Analysis of microbiota in black stain of children and its impact on caries risk. A systematic review

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Abstract

Black stain (BS) is a peculiar discoloration often observed on the teeth of paediatric patients, particularly prevalent in certain populations. This systematic review aims to analyze the composition of the microbiota associated with BS and its potential implications for caries risk. A acomprehensive search was conducted across multiple databases (PubMed, Scopus, Web of Science) to identify relevant studies published up to January 2024. Eligible studies were those investigating the microbiological profile of BS patients compared to non-black stain individuals, with a focus on cariogenic species and overall microbial diversity. Data extraction and synthesis were performed following PRISMA guidelines. The screening phase ended with selecting 14 publications for this work. The review explores the association between the microbiota of BS patients and caries prevalence or severity. Synthesising current evidence sheds light on the potential role of the microbiota in BS formation and its influence on caries susceptibility. Understanding these microbial dynamics may inform preventive strategies and therapeutic interventions tailored to mitigate caries risk in populations affected by BS. This review underscores the significance of further research to elucidate the complex relationship between BS, oral microbiota, and dental caries.

KEYWORDS Black stains, children, microbiota, caries.

Introduction

Dental discolorations represent one of the main aesthetic concerns for many individuals, significantly influencing the perception of smile and oral health [Adcock and Hogan, 2008; Bryan and Welbury, 2006; Pani et al., 2015]. They can be classified into extrinsic and intrinsic pigmentation, with different causes and clinical manifestations [Chen et al., 2014; Costa et al., 2012; Downs and Black, 1998]. Extrinsic pigmentation involves the tooth surface and is often associated with factors such as chromogenic bacteria, poor oral hygiene, consumption of staining foods and beverages, and the use of certain medications and dental products [Eriksen and Nordbø, 1978; Garcia Martin et al., 2013; Gayatri et al., 2018]. On the other hand, intrinsic pigmentation involves the hard tissues of the tooth and may result from genetic conditions, metabolic diseases, or the use of certain medications[Heinrich-Weltzien et al., 2014; Ibiyemi and Taiwo, 2011; Li et al., 2015]. Among extrinsic pigmentation, BS represents an interesting and relatively common phenomenon, characterised by linear BS extending over the dental surface [figure 1]. The development of BS is linked to the presence of chromogenic bacteria, particularly Actinomyces, and the chemical reaction between hydrogen sulfide produced by these bacteria and excess iron in saliva [Pani et al., 2015; Reid et al., 1977]. This process can be influenced by disorders of iron homeostasis and inflammatory processes that increase the concentration of iron in tissues and secretions [Cicciù et al., 2023; Rathi et al., 2023; Reddy et al., 2022; Saba et al., 2006; Slots, 1974; Theilade, 1977]. Diagnosis of BS has been a subject of discussion, with various criteria proposed by different authors to classify and identify this discoloration [Gayatri et al., 2018; Grenda et al., 2022; Ibiyemi and Taiwo, 2011; Zadro et al., 2017]. However, there remains a lack of standardisation in diagnostic criteria, which can complicate comparison between studies and assessment of prevalence and associated factors [Saba et al., 2006; Sangermano et al., 2019]. Numerous factors can influence the formation of BS, including age, dietary habits, oral hygiene, medication use, and genetic predisposition. Epidemiological studies have highlighted variable prevalence of BS in the paediatric population and adults, with some familial occurrence suggesting a possible involvement of genetic factors in their aetiology [Slots, 1974; Theilade, 1977]. Although BS pose an aesthetic problem for many patients, some studies have suggested that the presence of this discoloration might be associated with lower susceptibility to dental caries. However, understanding the underlying mechanisms of BS formation and their impact on oral health remains partial [Garcia Martin et al., 2013; Zadro et al., 2017].

