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Chromogenic bacteria in the oral cavity and social impact in pediatric dentistry - A Literature  
Review

Universidade Fernando Pessoa

Faculdade de Ciência de Saúde

Porto, 2020



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as part of the requirements for the obtention of  
Dental Medicine master's degree."

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**ABSTRACT**

**Objective:** To review the recent literature on the chromogenic bacterias' mechanism and the social impact that might result on children.

**Methods:** A literature review search comprised two databases: PubMed and B-on. Eligible studies were selected based on the inclusion criteria. The research resulted in a total of 650 articles, of which 18 articles were selected for a complete assessment, 9 about chromogenic bacterias and 9 about social impact. Subsequently, 23 were added.

**Topic covered:** The mechanisms of black stain formation are poorly understood. Chromogenic bacterias are an etiological factor in black stains, but the bacterial species are still little known. No studies on the social impact of chromogenic bacteria on children have been found. However, a different types of aesthetic defect of the anterior teeth (amelogenesis imperfecta; enamel hypoplasia; incisivo molar hypomineralization) can cause embarrassing situations for the child, including school bullying. Parents, who are responsible for the hygiene of their children, may also be subject to social judgments.

**Keywords:** Chromogenic bacteria; Black stain; Pediatric dentistry; Psychosocial impact; Judgment; Bullying; Aesthetic alteration

## INDEX

	<b>Page</b>
Index of acronyms and abbreviations .....	vii
<b>I Introduction</b> .....	1
1.1 Materials and methods .....	2
1.1.1 Focused question .....	2
1.1.2 Search strategy .....	2
1.1.3 Screening and selection .....	3
1.1.4 Results .....	3
<b>II Development</b> .....	4
2.1 Definition of Black Stain .....	4
2.2 Chromogenic Bacteria .....	4
2.3 Etiological factors .....	5
2.4 Treatment .....	5
2.5 Social impact .....	6
<b>III Discussion</b> .....	8
3.1 Black Stains formation .....	8
3.2 Social impact .....	9
3.3 The importance of treatment .....	13
<b>IV Conclusion</b> .....	15
<b>Bibliography</b> .....	16
<b>Attachments</b> .....	18
<b>1 . Figure 1</b> - Information flow with the different phases of the search and selection process for black stain.....	19
<b>2 . Figure 2</b> - Information flow with the different phases of the search and selection process for social impact.....	20
<b>3 . Table 1</b> - Summary of studies of chromogenic bacteria's and black stains...	21
<b>4 . Table 2</b> - Summary of studies of social impact.....	23

### **Index of acronyms and abbreviations**

BS	Black stain
BPB	Black pigmented bacteria
CPQ	Child Perception Questionnaire
MIH	Molar-Incisor Hypomineralization
OHRQoL	Oral Health Related Quality of Life
PCR	Polymerase Chain Reaction
PDT	Photodynamic therapy
TAS	Total Attribute Score

## **I. INTRODUCTION**

Discoloration, or teeth's pigmentation, is a common issue for people. There are variations of its etiology, appearance, composition, location, gravity and degree of adhesion (Hattab et al., 1999). Depending on the nature of the discoloration, it may result in clinical and/or aesthetic problems (Gasparetto et al., 2003). There are three main categories of dental discoloration: intrinsic, internalized and extrinsic (Hattab et al., 1999; Sulieman, 2005; Watts and Addy, 2001).

The black tooth stain that will be study here is extrinsic, meaning that it is a deposit of extrinsic agents located on the external surface of the tooth or in the acquired film (Hattab et al., 1999; Sulieman, 2005; Watts and Addy, 2001).

The pigments are firmly attached to the surface of the tooth and become encrusted in the retentive dental areas, such as grooves, hollows, cracks (Li et al., 2015). It is difficult to remove these external stains with conventional toothbrushing (Hattab et al., 1999).

The black stain – BS, is common for children, in primary and permanent teeth, but can also affect adults (Hattab et al., 1999). According to Chen et al. (2014) and Martin et al. (2013), both genders are equally affected. The prevalence of BS is estimated to lie between 1 - 20%, depending on the country and across studies (Ronay and Attin, 2011 cit. in Li et al., 2015).

The concept of beauty and aesthetics is specific to human beings and has emerged with the construction of civilisation (Da Silva et al., 2012). The smile has a crucial role in our perception of people's beauty. When children's smiles do not fit the aesthetics criterions, it can have negative social and psychological consequences (Olweus, 2011). Their guardians may also be affected as people could judge it as a bad oral health and question the care given to these children (Gomes et al., 2014).

It is surprising that the psychological impact of extrinsic BS is a largely unexplored area.

This literature review is motivated by the lack of studies in the current literature relative to the impact that this extrinsic colour change has on children. This work will focus on the chromogenic bacteria's mechanism and the social impact that might result on children.

## **1.1 – MATERIAL AND METHODS**

### **1.1.1 – Focused question**

What are the social impacts of the black stain produced by Chromogenic bacteria can have on children ?

### **1.1.2 – Search strategy**

To conduct this literature review, two systematic searches have been made, one on chromogenic bacterias for children and another on the social impact that the generated stains causes on them. These two searches were conducted separately because many articles were found on black stain, but none about the social impact of this pigmentation. The answer of the second question rely on the comparison with studies done on the social impact of other pigmentation issues and defects of the enamel.

#### **First digital search**

Regarding chromogenic bacteria and BS in children, done on PubMed and B-On, this following combination of keywords have been used: ((Extrinsic OR Pigment OR Pigmentation OR Stain OR Staining OR (Black pigmented)) AND (Bacteria OR Chromogenic OR Microbiota OR (Dental carie) OR Plaque) AND (Tooth OR Teeth OR Dentition OR Dentin OR Dentistry OR Dental) AND (Child OR Children OR Pediatric OR Primary OR Mixed OR Teenager)). In PubMed the following filters were applied: "5 years"; "Humans"; "Childbirth-18 years"; "Best match"; "Abstract", and in B-on: "AB Resumo", "2014 to 2020".

#### **Second digital search**

About social impact of esthetic defects in children, only done with PubMed, this following combination of keywords have been used: ((Bullying OR psychosocial OR judgments OR (psychological impact) OR (School-going children)) AND ((tooth abnormalities) OR amelogenesis OR (enamel defects) OR (anterior teeth) OR (Black pigmented) OR Pigmentation OR (Pediatric dentistry)). The search was made with the following filters: "from January 1, 2014 to February 31, 2020" and "Best match".

### **1.1.3 – Screening and selection**

The inclusion criteria considered published articles from January 2014, that were letters, reviews or investigations, in english, french and portuguese, related to child or teenage patients; patients with extrinsic BS linked to chromogenic bacteria; patients with visible and unsightly defects of the anterior teeth; and the social impact of any type of aesthetic defect in the anterior teeth.

While the exclusion criteria concerned studies published before 2014; adult patients; extrinsic dental stains due to the consumption of coloring food and articles about the social impact of dental pain associated with visible enamel defects.

