient-health professional in its educational aspect (6).

Various studies demonstrate that socio-economic and cultural aspects may influence the oral hygiene habits. The lack of information and knowledge about oral health behaviors and limited access to dental healthcare may explain the association between the higher risk of oral diseases and lower socio-economic status (7-12).

The degree of association between a number of social, economical and behavioral risk factors and the prevalence data for oral cancer, dental caries and destructive periodontitis have been determined in various studies. These associations should be interpreted with caution, but are suggestive of the need to take them into consideration when developing health promoting oral health policies (13-16).

Studies confirm that low social class increases the risk of developing high levels of dental caries (17). Parents’ low educational level and professional situation (employed/unemployed) also play an important role in the child/adolescent oral health status (18,19). When analyzing the acquisition of health behaviors, we must also consider the importance of values and behaviors of peers, parents and other family members who continue to be influential (19,20).

In the previous years, WHO has dedicated special attention to health-promoting programs applied in schools. Oral health education is an important issue that should be developed among the population with a view to decreasing the prevalence of oral illnesses (21).

The objectives of this study are:

- To characterize oral health behaviors among a sample of Portuguese adolescents and verify their association with socio-demographic variables.
- To introduce different strategies that allow the accomplishment of collective programs appropriate for the promotion of oral health at individual and community levels.

7.3. Materials and Methods

The first step for success in a program is the identification of each individual's educational needs, for it is only through knowledge of the individual characteristics of human behavior that it will be possible to outline an action plan for the benefit of the community (6).

A non-probabilistic convenience sample of 447 adolescents aged between 12 and 18 years old, attending a public school in Sátão, Portugal, was enrolled in this epidemiological observational cross-sectional study that was carried out from September to December of 2012. An interview to each adolescent in the classroom was held inquiring about socio-demographic variables, social and daily habits and oral health behaviors. The studied sample represents the entire school group of the area, and 88.6% of its pupils were enrolled in the study.

Socio-demographic variables such as gender, age, school grade at the moment of the survey, residence area (urban/rural), parents' educational level (indicating the higher educational level of parents) and father's professional situation (employed/unemployed) were taken into account. Oral health behaviors were assessed by questioning adolescents about various issues, such as: the daily frequency of toothbrushing; daily use of dental floss; number of dental appointments in the last 12 months and the reason for the last dental appointment; experience of at least one episode of dental pain during his/her lives; fear of the dentist; and consumption of sugary beverages or soft drinks. Data analysis was carried out using the *Statistical Package for Social Sciences* (SPSS 18.0 version). Prevalence was expressed in proportions and compared by the Chi-square test and continuous variables by the Kruskal-Wallis and Mann-Whitney tests. The significance level established the inferential statistics was 5% ($p < 0.05$).

This research involving human data has been performed in accordance with the Declaration of Helsinki and was submitted and approved by the Ethics Committee of the Health School and Research Centre for Education, Technology and Health Studies of the Polytechnic Institute of Viseu, Portugal (CI&DETS).

The information collected by questionnaires was provided voluntarily and confidentially. Anonymity of collected information was guaranteed since adolescents were told not to sign their names or write down any other form of identification in any part of the questionnaire. Data collection was made only for adolescents whose parents signed an informed consent that explained the objectives of the study.

Considering the obtained results, a revision of the literature was made in order to define different strategies that enable the accomplishment of collective programs appropriate for the promotion of oral health at individual and community levels.

7.4. Results

The final sample was composed of 447 adolescents, 38.3% males and 61.7% females, all between the ages of 12 and 18 years old, from a public school of Sátão, Portugal. When analyzing the parents' educational level, we verified that 4.3% had parents that only attended school until no later the 4th grade,

53.5% stayed in school from the 5th to the 12th grade and 15.0% proceeded to a higher degree after finishing the 12th grade. The analysis of the distribution of the sample by residence area showed that the majority of individuals lived in rural areas (rural=65.3% vs urban=34.7%).

In the sample under analysis, we verified that 13.3% of the adolescents considered having a very good oral health, 65.7% good oral health and 21.0% moderate/poor oral health. When assessing daily toothbrushing, 9.4% brushed their teeth only once a day, 67.3% twice a day and 23.3% 3 or more times a day.

When adolescents were asked if they also brushed their tongue during oral hygiene, 83.2% referred to brushing their teeth, not forgetting to brush the tongue, thus corresponding to a complete process of brushing. Adolescents were also questioned about their oral hygiene learning process, and in this situation we verified that 41.7% referred that their dentist never taught them how to toothbrush, while 58.3% said that their dentist had, at least once, talked about basic measures of daily oral hygiene.