Composition of Black Stain

The insoluble black ferric component that makes up BS pigment is most likely ferric sulfide which was formed as a consequence of the chemical interaction between hydrogen sulfide generated by chromogenic bacteria (anaerobic bacteria) and excess iron present in saliva [Eriksen and Nordbø, 1978; Mutsaddi et al., 2018]. Iron/copper and sulfur complexes are thus considered responsible for the dark color. It is now

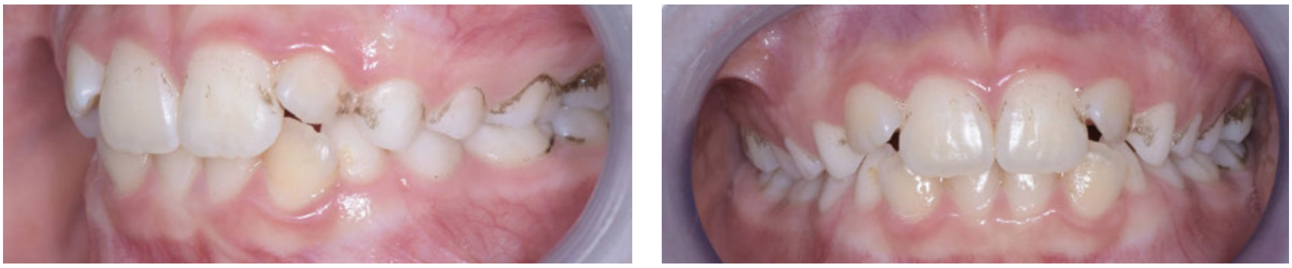

FIG. 1 Child's black stain in primary dentition in frontal and lateral view

established that the high concentration of available iron is due to disorders of iron homeostasis (consisting of iron overload in tissues and secretions and deficiency in circulation), also caused by inflammatory processes. In the presence of IL-6, a pro-inflammatory cytokine, iron is accumulated in cells and secretions [Adcock and Hogan, 2008; Ibiyemi and Taiwo, 2011; Pani et al., 2015]. The accumulation of ferric ions, besides causing bacterial multiplication, superoxide formation, and cellular damage associated with pathological destructive inflammation, can react with hydrogen sulfide synthesized by anaerobic bacteria, forming ferric sulfides. In physiological situations, the concentration of available iron in tissues and secretions is 10–18 μM and therefore far from that necessary for bacterial replication and consequent formation of ferric sulfides. In pathological situations, however, the available iron in tissues and secretions is greater than 10 μM and therefore sufficient for microbial replication and formation of pigmentation [Adcock and Hogan, 2008]. Regular consumption of iron-rich foods and vitamin supplements containing ferric ions during pregnancy or early childhood may favour the development of a chromogenic microbiota. Furthermore, black pigmentation, as well as the development of various colorations or pigmentation on the tooth surface, is also due to saliva proteins rich in proline [Saba et al., 2006]. The latter has a high binding affinity with phenolic and polyphenolic compounds widely present in plant foods and beverages including red wine. Ultrastructural examination of BS has revealed the presence of microorganisms incorporated within the matrix, mostly Gram-positive species. The microbiological composition of BS is believed to be dominated by Actinomyces [Heinrich-Weltzien et al., 2014]. Recent PCR analyses of plaque samples in children with BS have also shown a significantly higher number of Actinomyces naeslundii and a lower number of Lactobacillus spp. compared to non-pigmented plaque samples [Sangermano et al., 2019; Mummolo et al., 2014].

Standards for Black Stain Diagnosis

The criteria for diagnosing BS are not well-defined and vary among authors. Shourie has proposed criteria that include the presence of incomplete pigmented spot coalescence and a continuous line of pigmented patches [Żyła et al., 2015]. Other authors like Koch et al. [2001] have introduced new diagnostic criteria describing BS as dark spots forming a linear discoloration on smooth dental surfaces, involving at least two different dental elements, in the absence of cavitation on the enamel surface [Ortiz-López et al., 2018; Paredes Gallardo and Paredes Cencillo, 2005]. Further criteria, such as those proposed by Gasparetto et al. [2003], also include the extension of the affected dental area, with scores corresponding to the presence of pigmented spots or thin lines with incomplete coalescence, continuous pigmented lines, and pigmented areas that surpass the cervical third of the tooth's surface by half [Mousa et al., 2022].