Publication records and titles identified by the electronic search were screened based on the inclusion criteria. A second selection was made by screening the abstracts. Finally, a reading of the full texts of the selected articles was done. Then, articles that met the inclusion criteria were processed for data extraction.

### **1.1.4 – Results**

#### Study selection

The figure 1 shows the information flow with the different phases of the search and selection process for black stain (Attachment 1). During the research about chromogenic bacteria, the results were initially filtered by eliminating 26 repeated articles in the different databases, then 167 were rejected by the title. Into the 44 remaining, a thorough reading of the abstracts was made and 28 articles were eliminated. Finally, a complete reading of the 16 articles was carried out and 9 articles, including 2 added manually were selected to study the role and mechanism of chromogenic bacteria in BS in children.

Regarding research on social impact, figure 2 (Attachment 2) shows the information flow. 389 articles were eliminated after reading the title, among the 413 initials. After reading the abstract of 24 remaining articles, 13 studies were eliminated. 2 articles were initially added manually. So, after doing a full read 9 articles were finally selected to study the social impact of esthetic defects (of any type) on the children's anterior teeth.

Furthermore, to support and develop information indirectly related to the objectives of this study, 23 articles were added to the bibliographic search. Using a total of 41 articles.

## **II. DEVELOPMENT**

### **2.1 – Definition of Black Stain**

The BS is a specific type of extrinsic stain. Clinically, it is diagnosed as a dark, pigmented line, parallel to the gingival margin, or as an incomplete "line", "coalescence" of dots ( $\leq 0.5$  mm in diameter) or dark areas, which generally extends at the level of the gingival margin (at the level of the contour of the marginal gingiva), and which rarely extends to the incisal third of the crown (Bircher, 2008). According to Gasparetto et al. (2003), BS are usually present on the posterior teeth, but can also be found on the vestibular, lingual, or palatal surfaces of the anterior teeth. For Zhang et al. (2017) these stains can also extend over diffuse areas, covering part of the crown. For the same author, it can occur in the primary and permanent dentition. However, many studies reported that BS were more frequently found on primary teeth, as the pigments disappear when the transition from primary to permanent dentition is complete (McDonald and Avery, 2011; Duggal et al., 2014 cit. in Durand and Sotomayor, 2018). This is believed to be due to certain characteristics of the primary tooth, such as permeability and porosity, which are greater than in permanent teeth (Banda et al., 2011).

### **Epidemiology**

Among the publications reviewed, prevalence ranged from 1% to 20% (Ronay and Attin, 2011 cit. in Li et al., 2015). For instance, in Potenza, Italy, 6.3% of children from 6-12 years old had BS (Koch et al., 2001), 14.8% in Brazil (Gasparetto et al., 2004) and 6.72% in Peru (Durand and Sotomayor, 2018). According to several authors gender has no impact on BS (Chen et al., 2014; Martin et al., 2013).

### **2.2 – Chromogenic Bacteria**

The bacterial etiology of BS is not clearly defined. In 2015, Li et al. hypothesized that differences in microbiota, observed in children with BS, favour an environment conducive to pigment formation. There is still a lack of evidence about the species and the specific role of bacteria regarding BS formation. Nonetheless, in the literature review of Zyla et al. (2015), Gram-positive rod type, anaerobic and facultative anaerobic were predominant in the microbiota of children with BS. Tree Polymerase Chain Reaction - PCR studies, found that *Actinomyces* was more abundant in microbiota of children with BS (Saba et al., 2006,

Heinrich-Weltzien et al., 2014 and Li et al., 2015). It suggests that *Actinomyces* might be responsible for the deposition of BS. The information from the recent literature about the responsibility of chromogenic bacteria regarding the BS formation will be detailed in the discussion section of this study.

### **2.3 – Etiological factors**

Unclear etiological factors are associated with the formation of BS. In 2014, Chen et al. observed more severe BS on the lingual surfaces of antero-inferior teeth, where salivary secretion is the most abundant which suggests that saliva plays a role in the development of BS. For Chen et al. (2014) and Martin et al. (2013), consumption of vegetables, fruits dairy products, eggs and soy sauce may facilitate BS' formation. However, Durand and Sotomayor (2018) in their cross-sectional study, found no significant association between diet and the formation of BS ( $p=0.406$ ). In a literature review, Zyla et al. (2015) found no relationship between oral hygiene; socioeconomic environment; age of the child and the formation of a chromogenic microbiota. Chen et al. (2014) found that children with a history of pneumonia had more BS teeth than those without ( $p<0.001$ ).

### **2.4 – Treatment**

There is currently no consensus in the literature on the treatment protocol for pigment removal (Kharkwal et al., 2011; Theodoro et al., 2012). The treatment is difficult because of the high quantity/proportion of calcium and phosphate in the biofilm, making manual toothbrushing insufficient to remove the stains (Theilade et al., 1973 cit. in Pessoa et al., 2015).

The BS are usually removed by professional prophylaxis and polishing, however, its tend to re-form within 30 days with this type of treatment (Ronay and Attin, 2011 cit. in Pessoa et al., 2015; Huamán, 2013 cit. in Durand and Sotomayor, 2018).

In 2015, Pessoa et al. treated one patient with photodynamic therapy - PDT, that consists of applying a non-toxic photosensitizer (toluidine blue) to the tooth surface to be treated and then exposing it to light with a wavelength of 660nm. This generates singlet oxygen and free

radicals, which are cytotoxic to the target tissue, and reduce the number of microorganisms. After PDT, the remaining stains were removed with a Gracey curette and professional prophylaxis. This treatment was carried out once a week for 5 weeks, until the pigments had completely disappeared. The results showed no recurrence within 7 months. Moreover, after treatment, the authors noted a net reduction in the overall prevalence of bacteria (22%). In 2012 Theodoro et al. conducted a study on PDT, however, failed to show statistically significant benefits for this therapy in their clinical results.

## **2.5 – Social impact**

### **Importance of beauty**

It is recognized that physical appearance plays a role in the way people perceive an individual. In 1972, Dion (cit. in Craig et al., 2014) showed that nice looking adults and children are judged more positively for a range of personal characteristics while those that are "unattractive" are perceived as being more mean or dishonest. The face has a predominant role in physical appearance and is partly responsible for positive or negative social judgments (Willis and Todorov, 2006).

The mouth is the communication center of the face, attracting the attention of the speaker (Van der Geld et al., 2007). The anatomy, colour and harmony of the teeth are particularly important to the appearance of the face, and subtle deformities can lead to taunting and mockery (Cunningham, 1999).

For children, the smile is a predictor of quality of life, and the way he/she interacts with the environment (Filstrup et al., 2003). It reveals self-esteem, self-confidence and well-being (Källestal et al., 2000).

In 2001, the influence of tooth decay and tooth discolouration on social judgments has been studied on a Chinese population in the UK (Feng, 2001). Kershaw et al. (2008) showed that tooth colour has an impact on how others perceive an individual. People with coloured teeth are judged more harshly for a range of personality traits such as "intellectual ability", "social competence", "satisfaction in relationships" and "psychological adjustment". This is because damaged or discoloured teeth are frequently equated with poor oral hygiene. In addition,

enamel defects such as imperfect amelogenesis, dental fluorosis and MIH are confused with problems of hygiene and carelessness of appearance.