When assessing the use of dental floss, our study showed that only 5.8% used dental floss daily, while 33.9% referred using dental floss sometimes and 60.2% said that never used dental floss during their oral hygiene.

The number of dental appointments was another important analysed variable and our results demonstrated that 67.0% had a dental appointment in the last twelve months which demonstrates that a high prevalence of adolescents do not have a dental appointment at least twice a year. When assessing the main reason for the last dental appointment, 85% referred having regular dental appointments, 35.8% visited a dentist due to dental pain and had a dental appointment in an emergency situation and 58.2% for dental caries treatment.

The prevalence of dental fear among adolescents was of 15.3%, a fact that can compromise regular dental appointments these adolescents should schedule at least twice a year. Finally, and after analyzing of the variable referring to the consumption of sweet beverages and soft drinks, the prevalence among adolescents was of 92.5%.

By analyzing the association between socio-demographic variables and dental appointments in the last twelve months, we verified significant statistical differences among dental appointments in the aforementioned period and father's unemployment (unemployed=41.5% vs employed=68.1%, $p<0.001$) and crowding index ($\leq 1=67.4%$ vs $>1=55.0%$, $p=0.04$) (Table 7.1).

Table 7.1: Prevalence of dental appointments in the last twelve months and its association with socio-demographic variables.

Dental appointment (last twelve months)	No		Yes		<i>p</i>
	N	%	N	%	
Parents' educational level					
≥4 grade	5	26.3	14	73.7	0.16
5-12 grade	83	35.0	154	65.0	
>12 grade	15	23.1	50	76.9	
Father's professional situation					
Employed	100	31.9	213	68.1	<0.001
Unemployed	24	58.5	17	41.5	
Gender					
Male	62	36.9	106	63.1	0.1
Female	84	30.5	194	69.5	
Age					
≤15 years	75	33.7	150	66.7	0.5
>15 years	65	34.0	126	66.0	
Residential area					
Rural	78	34.2	150	65.8	0.4
Urban	48	35.6	87	64.4	
Crowding index					
≤1	114	32.6	236	67.4	0.04
>1	9	45.0	11	55.0	

When associating the consumption of soft drinks and socio-demographic variables we observed that the prevalence of sweet beverages consumption was higher among younger adolescents (≤ 15 years=97.8% vs >15 years=86.7%, $p<0.001$) and those living in rural areas (rural=93.8% vs urban=88.2%, $p=0.04$).

Taking into account these results, various oral health promotion strategies should be developed and should be structured based on the following main points:

- Oral health education for children and adolescents in schools and public institutions;
- Oral health promotion for teachers and parents;
- Technology application in oral health education;
- Education and motivation for oral health behaviors given by health professionals.

Oral health education for children and adolescents in schools and public institutions

Countries have been developing comprehensive programs involving health and educational sectors (3). Oral health education is a major public health issue that must be taught to children and adolescents within

the family and school environment. Oral health promotion is very important in order to insure the application of primary prevention methods such as daily tooth brushing at least twice a day, daily use of dental floss and regular visits to dentists to prevent and detect oral diseases at an early stage. Oral health education is the first step in the prevention of oral diseases in order to decrease socio-demographic differences and to give equal opportunities of oral health, thus promoting measures necessary for the improvement of the population's quality of life (22-24).

Oral health education should also cover food hygiene comprising the control of the consumption of cariogenic foods with a high content of carbohydrates, which is fundamental to avoid dental caries development (16,25,26).

We can still verify the existence of numerous schools with vending machines selling foods and beverages with a high sugar intake. It is strictly necessary to promote the decrease of sweet foods and beverages in schools and around school buildings. Therefore, school policies should have into account the necessary change with a view to preventing the consumption of sweets and to motivating the consumption of healthy foods and drinks (27,28).

Another important issue that should be acknowledged in oral health education is the unnecessary fear of dental appointments. Studies demonstrate that this is one of the main reasons for avoiding a visit to the dentist, especially among children and adolescents (29,30).

The best way to teach the main aspects of oral health to children and adolescents is going to their school environment to explain the importance of:

- Ensuring proper oral hygiene habits;
- Fluoride application at home and during dental appointments;
- Fissure sealant application and regular reassessment;
- Regular check-up dental appointments at least twice a year;
- Decreasing sugar intake and maintaining a well-balanced nutritional intake to prevent dental caries;
- Consuming fruit and vegetables that can protect against oral cancer;
- Stopping tobacco use and decreasing alcohol consumption to reduce the risk of oral cancers, periodontal disease and tooth loss;
- Using protective sports and motor vehicle equipment to reduce the risk of facial injuries (31,32).