Factors Associated with Black Stain

It is challenging to identify the various elements that contribute to the creation of BS because its aetiology is yet unknown. Several writers have looked for relationships between the prevalence of BS and factors such as age, gender, nutrition, oral hygiene, medications, and socioeconomic status. [Muthu et al., 2019; Mutsaddi et al., 2018]. However, results are often conflicting. For example, some studies show a higher prevalence of BS in subjects with a low level of parental education, while others report opposite results. Additionally, age seems to play a role in the occurrence of BS, with an increase observed with advancing age, although this correlation is not always statistically significant [Janjua et al., 2022; Natoli, 2019; Sulieman, 2005]. Studies have shown that permanent dentitions is more frequently affected by BS than deciduous dentitions. Dietary practices, such as consuming soy sauce, dairy products, eggs, vegetables, and fruits seem to favor the development of this discoloration. Interestingly, children who have never been fed with a bottle seem to have a lower prevalence of BS, suggesting a possible role of bottle-contained liquids in the formation of this discoloration. Furthermore, oral hygiene may play a role in the etiology of BS, although data are conflicting. Some studies have suggested that the use of fluoride-containing toothpaste and mouthwashes may increase the formation of stains, while others have found no correlation between BS prevalence and the type of toothpaste or brushing frequency. Pregnancy and infancy iron supplementation has also been linked to the development of BS.

Black Stain Prevalence

Most studies on BS have been conducted in the paediatric population, as this discoloration is more common in youth, although it can also occur in adults. The prevalence of BS varies considerably among studies, with reported rates ranging from 2.4% to 18%. This variability can be attributed to the lack of standardisation in diagnostic criteria and the diversity of populations studied [França-Pinto et al., 2012; Ravikumar et al., 2018]. Studies have shown a similar prevalence of BS between genders, suggesting that this discoloration is not influenced by sex. However, a higher prevalence of BS has been observed in subjects with a low level of parental education and older age [Hattab et al., 1999; Nokhbatolfoghahaei et al., 2023; Primozic et al., 2021; Reid and Beeley, 1976].

Familiality of Black Stain

The origin of BS has been a subject of debate for over a century. Some studies suggest a possible genetic predisposition with family members exhibiting a high specific severity of BS [Mutsaddi et al., 2018; Quinzi et al., 2020]. However, other authors emphasise the importance of environmental factors in the formation of BS, suggesting that not all family members with the same dietary habits develop this discoloration.

Treatment of Black Stain

Treatment of BS poses significant challenges, as conventional oral hygiene methods offer only temporary relief and fail to address the root causes of pigmentation. Nonetheless, various treatment modalities exist, such as professional oral hygiene sessions involving ultrasonics, air flow, polishing with pastes, and rubber cups [Ronay and Attin, 2011].

Despite the available treatments, BS tends to recur post-treatment, underscoring the need for more effective and targeted therapies. BS, characterised by linear marks on the teeth, results from chromogenic bacteria like Actinomyces and chemical reactions involving hydrogen sulfide and excess saliva iron [Nokhbatolfoghahaei et al., 2023].

While BS is aesthetically concerning, studies suggest it might indicate reduced susceptibility to dental caries due to favorable salivary parameters. However, the diagnose of BS is challenging due to the absence of standardised criteria, emphasising the need for precise diagnosis to guide appropriate treatment [Ravikumar et al., 2018].

Current treatments provide only temporary relief, leaving the underlying pigmentation causes unaddressed. Therefore, research is crucial to comprehend BS formation mechanisms, its relationship with dental caries, and other oral health factors. Advancing understanding is key to developing more effective therapies for managing dental pigmentations, ultimately enhancing dental care quality [Janjua et al., 2022].