### Bullying

Bullying is described as an antisocial behavioural phenomenon that violates the rights of another person and reflects intentional and repeated aggression, verbal or physical, against anyone unable to defend him/herself and can occur in any social context (Olweus, 2011). Bullying of schoolchildren is a worldwide phenomenon, and dentists regularly meet children or adolescents who are or have been victims of it (Fekkes et al., 2006). Bullying victims can have serious psychological consequences, such as isolation, depression, anxiety, and can affect performances and learning, in the short and long run (Bond et al., 2001). According to child developmental psychology, abstract thinking, self-image and self-concept begin around 6 years old (Abanto et al., 2011). This is the age at which children begin to judge and compare their physical characteristics and individual qualities with those of other children. However, several authors have observed that most children that are victims of bullying are at least 12 years old (Fleming and Jacobsen, 2010). Bullying of schoolchildren is a global phenomenon, and its effects can last for months and result in poor learning performance, low self-esteem and introspection (Fekkes et al., 2006).

### **III. DISCUSSION**

#### **3.1 – Black Stains formation**

Table 1 (Attachment 3) summarizes the selected studies about BS.

The black pigmentation may be due to chromogenic bacteria in association with chemical compounds, such as ferric sulfide, resulting from the reaction between hydrogen sulfide produced by chromogenic bacteria and iron, present in saliva or gingival fluid (Zyla et al., 2015). Indeed, a higher amount of iron than usual has been observed in the dental plaque of children with the pigmentation ( $p < 0.001$ ) (Zhang et al., 2017) (Attachment 3). Copper has also been suggested by Zyla et al. (2015) to play a role in the formation of BS. However, Zhang et al. (2017) found no statistically significant difference in copper content between children with and without BS ( $p = 0.912$ ). Those different findings may be due to the metallic cures used to collect the samples (Zyla et al., 2015).

The importance of the saliva for the BS development could be explained by the higher amount of calcium and phosphate ions observed by Noorkhakim et al. (2018) in children with BS (Attachment 3). Phosphate has a buffering capacity that maintains a high salivary pH and would affect the plaque microbiota. This less acidic pH could be a favourable factor for the development of chromogenic bacteria (Noorkhakim et al., 2018). Furthermore, a more basic pH restrains the development of cariogenic bacteria, such as *Streptococcus mutans*, found to be less present in children with BS and so, may protect against the formation of dental caries (Heinrich-Weltzien et al., 2014). This is consistent with Zyla et al., 2015, who in their literature review, find that children with BS have lower dental caries experience.

Regarding the exact species of chromogenic bacteria responsible for BS formation, *Actinomyces* found in greater quantities in children with BS than without, may have an important role in the formation of pigments due to its production of hydrogen sulfide (Saba et al., 2006; Heinrich-Weltzien et al., 2014 and Li et al., 2015). However, other microbiota alterations between children with and without BS were observed. Saba et al. (2006) detected a higher frequency of *Aggregatibacter actinomycetemcomitans* in the BS group but not Heinrich-Weltzien et al. (2014) and Li et al. (2015) (Attachment 3). Alteration in the abundance of other species like *Tannerella*; *Treponema*; *Corynebacterium*; *Cardiobacterium*

and *Haemophilus* was observed by Li et al. (2015). Those results may be explained by the different techniques used to determine the number of selected bacterias in the samples: Saba et al. (2006) used PCR and Electrophoresis in agar gel; Heinrich-Weltzien et al. (2014) used real-time PCR with specific chosen primers; and Li et al. (2015) used PCR and 16S rRNA gene sequencing with the pipeline tool MOTHUR. Furthermore, Li et al. (2015) analysed samples collected from tooth pigmented surface, saliva and supra-gingival plaque, whereas Saba et al. (2006) analyzed samples collected from tooth pigmented surface only and Heinrich-Weltzien et al. (2014) from supra-gingival plaque only.

It is the authors believe that the existing literature demonstrates the importance of the microbiota alteration for BS formation but that more studies are needed to fully understand the role of chromogenic bacteria in the etiology of BS.

### **3.2 – Social impact**

In the literature, there are no any study on the social impact of dental stains due to chromogenic bacteria in children. Therefore, in this literature review, studies on the social impact of aesthetic disorders of the anterior tooth enamel, such as fluorosis, amelogenesis imperfecta, enamel hypoplasia and opacity and molar-incisor hypomineralization – MIH were used to understand what may be the social impact of BS due to chromogenic bacteria on the children's lives. The Table 2 shows the selected studies (Attachment 4).

#### Characteristics of children who judge

Several authors, (Craig et al., 2014; Nayak et al., 2018; Siddiq et al., 2020) have studied the ways in which children judge their peers with visible enamel defects. They asked children of different gender and ages in different schools to judge a girl and a boy, with or without enamel defects, from a photograph. The same questionnaire was used in the 3 studies allowing result comparison. The participants gave a rate for each personal attribute, such as "naughty", "clever", "rude", "kind", "honest", "he/she does not care about appearance", "careful", "lazy", "confident", "helpful" and "stupid". The result is an overall score, the "Total Attribute Score" – TAS, which can vary from 11 (the most negative) to 44 (the most positive). The studies by Craig et al. (2014) and Siddiq et al. (2020) found that a lower mean TAS was attributed for subjects (girl and boy) with enamel defects (29,2 and 25,9±4,73, respectively)

than without (31,45 and 34,8±4,83, respectively), and this difference was significant ( $p<0.001$  for both studies). Similar results were observed by Nayak et al., (2018) with a mean TAS lower in children with enamel defects (27.5) than without (30.59) (Attachment 4).

According to the study by Nayak et al. (2018) and Siddiq et al. (2020), boys and girls make similar social judgments. However, in the studies by Craig et al. (2014), boys attributed lower TAS ( $p<0.002$ ).

Studies evaluating child-attributed TAS have compared different age groups. Craig et al. (2014), interviewed 11/12 and 14/15 years old children; Nayak et al. (2018), 13/14 and 15/16 years old children; and Siddiq et al. (2020), 12/13 and 14/15 years old children. Each of these studies found similar scores between the two age groups. Thus, age does not seem to influence the social judgments regarding enamel defects.

In the study by Nayak et al. (2018), among different criteria (gender, age and type of school), the only significant difference found among the participating children was the type of school ( $p=0.019$  for boys and  $p=0.056$  for girls) (Attachment 4). Students in the urban school, attended by a relatively well-to-do population, made more negative judgments than those in the rural school when looking at the male photographic subject with an enamel defect. The authors of this study suggest that it is possible that children of lower socioeconomic status, who are likely to have poorer dental health, may have lower expectations for treatment and thus be more generous in their social judgments than children from higher socioeconomic environments. According to the results of Craig et al. (2014) and Siddiq et al. (2020), the type of school (affluent or socially disadvantaged population) did not have a significant effect on mean TAS.