These important aspects can be explained during simple oral health education sessions using audiovisual equipment, showing the best toothbrushing techniques with the use of mouth and teeth macromodels. The demonstration of simple and comprehensive movies, interactive plays that can be used in mobile phones, theatrical plays, puppet plays and fairy tales help understand the importance of oral hygiene. The advertisement in *facebook* and other digital platforms and the distribution of pamphlets with a summary of the oral health education session implemented by dental and health professionals can also become also be an efficient method for teaching and motivating children and families to develop better oral health behaviors.

Oral health promotion for teachers and parents

Training of schoolteachers should also be developed in Portugal aiming at increasing national capacities in relation to the integration of oral health promotion in schools. It is important that oral health education does not become limited to children and adolescents. It should also reach teachers and parents. The suggestion of developing oral health education meetings is essential to explain the importance of adequate oral health behaviors for their children and students, and even for themselves, since parents may not have a wide knowledge about oral health, thus being unable to transmit it to their children (19,33).

Children tend to copy the habits and attitudes of their own parents and teachers. The same can be verified when analyzing oral health behaviors. If other family members practice adequate oral hygiene habits at home, when children become adolescents, brushing has been converted into an integral part of their hygiene and self-care practices (6,34).

The application of technology in oral health education

Developments in science and technology are providing patients with better quality and more convenient oral health care, namely in the field of oral health education. Knowledge of technologies and associated skills enable the development of new pathways to teach oral health. Bearing in mind the importance of technology nowadays, the implementation of oral health education and its association with new technologies are essential to draw the community's attention and to reach the public, namely children and adolescents (35).

One of the objectives still to be accomplished is the design of a web-based program specifically for oral health education that would be comprehensive in scope and relevant to the target audience. This web-based program should address the following aspects:

- Promote oral hygiene practices that should be developed at home and integrated in school health programmes, such as regular daily toothbrushing, daily use of dental floss and the application of fluoride;
- Encourage healthy dietary habits;
- Demonstrate the necessity of regular check-up dental appointments and preventive measures applied in a dental office, such as fissure sealant application.

Development and implementation of specific videogames and programs involving oral health aspects can also become an interesting measure to be implemented and the to teach oral health in schools and even at home for children and adolescents in the presence of their family and teachers.

Health professionals: education and motivation for oral health behaviors

The role of health professionals is crucial to the struggle against the development of oral diseases. Health

promotion and oral health education programs should be continuously repeated with a view to achieving long-term favorable results and acceptable levels of motivation for change in oral health behaviors (6,36). Health professionals have an important role in the development of global policies in oral health promotion and oral disease prevention as far as the following aspects are concerned:

- Building oral health policies towards effective control of risks to oral health;
- Stimulating development and implementation of community-based projects for oral health promotion and prevention of oral diseases, with a focus on disadvantaged and poor population groups;
- Encouraging national health authorities to implement effective fluoride programmes for the prevention of dental caries;
- Advocating for a common risk factor approach to simultaneously prevent oral and other chronic diseases;
- Providing technical support to countries to strengthen their oral health systems and integrate oral health into public health (37-39).

It is necessary that the services and health interventions are programmed appropriately bearing in mind the economic and social status. It is worth pointing out that proposals do not inadvertently undermine communities' health or reinforce social inequalities (40).

On a more individual and clinical level, the dental professional must assess the oral health status of his/her patient in order to understand the needs and identify the instructions that should be given. Understanding the oral health behaviors of a patient is essential to orientate the health promotion methods that can be applied (33).

The health professional and even dental students, in collaboration with universities, can also provide less expensive dental appointments and even free primary preventive appointments for those who present more socio-economical difficulties (41).

7.5. Discussion

The present study demonstrates that measures must be taken in order to improve oral health behaviors among Portuguese adolescents. The daily routine of toothbrushing has continuously been well established, but there is still a lack of comprehension of the importance of completing oral hygiene with other methods, such as the use of dental floss and regular dental appointments (42,43). This study also reflects the relation between oral health behaviors and socio-demographic factors already referred in other studies (8,9,44,45).