The microbiome in Black Stain

The microbiome in BS has garnered increasing attention within dental research circles due to its potential to offer valuable insights into the pathogenesis and management of this condition. A recent study sought to evaluate the prevalence and epidemiological characteristics associated with BS among preschool children in China [Adcock and Hogan, 2008; Costa et al., 2012]. Employing a structured questionnaire, researchers objectively collected data on sociodemographic characteristics, lifestyle factors, and oral hygiene habits of the participating children. Additionally, they analysed saliva and microbial plaque samples to ascertain the microbial features linked with BS. Given the relatively low incidence of oral diseases like caries and periodontitis among preschoolers and the largely unexplored microbial underpinnings of BS in the oral microbiome, children in early childhood (ages 3–4) were deemed suitable subjects for investigating BS and extracting pertinent scientific insights [Adcock and Hogan, 2008; Chen et al., 2014; D'Apuzzo et al., 2019]. The study revealed that BS was clinically evident in 12.4% of the 250 preschoolers examined, with the majority of affected children exhibiting BS on more than eight teeth. This prevalence rate aligned closely with findings from previous studies and surpassed the prevalence reported in an earlier investigation [Alkhtib et al., 2016]. Interestingly, dental caries indicators were markedly lower in the BS group, consistent with prior research. Analysis of sociodemographic factors and oral hygiene practices revealed a reduced risk of BS among children with less frequent consumption of jam and honey, as well as those who received regular fluoride application [Garcia Martin et al., 2013; Gayatri et al., 2018]. These findings underscore the impact of oral hygiene behaviour and dietary habits on BS occurrence in children. Furthermore, a protective role of BS against dental caries was observed, positing that regular fluoride application might favour a higher pH in the oral cavity, thereby facilitating BS deposition. BS represents a distinctive form of dental plaque deposited by oral microorganisms, with saliva playing a crucial role in its formation. Consequently, researchers analysed plaque and saliva samples to elucidate additional microbial characteristics. Microbiome analysis unveiled notable differences in plaque microbial structure between the BS and control groups [Ronay and Attin, 2011; Sulieman, 2005]. Eight microbial biomarkers, including Actinomyces and Rothia aeria, delineate BS. Actinomyces can create pigments that are either black or brown and is closely associated with BS in children. Notably, the abundance of Actinomyces naeslundii may mitigate caries development, whereas Rothia aeria usually occurs more frequently in BS. Moreover, bacterial species like Aggregatibacter sp. exhibit higher abundance in BTS samples. Metabolic pathways implicated in BS, such as glycolysis, cysteine and methionine metabolism, are markedly enriched in plaque samples featuring BS. An analysis of microbial functions disclosed a hyperactive metabolic state on dental surfaces with BS plaques [Costa et al., 2012; Reid et al., 1977].

Materials and Methods

Protocol and Registration

This systematic review was conducted by the standards of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2020 statement [Liberati et al., 2009]. The review protocol was registered at PROSPERO under the unique number 523242.

Search Processing

The search period started on December 12, 2023 and ended on January 25, 2024. "Black Stain", "caries", and "microbiota" were the search terms utilised on the databases (PubMed, Web of Science, and Scopus) to select the papers under evaluation, with the Boolean operator "AND" and "OR".

The search was restricted to papers published in English during the previous ten years (January 2014 – January 2024) (Table 1).

Eligibility Criteria

The reviewers, who worked in pairs, chose works that satisfied the following criteria for inclusion: 1) human subjects-only research; 2) clinical studies; 3) research done on children in primary or mixed dentition, both male and female with tooth decay or BS.

Exclusion criteria were: 1) in vitro studies; 2) animal studies; 3) systematic reviews, narrative reviews and meta-analyses; 4) case reports.

Duplicate studies were removed manually.

The review was conducted using the PICO criteria:

- Population: children in primary or mixed dentition, both male and female with tooth decay or BS
- Intervention: study of the microbiota in patients with BS
- Comparison: analysis of microbiota associated with caries
- Outcome: evaluation and analysis of the difference between the two types of microbiotas.

Data Processing

Working separately, two reviewers (F.I. and G.D.) used the predetermined inclusion and exclusion criteria to filter the data that was taken from each database. Individual choices were hidden from the researchers. Both reviewers converged on their findings in the last meeting. A paper's entire text was retrieved and examined when a reviewer thought it might be accepted. This took place twice, separately. Every qualifying primary study's data extraction includes the authors and publication date, study type, purpose, materials and techniques, and findings. Disagreements between reviewers regarding the articles' selection were discussed and settled.

Article screening strategy	Database: Scopus, Web of Science and PubMed
	Keywords: Black Stain; caries; microbiota
	Boolean variable: "AND" and "OR"
	Timespan: 2014-2024
	Language: English

TABLE1 Database search indicators

Quality Assessment

Two reviewers, IT and RS, evaluated the quality of the included papers using the ROBINS method, which was created to evaluate the risk of bias in the findings of non-randomised studies that compare the health effects of two or more interventions. A bias degree was assigned to each of the seven assessed points. In the event of a disagreement, a third reviewer (FI) was consulted until a consensus was obtained.