These 3 studies used computers modified photographs to be able to compare the same child with or without tooth aesthetic defect. The colours of the addicted defect were bright white or light brown. BS are dark and when located on the vestibular surface of the anterior tooth are easily visible. It is the author point of view that the results showing a lower TAS among children with brighter enamel defect (Craig et al., 2014; Nayak et al., 2018; Siddiq et al., 2020) could also be associated with BS.

### The consequences of the judgment

In 2014, Parekh et al. interviewed 13 to 16 years old children from the UK with amelogenesis imperfecta to determine the social impact of tooth esthetical defect. A questionnaire was developed based on in-depth interviews and a modified short version of the Child Perception Questionnaire - CPQ. The results showed that most children with amelogenesis imperfecta received comments about the colour and appearance of their teeth that caused feelings of isolation and affected their self-confidence. This makes it harder for them to smile and talk to others:

*"If someone sees it, they go 'Oh, don't you brush your teeth?' and stuff like that"* [P2, 13 years]

*"I'm not feeling well, I'm feeling a little bit bad, because they can see that my teeth look like dirty"* [P7, 13 years]

*"If the colour was fine, I'd feel a lot more confident"* [P2, 13 years]

*"The colour just knocks my confidence"* [P1, 16 years]

*"When all my friends are talking, I'd want to join in but I don't want to show my teeth"* [P3, 16 years]

This study also showed that 50% of the participants reported being annoyed at least "occasionally". Boys were 24% to report being teased "often", while girls were 11%. In addition, the smile ratio worsened for these children, as 30% of participants reported avoiding smiling "occasionally" and 23% reported avoiding smiling "often" or "all the time". Girls appear to avoid smiling "all the time" more often than boys (32% vs. 14%) (Attachment 4).

Another study tried to determine the relationship between imperfections in the anterior teeth and emotional and social well-being (Gupta et al., 2019). 528 Indian children between 10 and 15 years old were interviewed. Children with dental caries on the anterior teeth avoided school or leisure activities significantly more than the other children without unsightly dental caries. Children with trauma to the anterior teeth were socially less confident because they were often teased by others. Conversely, children who had never experienced a traumatic incident and who had no aesthetic defect were socially more skillful and stronger. This difference was statistically significant ( $p=0.000$ ). The results also revealed that 49.2% of the

children were shy if they had an aesthetic defect, and 37.5% were concerned about their appearance if they did not have healthy front teeth. Furthermore, among children with an unsightly dental problem, 48.7% did not want to speak or read loudly in class, 88.4% avoided school or leisure activities, 89.2% did not want to spend time with other children, 66.7% avoided smiling or laughing, 77.1% were afraid of being teased by other children and 71.2% were afraid of being questioned by other children. This clearly shows that dental appearance may have a considerable impact on a child's social well-being.

Oral health-related quality of life - OHRQoL, is defined as the absence of negative effects of oral conditions on social life and a positive sense of dento-facial self-confidence. Gomes et al. (2014) in Brazil used The Early Childhood Oral Health Impact Scale (ECOHIS) to determine the impact of dental caries and traumatic dental injuries on the OHRQoL of 843 children between 2 and 5 years old. It results that 32.1% of them had their OHRQoL negatively impacted.

At school, the judgment of others may extend to bullying and harassment. In 2014, Al-Omari et al. used a short form of CPQ to determine the relationship between OHRQoL and bullying for 920 Jordanian children between 11 and 12 years old. This cross-sectional study showed that 41.2% of the 920 children reported being bullied about their teeth and their OHRQoL were more affected in regard of "oral symptoms"; "functional limitations"; "emotional well-being"; "social well-being", than children not being bullied ( $p < 0.001$ ). However, in this study, no information was reported about the nature of the dento-facial alteration that created the bullying. Scheffel et al. (2014) report testimonies of child victims of bullying (Attachment 4). One child with enamel hypoplasia was nicknamed the "vampire", another with MIH was often mocked with his "rotten teeth". Even worse, a child with amelogenesis imperfecta was discriminated during breaks and group activities. Negative comments about teeth can be routine, interfering with the child's social interaction.

Even if these selected studies did not use the same questionnaire protocol it is clear that aesthetic oral alteration may have an impact on the quality of life of children. From the point of view of the author, visible BS should be taken into account to minimize the risk of bullying due to orofacial alteration.

Gomes et al. (2014) also questioned the impact than can have the child oral health conditions on the family quality of life (Attachment 4). It showed that 26.2% of the parents of the 843

children, between 2-5 years old, had lower OHRQoL. It is less than children themselves (32.1%). Cavitated lesions, traumatic dental injuries and parent's/caregiver's perception of child's oral health as poor were significantly associated with a lower family's OHRQoL ( $p < 0.001$ ). The ECOHIS mean score showed that "reported pain" from children was the main cause of negative impact on parent's quality of life. Frequently they "felt guilty" and "been upset". Dantas (2019) evaluated the social impact of HIM on children and their family. 30 portuguese children between 5-14 years old with HIM - with a minimum of an anterior tooth affected - and their parents were asked to fill a questionnaire. For 76.7% of the questioned children, HIM was not an issue and only 10% received bad comments at school. However, when the child was concerned about the dental alteration, parents were less preoccupied ( $p < 0.001$ ); 66.7% of parents did not know that their child was embarrassed by the structural defect. This study showed that 80% of parents are more concerned with tooth defects when it occurs on anterior teeth. Finally, it seems that the family received more frequent questions from others about their child's tooth aspect than the child himself.

Those two studies are difficult to compare due to the difference among samples (number and age of children), aesthetical alteration and questionnaire protocol. Nevertheless, it seems that the family is less impacted with the children's dental aspect than the children themselves. Parents are more concerned when tooth pain is present. It seems frequent that parents are not aware of the social impact that can have oral aesthetic alteration on children at school. However, some received comments from other adults.

Visible BS may be easily perceived by others as an oral hygiene deficiency. Parents are responsible for child hygiene especially at an early age. It is possible that this misunderstanding about the BS etiology may provoke interrogations and judgments from friends and relatives and eventually create a negative impact on the parent's quality of life.

### **3.3 – The importance of treatment**

It is not uncommon for adults to seek dental treatment for aesthetics reasons. For children, dentists may be more reluctant to provide a treatment for aesthetic reasons such as enamel opacities. In the study by Parekh et al. (2014), children (with amelogenesis imperfecta) were asked whether it was important for them to improve the colour, shape and size of their teeth

and to improve their smiles. In 63% of the cases, improving the colour of the teeth was the most important. For Dantas (2019), 16.6 % of children between 5-14 years old with HIM asked for a colour alteration of their anterior tooth. In addition, in their reported case study, Scheffel et al. (2014) found that after completion of dental treatment and participation in family and school psychoeducational counselling, children were no longer verbally abused. Significant improvements in self-esteem, self-confidence and socialization of all patients began within a few days after completion of treatment. In addition, parents' satisfaction with their children's appearance improved significantly.

In this context, treatment of BS consisting of regular pigment removal seems important to improve the quality of life of children and avoid discriminatory behaviour.