Portugal was identified in the Portugal Health System Performance Assessment as one of the European countries in which there were proportionally more difficulties to improve the population's oral health. Even if there are no significant studies on the prevalence of oral health problems on the Portuguese adult population, there are some studies about the school population that identify moderate rates of dental caries (46,47). Unfortunately, in Portugal nowadays we observe an increase of unemployment that limits the access to medical and dental healthcare due to the economic and social crisis. In Portugal, the

population's unequal access to oral healthcare is evident. It is widely known how difficult it is to individuals facing socio-economic disadvantages to have access to oral healthcare. This situation results from the fact that the vast majority of oral healthcare is currently provided by the private sector, involving funds that not everyone is able to support (48).

The lack of information about the importance and the need of a dental appointment should be a part of oral health education in order to decrease the fear and anxiety of visiting a dentist (41,49). Another major risk factor related to the development of oral diseases is the consumption of sugary foods and drinks. In this study, we verified that a high proportion of adolescents consume sugary beverages on a regular basis. This proves the necessity of alerting the population, namely children/adolescents and their parents, for the negative effects of excessive sugar intake on oral health (26). The enterprises that make and sell softdrinks and sweet foods should also be advised in the reduction of the percentage of sugar incorporated in their beverages and snacks that are of easy access to children and adolescents.

By considering the results obtained in the present study, it is clear that it is urgent to develop and implement oral health education in effective terms, so that oral health knowledge can improve and the oral hygiene status of children and adolescents change (16,50).

Schools are an excellent starting point for preventive action in the field of oral health. Education is the backbone of development in any country, and in order to be really fit for school, children first need to be healthy. Those who suffer poor health cannot concentrate or actively participate in school activities. Healthy children, on the other hand, attend school more regularly and can benefit fully from what the education system has to offer. School health programmes, therefore, have the potential to combine resources for education, health, nutrition and sanitation at the same venue: the school (4).

Dental and health professionals and dental medicine students in association with schoolteachers should seek to mobilize and strengthen health promotion and education activities at local, national, regional and global levels. The initiative is designed to improve the health of children, school personnel, families and other members of the community through schools. Promoting health in schools reinforces the importance of school as a healthy setting for living, learning and working (39).

Health promotion and oral health education programs should be continuously repeated in order to achieve long-term favorable results and acceptable levels of motivation for change in oral health behaviors (51).

With the improvement of knowledge of oral and physical manifestations associated with oral health behaviors and inadequate eating habits, the dental practitioner make more accurate assessment, and identify mechanisms for decreasing the potential for further damage to the teeth and the oral cavity, thereby improving their patients' quality of life (52).

An oral health program should also constantly be monitored and evaluated. It is the only way to assess the quality and the effectiveness of an oral health program applied to a determined community. The planning, monitoring and evaluation of a proposed program must take into consideration all the steps outlined, such as identifying needs, assessing resources, determining priorities, setting new goals and remodeling strategies in order to become adequate to the community where the program is applied (32).

7.6. Conclusions

Community programs should be considered and developed in order to improve knowledge and behaviors related to adolescents' oral health, drawing special attention to the intervention of various health professionals, teachers and parents in the oral health education that should be transmitted to children and adolescents.

Therefore, oral health education activities directed towards the prevention of risk factors for developing oral diseases should involve both parents and their children, because parental behavior is a significant indicator of children's oral health.

Good health is a major resource for social, economic and personal development. Health promotion, therefore, goes beyond health care. It puts health on the agenda of policy makers in all sectors and at all levels, directing them to be aware of the health consequences of their decisions and to accept their responsibilities for health. Promoting health in the social and cultural settings where people live is the most creative and cost-effective way of improving oral health and, consequently, the quality of life in a target community.

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7.9. Competing interests

The authors have declared that no competing interests exist.

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Chapter 8

Concluding remarks and future trends

In Portugal, the last years have been characterized by a serious socio-economic crisis that has caused a decrease in the level of development of health promotion and disease prevention strategies. The increase of unemployment limits the access to medical and dental healthcare. This situation has created an inequality access to oral healthcare among the population. It is known the difficulty of access to oral healthcare by individuals socio-economically disadvantaged. This occurs due to the fact that the vast majority of oral healthcare being provided by the private sector, involving funds that not all people are able to support.

The results of the present research developed demonstrate the improvement in oral healthcare in Portugal in the last years in which children and adolescents have easier access to dental appointments. However, the oral health indicators show the need of continuous improvement mainly towards primary prevention, specifically better oral hygiene habits.