#### **IV. CONCLUSION**

No studies evaluating the impact of BS on the child's social life were found. However, in the literature, many studies show that tooth aesthetic alterations have an impact on the social life of children/adolescents, and could lead to school bullying. Being a pigmentation responsible for dental aesthetics alterations, it is possible that BS may have the same negative impact. Although the comparison cannot be direct because, unlike intrinsic alterations such as MIH, BS is a visible extrinsic alteration and can be easily removed by professional prophylaxis.

BS is quite frequent in primary dentition and the negative impact falls on the parents/caregivers for the children as they are, at this stage, the responsible for their children's oral hygiene.

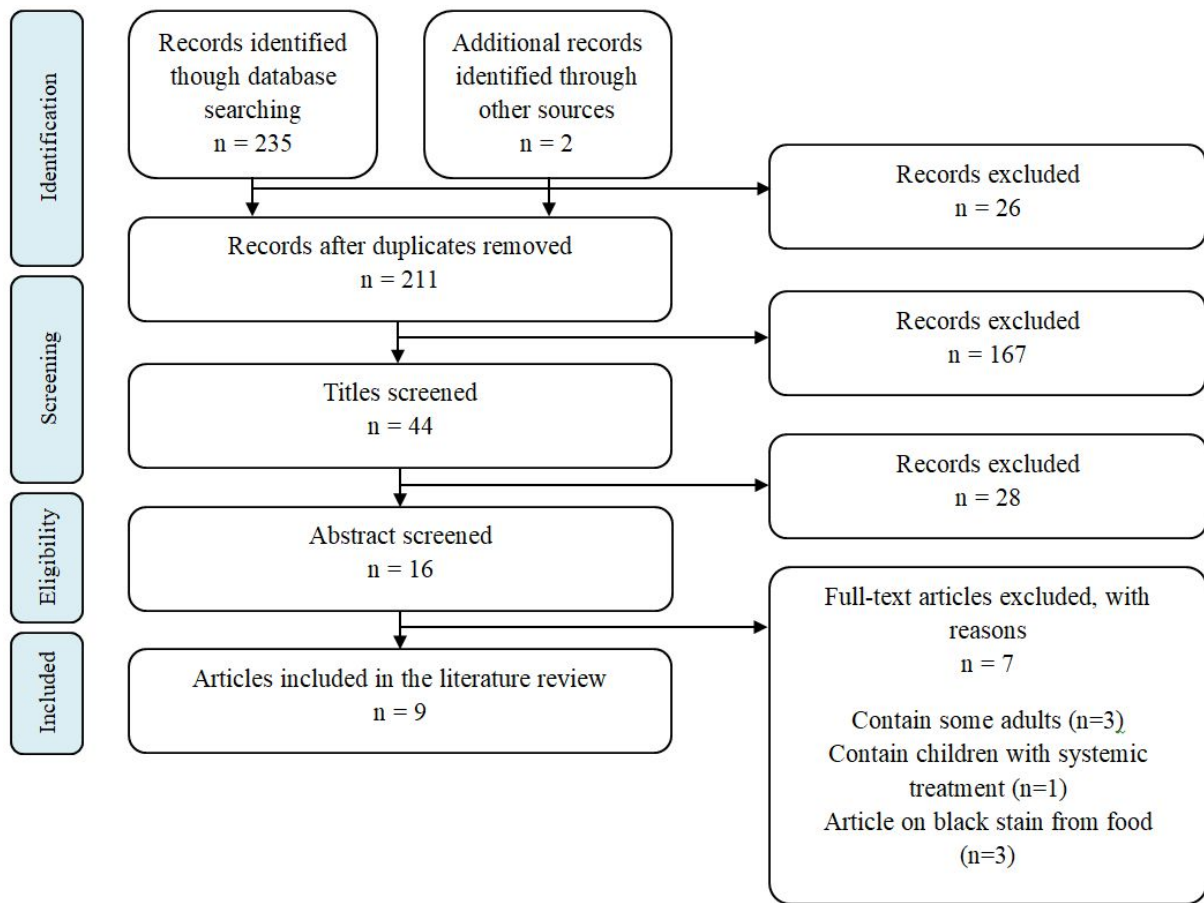
BS is not considered to be important for public health by dental health professionals although it does result in an observable colour difference. That being said, it is important for dentists to be aware that dental treatments such as PDT or a regular professional prophylaxis can offer significant psychosocial benefits to young patients with BS.

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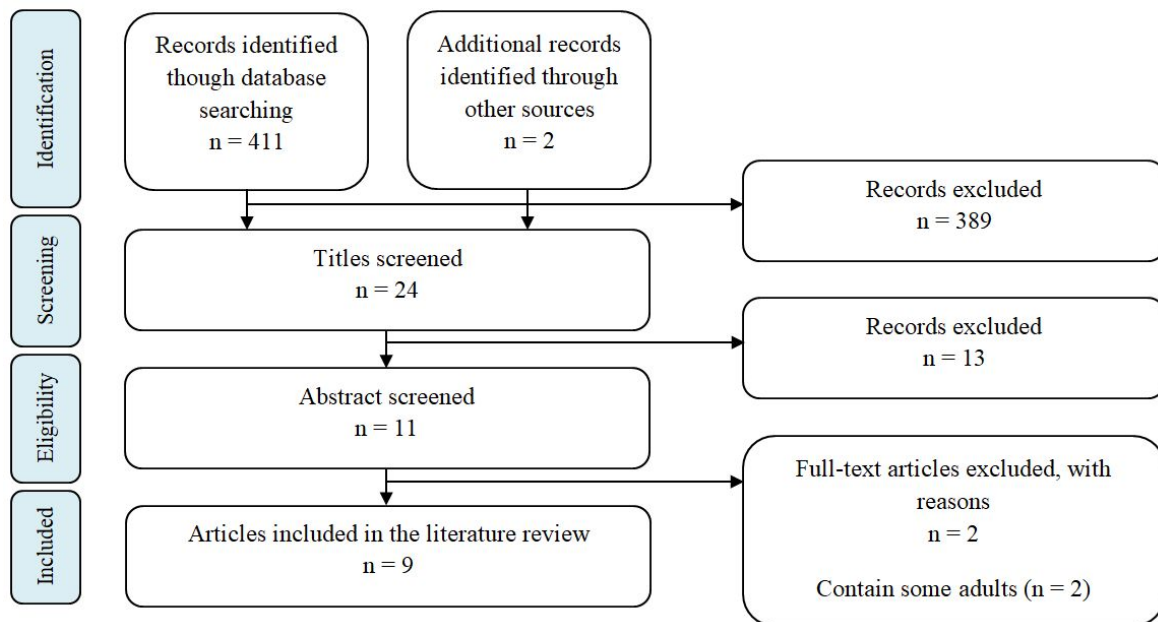
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**ATTACHMENTS**



Attachment 1. Figure 1 - Information flow with the different phases of the search and selection process for black stain



Attachment 2. Figure 2 - Information flow with the different phases of the search and selection process for social impact

Reference, year of publication, study type	Country, sample size, age	Material and methods	Aim	Colouring elements	Microbiological result, dental cavity, saliva	Conclusion
Durand and Sotomayor, 2018 Cross-sectional study	Peru n=714 6-7 y	Epidemiological analysis with cases and controls.	To determine the prevalence and risk factors associated with extrinsic discolouration of primary dentition.	Other risk factors were assessed; however, no statistical significance was found: - poor oral hygiene - food consumption - administration of postuption drugs - consumption of nutritional supplements and natural water	N/I	The prevalence of extrinsic BSs was 6.72%. There was a significant association between the presence of extrinsic BSs and poor experience of caries.
Noorkhakim et al., 2018 Investigation article	Indonesia n=32 4-8 y	Saliva samples analyzed by UV-Vis spectrophotometer.	To determine the levels of calcium and phosphate in the saliva of children with and without BS.	There is more Calcium in the children's saliva with BS than without BS. The difference is significant. There is more Phosphate in the children's saliva with BS than without BS. The difference is significant.	N/I	The calcium and phosphate levels in the saliva of children with BS > without BS.
Zhang et al., 2017 Investigation article	China n=46 3-6 y	BS and DP sample analyzed by inductively coupled plasma-mass spectrometry. Raw sequencing of the bacterial 16S rRNA gene.	To study on the relationship between iron and BS.	Iron and bacteria: In BS: ferrous compounds In PB: ferric compounds Iron levels: BS>DP (p<0.001) Copper levels: BS=DP (p=0.912) Iron plays an important role in transportation. Increased magnesium/iron transport activity in BS groups.	In BS, the colour alterations are associated with: <i>Actinomyces</i> (+ abundant in BS). METABOLISM: Glycolysis, metabolism of starch, sucrose, vitamin B6: + abundant in BS + metabolism: teeth with BS + ion transport and transport activity in the BS	There was significantly more iron in the BS > DP.
Zyla et al, 2015 Review Article	Poland N/I	Digital search: MEDLINE Results: n=10 articles	To investigate the link between BS and the experience of tooth decay.	High calcium and phosphate is content in BS. The black compound is certainly ferric sulfide. The sulfur complex and metal ions are probably responsible for the BS's black colour.	The majority of bacteria are Gram positive rods (anaerobic and facultatively anaerobic).  <u>SALIVA</u> : Significantly higher levels of inorganic phosphates, calcium, sodium, copper, and protein; and significantly lower in glucose, in patients with BS than without. The buffering capacity and salivary pH are higher in children with BS.	Compared to nondiscoloured DP, the BSs contains fewer cariogenic bacteria. Calcium concentrations and saliva buffering capacity, as well as non-cariogenic DP may explain the association between a lower caries experience in children with BS.
Li et al., 2015 Investigation article	China n=25 4-5 y	BS, saliva and supra-gingival DP samples analyzed by PCR and 16S rRNA gene sequencing with the pipeline tool MOTHUR.	To analyze the microbiota of BS in primary dentition (by sequencing of the 16S rRNA gene), by comparing the microbial composition of DP and saliva in children without cavities, and with or without BSs.	N/I	60 samples: 10 phyla, 19 classes, 32 orders, 61 families, 102 genera.  <b>On the 10 phyla:</b> DP (with or without BS, BS's sample): Contain the 3 most abundantly 10 "Phyla" bacteria: <i>Firmicutes</i> , <i>Proteobacteria</i> and <i>Bacteroidetes</i> (> 95% of the total sequences). <u>SALIVA</u> (with or without BS): Contain the 3 most abundantly 10 "Phyla" bacteria: <i>Firmicutes</i> , <i>Proteobacteria</i> and <i>Bacteroidetes</i> ( <i>Firmicutes</i> : > 50% of the total sequences). <b>Out of the 120 genera:</b> DP (in plaque and BS): The 5 most abundant are: <i>Streptococcus</i> , <i>Neisseria</i> , <i>Burkholderiales unclassified</i> , <i>Capnocytophaga</i> and <i>Neisseriaceae unclassified</i> (> 60% of the total sequences). <u>SALIVA</u> (with or without BS): <i>Streptococcus</i> , <i>Neisseria</i> and <i>Capnocytophaga</i> (> 60% of the total sequences).  More abundant bacterial genera in children with BSs: <i>Actinomyces</i> ; <i>Tannerella</i> ; <i>Treponema</i> ; <i>Corynebacterium</i> ; <i>Cardiobacterium</i> ; <i>Haemophilus</i> .	Alterations in the oral microbiota may be associated with the formation of BSs. Children with black colouring present a reduced microbial diversity, and the formation of BSs may be linked to an alteration of the plaque microbiota.
Pessoa et al., 2015 Case report	Brazil n=1 10 y	BS and DP's microbiological analysis before and after PDT, by RT-PCR.	To describe the clinical case of a child with BSs on the teeth, and offers an alternative therapy aimed at eliminating black pigmentation, based on photodynamic therapy.	N/I	Analysis of DP samples from 10 sites: The bacterial species identified by RT-PCR were: <i>A. actinomycetemcomitans</i> ; <i>P. gingivalis</i> ; <i>P. intermedia</i> ; <i>P. melanogenica</i> ; <i>P. nigrescens</i> .  Overall prevalence of 64% of the bacteria studied, ↑ prevalence of the species <i>P. melanogenica</i> and <i>P. intermedia</i> , present in 80% of the samples, while <i>A. actinomycetemcomitans</i> was found in 20% of the samples.  After treatment, a marked reduction in the overall prevalence of bacteria was observed (22%), in particular for <i>P. intermedia</i> , which was not identified in any of the samples after treatment.	Significant reduction in the levels of black pigmented bacteria after treatment, without recurrence during a follow-up period of 7 months.
Chen et al., 2014 Investigation article	China, Shanghai n=1397 N/I (preschool)	Examination of the presence of BS and DP. Questionnaire addressed to parents/guardians.	To study the prevalence and associated factors of BSs.	N/I	<b>CARIES</b> Children with BS < without BS and the average dmft too. <b>DP</b> The Visible Plaque Index = significantly: children with BS < without BS. DP and cavities → less in BS.	The rate of black colouration in this sample was 9.9%. Children with BSs had a significantly ↓ caries. The factors associated with the BSs were: age; born in Shanghai; a high level of parent education; a low visible DP index; a low dmfs; a good oral hygiene; a low use of baby bottles; a diet with lots of soy sauce; a medical history of pneumonia.