The social, economical and cultural barriers towards oral disease prevention can be easily surmountable through training and motivation for the need of visiting the dentist, through programs of oral health promotion and efficient dissemination of information on adequate oral health behaviours. A person must have the adequate knowledge to understand and have a self-perception of the state of his or her own oral health and also the dentist should be aware of the need to educate and motivate their patients to have adequate oral health behaviours.

Furthermore, our results demonstrate a very low prevalence of *H. pylori* in the oral/salivary niche, which leaves the question if the young age of the sample studied can justify such a low prevalence of *H. pylori* present in the oral cavity. One of the possible explanations may be the fact that adolescents do not have gastric pathology diagnosed nor they have symptoms compatible with *H. pylori* infection, which contrasts with some studies that use adults as study sample, and mainly that present a clear diagnosis of gastric pathology associated with *H. pylori* infection. The differences observed in the prevalence of oral *H. pylori* may also be justified by the diverse methodologies/techniques employed in the different studies to detect the bacteria.

The influence of the oral microflora in the development of dental caries was also monitored. The enormous diversity of oral microbiota allows a better understanding of the oral micro-ecosystem, and these pathogenic populations present in the oral cavity provide new insights into the etiology of dental caries and suggest new targets for interventions of the disease. Therefore, no direct and independent association between bacterial strains and dental caries was found. The presence of oral microflora is clearly one of the main etiological factors for dental caries development, but cannot be considered in an isolated manner. To develop oral disease, various other factors need to be present such as high and daily sugar intake, inadequate oral hygiene habits and even genetic susceptibility. The imbalances in the resident microflora may be the ultimate mechanism of dental caries development. Oral diseases can appear in the presence of changes of the oral bacterial communities' structure and that may be related with the shift from health to disease. In the present study, we must have a special attention to the fact that *A. actinomycetemcomitans* infection was found to be associated with socio-demographic aspects. These

findings may reveal that the identification of this specific bacterial strain in early ages may be important to prevent serious oral diseases in the future and during adulthood, such as periodontal diseases.

The need for improvement of the biomaterials applied in dental medicine is also essential to prevent the initiation and development of oral diseases. Therefore, we complemented an epidemiological approach with a laboratorial component with the main goal of demonstrating, in an initial stage, the possibility of improving the antibacterial effect of a primary prevention biomaterial, in this case, a resin-based fissure sealant.

In this study, the entrapment of SDF in the resin-based fissure sealant was not found to be an effective method to potentiate the antibacterial effect of the fissure sealant, and avoid the immediate development of dental caries. The development of more laboratorial research and, afterwards, long-term clinical data are necessary in order to verify if this association between biomaterials is effective and can be considered potential assets in the field of oral health management. However, it is important to refer that more studies towards the development of the incorporation of SDF in a resin-based fissure sealant must be done in order to understand if the association of both biomaterials can become clinically viable and applied in daily clinical practice.

The association of different biomaterials with complementary physic and chemical properties can help potentiate the clinical capacities of dental materials and avoid, at least, during a longer period of time, the initiation and development of oral diseases.

Considering the last objective defined in the present research we should refer that Portugal was identified in the Portugal Health System Performance Assessment as one of the European countries in which there were proportionally more difficulties to improve the population's oral health. Even if there are no significant studies on the prevalence of oral health problems on the Portuguese adult population, there are some studies about the school population that identify moderate rates of dental caries during the last years. By considering the results obtained in the present study, it remains clear that it is urgent to implement and develop oral health education in effective terms, so that oral health knowledge can improve and the oral hygiene status of children and adolescents can change for the better. Schools are an excellent starting point for preventive action in the field of oral health. Education is the backbone of development in any country, and in order to be really fit for school, children first need to be healthy.

The present research may be considered an opening to establish more future research in order to:

- Develop better methodologies to potentiate the antibacterial effect of biomaterials and understand the effect that the association of different biomaterials can have in reducing the risk of oral disease development, such as dental caries.
- Better understand the role of the oral cavity in the detection of certain bacterial strains and its influence in oral health and in the development of other diseases, namely, of the gastro-intestinal tract.

CONCLUDING REMARKS AND FUTURE TRENDS

- Perform epidemiological studies with the assessment of etiologic risk factors that should be considered as crucial tools for oral health planners and clinicians in order to implement and institute a risk-based preventive strategy.
- Potentiate primary prevention methods directed towards oral health promotion and disease prevention that should be considered as the basis of health, more than dental rehabilitation that occurs in an advanced phase of disease development.