Reference, year of publication, study type	Country, sample size, age	Material and methods	Aim	Colouring elements	Microbiological result, dental cavity, saliva	Conclusion
Heinrich-Weltzien et al., 2014 Investigation article	Germany n=93 3-10 y	Supragingival DP or BS samples analyzed by using RT-PCR with selected species.	To assess the experience of caries and the microbiota in systemically healthy children with and without BSs.	N/I	<p>Lower overall caries prevalence in children with BSs (primary dentition) than children without BSs (dmft: p=0,013; and dt: p=0,005).</p> <p><u>Bacteria with higher number in BSs than without:</u> <i>A. Naeslundii</i> (p=0,005) and <i>P. gingivalis</i> (no significant).</p> <p><u>Bacteria with lower number in BSs than without:</u> <i>F. nucleatum</i> (p=0,001) and <i>Lactobacillus sp.</i> (p=0,001).</p> <p><u>Bacteria without difference between samples with and without BSs:</u> <i>S. mutans</i>, <i>S. sobrinus</i>, <i>A. actinomycetemcomitans</i> and <i>P. intermedia</i>.</p> <p>Caries-free children with BSs has more <i>A. naeslundii</i> (p=0.013) than caries-free children without BS.</p> <p>With or without caries, <i>Lactobacillus sp.</i> is more present in children without BSs.</p> <p>Caries and BSs affected children present less <i>F. nucleatum</i> (p=0,007) and more <i>P. gingivalis</i> (p=0,085) and <i>A. actinomycetemcomitans</i> (p=0,061).</p>	The different microbial composition of BSs might be associated with a lower caries experience in children with BSs. The role of chromogenic bacteria associated with periodontitis needs further studies.
Saba et al., 2006 Investigation article	Italia n=200 6-12 y	BS samples analyzed by using PCR process and electrophoresis gel on the agar gel.	To determine the agent involved in the formation of BSs using modern techniques, and propose preventive and therapeutic protocols which, along with the solution of the aesthetic problem, do not modify the balance of the oral microbiota.	N/I	<p>50% of the samples with BSs are positive for <i>Actinomyces spp.</i> against 20% of the control samples. Statistical results show that patients with <i>Actinomyces spp.</i> are 4 times more likely to have BSs than patients without this bacteria (OR = 4.0, 95% CI).</p> <p>70% of the samples with BSs are positive for <i>A. actinomycetemcomitans</i> against 20% of the control samples.</p> <p>According to the statistical results, patients with this bacterium are 9 times more likely to have BSs than patients without <i>A. actinomycetemcomitans</i> (OR = 9.3, 95% CI).</p> <p>DNA from <i>P. gingivalis</i> and <i>P. melaninogenica</i> was absent from the samples with and without BS.</p> <p>Logistic regression analysis reveals that the two bacterial species (<i>Actinomyces spp.</i> and <i>A. actinomycetemcomitans</i>) are independent.</p>	<p><i>P. gingivalis</i> and <i>P. melaninogenica</i>, were not involved in both the black stained subjects and the control group.</p> <p>However, <i>Actinomyces spp.</i> could be implicated because its presence was demonstrated in 50% of patients with BS and in 20% of patients without BSs. As well as <i>A. Actinomycetemcomitans</i> with its presence in 70% of patients with BSs and in 20% of patients without BSs.</p>

BS – black stain; DP – dental plaque (without BS); N/I – no information; OR – odds ratio; PCR – polymerase chain reaction; RT-PCR – real-time PCR.

Reference, year of publication, study type	Country, sample size, age	Material and methods	Aim	School harassment, intimidation, social judgments	Aesthetic disorders and self-confidence, treatments	Consequences on the smile	Conclusion
Siddiq et al., 2020 Cross sectional study	India n=437 12-15 y	TAS with computer modified photographs (♂ and ♀) 12/13 and 14/15 y Public and private school	To evaluate children's perception compared to other children with visible enamel defect (dental fluorosis).	TAS average photos with fluorosis < without. There is no significant difference in relation to the age, gender, social class, type of school (private or public) of the children.	N/I	N/I	Children in this study perceived that fluorosis could have an impact on their own appearance. Children make negative judgments about other children with dental fluorosis.
Dantas, 2019 Investigation article	Portugal, n=30 children n=30 parents 5-14 y	2 questionnaires, 1 for children and 1 for parents.	To evaluate the impact of the HIM on anterior teeth on children and if they have the same perceptions than their parents.	N/I	46.7% of children asked their guardian the reason for their dental deterioration. Among these 14 children, only 5 sought treatment to change the colour, and they were in the older age group (7 to 13 y). The 8 children concerned about the alteration were the same ages.  It seems that ♀ feel more uncomfortable than ♂, because among the 7 children feeling uncomfortable, there were 5 ♀ and 2 ♂. 73.3% of children were not concerned about their dental defect.	66.7% of guardians did not know that their child was embarrassed by the structural defect. 76.7% of the children were not bothered by their structural defect, but in the event of discomfort felt, this is perceived as being more important by the children, than by their guardians. No child cared "very little", against 12.5% of those responsible. 80% of guardians were more concerned with an alteration if it was present on an anterior tooth, while 26.7% of their children were with the same opinion. Most children and guardians agree that at times the child has stopped smiling, and that friends have noticed the alteration. Most agree that those around them do not ask children or their guardians what they have on their teeth. However, when the children answered "yes" and it was "very often", and their guardians did not respond.	The opacity in anterior teeth has an insignificant role in the social and personal children's life. Children and their caregivers have different perceptions of HIM that affect children about: concerns they have, other people's comments, and whether others have noticed the aesthetic defect. Gender and age of the children did not have a significant influence on the answers given to the questions asked. Additional studies are needed to assess the impact of HIM and dental aesthetics on psychosocial aspects for children.
Gupta et al., 2019 Cross sectional study	India n=528 10-15 y	Questionnaire	To determine whether imperfections in anterior dentition affect children's emotional and/or social well-being.	N/I	Shy/same age with aesthetic defect ♀>♂ ♀ lower socio-economic status and dental aesthetic problems = emotionally insecure. Children with visible dental caries (on the front teeth) significantly avoided school or leisure activities compared to children without previous caries. Children with trauma to the anterior teeth were very significantly less socially confident, and often teased, than children without any visible dental defect, who were socially more skilled and stronger (p=0.000).	49.2% of children were shy if they had an aesthetic defect, and 37.5% were concerned by their appearance if they did not have healthy anterior teeth. Regarding social well-being, 48.7% don't want to speak/read loudly in class; 88.4% avoid school or leisure activities; 89.2% do not want to spend time with other children; 66.7% avoid smiling or laughing; 77.1% feared being teased by other children and 71.2% feared being questioned by other children if they did not have an aesthetic dentition.	Whatever the cause of the aesthetic defect, 53.8% of children were concerned with what other would think of them if they did not have a proper anterior dentition. This % of children was found to be lagging behind their counterparts who had a proper anterior dentition, irrespective of the gender or the socioeconomic status, or the level of their parents' education and their jobs. This difference was statistically highly significant. Imperfections of the anterior teeth have a negative impact on the children's psychological and social well-being. Children and adolescents may have value judgments influenced by dental appearance. Socioeconomic status can be a factor affecting judgment.
Nayak et al., 2018 Cross sectional study	India, Rajasthan n=200 13-16 y	TAS with computer modified photographs (♂ and ♀) 13/14 and 15/16 y Rural and urban school	To determine if adolescents make social judgments about other children who have notable enamel defects.	<u>WEAKEST EVALUATION (TAS):</u> made by ♂ aged 15 to 16 from the urban school, concerning the defective ♀ <u>HIGHEST EVALUATION (TAS):</u> for ♂15 to 16 y of the rural school, for ♂ without enamel defects. <u>GENDER:</u> TAS with/without defect ♂ = ♀ with (27.5) < without (30.59). <u>SOCIAL ENVIRONMENT:</u> ♂ from rural school with enamel defect gave significantly better TAS than the children in the urban school.	N/I	N/I	Children and adolescents may have value judgments influenced by dental appearance. Socioeconomic status can be a factor affecting judgment.
Al-Omari et al., 2014 Cross sectional study	Jordan n=920 11-12 y	Short form of the CPQ	To determine whether there is a relationship between self-reported bullying because of dento-facial characteristics and quality of life related to oral health in children.	Regarding oral symptoms and social well-being: ♂ reported higher scores, therefore more negative effects on their OHRQoL. There is a significant difference between the gender. Regarding bullying: children who report being bullied have significantly more negative effects on their overall OHRQoL. 41.2% reported being bullied (p<0.001). The OHRQoL subscales more affected where: "oral symptoms"; "functional limitations"; "emotional well-being"; "social well-being". Effects on overall OHRQoL by separating ♀ and ♂ who are victims of bullying: Significantly: Bullied ♂ > non-bullied ♂; Bullied ♀ > non-bullied ♀ Effects on their quality of life: ♂~♀	N/I	N/I	There is a significant relationship between bullying due to dento-facial characteristics and negative quality of life effects related to oral health.

Reference, year of publication, study type	Country, sample size, age	Material and methods	Aim	School harassment, intimidation, social judgments	Aesthetic disorders and self-confidence, treatments	Consequences on the smile	Conclusion
Craig et al., 2014 Cross sectional study	United Kingdom, Sheffield n=547 11-15 y	TAS with computer modified photographs (♂ and ♀) 11/12 and 14/15 y School with high educational attainment (A) and school with low educational attainment (B)	To determine whether children make judgments about other young people with aesthetic defects in anterior teeth (visible enamel opacities).	Social judgment: <b>GENDER</b> : with/without defect: average TAS ♀ = ♂ <b>ENAMEL DEFECTS</b> : Average TAS (♀ and ♂) with defect > without defect. <b>LOWEST EVALUATION</b> : ♂ (10 y from school B), judging the ♂ with enamel defect. <b>HIGHEST EVALUATION</b> : ♀ (7 and 10 y from school B), judging the ♂ without enamel defect.	N/I	N/I	♀ judged significantly more positively than ♂, for the subjects photographed, with and without enamel defect. The age group and the school (private / public) had no significant effect on the TAS average. Children can make negative psychosocial judgments based on the appearance of the enamel. ♂ had significantly more negative judgment than ♀.
Gomes et al., 2014 A cross-sectional study	Brazil, n=843 children n=843 parents 2-5 y	ECOHis questionnaire	To evaluate the impact of oral health conditions on the OHRQoL of preschool children and their families in a representative, preschool-based sample.	N/I	By using the ECOHis, the prevalence of negative impact on OHRQoL was 32.1% among children and 26.2% among families. 58.6% of children and 69.6% of families reported no impact on the ORHQoL, with a score of 0. The items with the highest averages were "reported pain" (child impact); "Felt guilty" and "been upset" (family impact). In children, the variables significantly associated with a negative impact on the OHRQoL were: child's order of birth, parent's/caregiver's perception of child's oral health as poor, cavitated dental caries and TDI. Among families, the variables significantly associated with a negative impact on the OHRQoL were: parent's/caregiver's perception of child's oral health as poor, cavitated dental caries and greater severity of TDI.	N/I	The factors leading to a negative impact on the OHRQoL for children and their families are: carious lesions on the anterior and posterior teeth; traumatic dental injuries; and the perception by parents/guardians of their child's oral health as being mediocre.
Parekh et al., 2014 Cross sectional study	United Kingdom, London n=7 13-16 y	Semistructured in-depth interviews and CPQ.	To explore the impact of visible AI on children and adolescents through in-depth interviews.	50% was annoyed at least "occasionally". It was often for 24% of ♂ and 11% of ♀. Some patients have received comments and thoughts from those around them about their teeth: "If someone sees it, they go 'Oh, don't you brush your teeth?' and stuff like that". "I'm not feeling well, I'm feeling a little bit bad, because they can see that my teeth look like dirty".	For 90% colour of teeth is important, 77% think improving smile is reason to continue treatment. For 63%, the most important thing from treatments is changing the colour, and for 18% it is the improvement of the smile. "If the colour was fine, I'd feel a lot more confident". "The colour just knocks my confidence". "When all my friends are talking, I'd want to join in but I don't want to show my teeth".	30% avoided smiling "occasionally" and 23% "often" or "all the time" (32% for ♀ and 14% for ♂). Patients with IA: Their main aesthetic problem is colour: "...if they were just a little more nice colour, not discoloured". "The colour, like when I say 'Shhh' my front teeth show on there, they're yellow ones". "I don't like smiling with my teeth because I don't like them. If I had nice teeth maybe I would be able to smile more". "I don't like smiling with my teeth because I don't like them". "I will smile when all of my teeth are going to be white, nice shape".	AI can have a significant impact on children and adolescents. Patients interviewed expressed concerns about aesthetic defects in their teeth, in relation to comments from other people. They were also very concerned about their self-image, and the results show that psychosocial is also impacted.
Scheffel et al., 2014 Case report	Brazil, n=3 8-10 y	A discussion with the patient. 3 cases: AI; HE and MIH.	To present and discuss about patients who have been bullied at school and family environment due to anomalies aesthetic related, and to observe the consequences of treatment aimed at correcting aesthetics.	AI: Dental appearance: source of teasing at school. Discrimination by classmates during the break times and group activities. Routine negative comments. EH: Growing up, the child's social behaviour changes. Mockery at school. The child is nicknamed "vampire" at school. MIH: Verbal mockery at school. They say that she did not brush her teeth, and that her teeth are rotten. Family members (cousins, aunts) talk about her dental appearance, they get involved. ⇒ Direct or indirect discrimination, exposing them to bullying (at school, or in the family environment).	AI: Poor academic performance; low self-esteem; introspection. Annoying social interactions. EH: The child became calm and isolated as he grew up, and stopped talking and participating in collective school activities. The child does not feel emotionally supported, and is always the subject of comments about the difference in teeth. Sense of social exclusion, punishment and inferiority.	During the clinical evaluation, the child hid his mouth and avoided speaking, did not smile. <b>AFTER TEMPORARY TREATMENT</b> : They are all shown greater self-esteem, more happiness, more freedom, and better social acceptance.	By treating teeth with aesthetic abnormalities, children regained self-esteem, self-confidence, and this resulted in greater socialization at school and in their family environment, and reduced their exposure to bullying and its consequences.

AI – amelogenesis imperfecta; CPQ – child perceptions questionnaire; ECOHis – early childhood oral health impact scale; HE – enamel hypoplasia; MIH – incisivo molar hypomineralization; N/I – no information; OHRQoL – oral health related quality of life; TAS – total attribute score; TDI – traumatic dental injury; ♀ – female; ♂ – male.