Results

Keyword searches of the Web of Science (88), Scopus (51) and PubMed (89) databases yielded a total of 228 articles. After that, 96 duplicate articles were removed, leaving 132 items total. Of these 132 studies, 118 were excluded because they deviated from the previously defined inclusion criteria.

After the screening process, 14 publications were chosen for this work. The PRISMA flow-chart of this review is summarised in Figure 1 and the data of each selected study (author(s), type of the study, aim of the study, materials and results) are reported in Table 2.

Quality Assessment and Risk of Bias

The risk of bias in the included studies is reported in Figure 2. Bias resulting from confounding the majority of studies is a high-risk form of bias, while that arising from measurement parameter is a low risk form of bias. Many studies have a low risk of bias due to bias in the selection of participants. The significant heterogeneity makes it impossible to calculate bias resulting from post-exposure. Many studies show insignificant bias as a result of missing data. There is little bias resulting from outcome measurement. In most studies, there is a significant bias in the selection of the published results. Five studies have a high risk of bias, two have a very high risk of bias, and two have a low risk of bias, according to the final results.

Discussion

Microbiota Composition in Black Stain Formation

The microbial makeup of dental plaque and saliva in kids with BS has been the subject of numerous studies. Li et al. [2015] compared the microbial diversity of BS and non-BS groups using 16S rRNA gene sequencing. Children without caries and those with BS had different oral plaque and saliva microbiomes. MOTHUR software was utilised for data analysis. Children with BS had saliva with a less variety of microorganisms, and microbial diversity was lower in BS compared to plaque. In plaque samples from children with BS, some bacterial genera—including Actinomyces, Cardiobacterium, Haemophilus, Corynebacterium, Tannerella, and Treponema—were more prevalent, indicating a possible role in the developement of BS. More research is needed to determine the relationship between periodontitis and caries and BS. While the study's use of 16S rRNA gene sequencing allowed for a more comprehensive understanding of the microbiota, more investigation is required to confirm the processes underlying stain formation and the roles played by the participating bacteria. The sample size is limited, thus additional samples are required to validate the conclusions.

Similarly, Heinrich-Weltzien et al. [2014]found differences in microbial composition between BS and non-discolored plaque, with higher counts of certain bacteria in BS plaque samples. Forty-six BS and 47 non-discoloured plaque children, aged 7.9 ± 1.3 years, were examined. Children with BS exhibited reduced dental caries prevalence ($p = 0.061$), as well as lower DMFT ($p = 0.013$) and DT values ($p = 0.005$). A. naeslundii ($p = 0.005$) and F. nucleatum ($p = 0.001$) as well as Lactobacillus sp. ($p = 0.001$) counts were higher in the BS plaque.

Caries-free BS children had higher A. naeslundii counts ($p = 0.013$), while caries-affected had lower F. nucleatum counts ($p = 0.007$). Microbial differences may relate to lower caries in BS children. These findings suggest a potential role of specific microbial genera in BS formation, although further research is needed to confirm mechanisms and functions [Heinrich-Weltzien et al., 2014]. The study of Young Hwang et al. [2020] investigated the oral microbiome in children with BS on teeth using pyrosequencing to understand the microbial aspects of BS formation. Six children with BS and four without were studied. BS group exhibited higher microbial abundance and diversity compared to controls. The BS group showed significant differences in six genera, including Abiotrophia, Eikenella, Granulicatella, Neisseria, Porphyromonas, and Streptococcus. Porphyromonas spp., known for producing black pigments, were abundant in BS samples, potentially contributing to BS formation. Streptococcus spp. were also abundant in BS samples, possibly facilitating microbial interactions leading to BS. Veillonella spp. and Actinomyces gerencseriae were identified as potential contributors to BS formation. Despite limitations in sample size, this study provides insights into the microbial dynamics associated with BS formation, highlighting Neisseria, Porphyromonas, and Streptococcus as key players. Further research is needed to confirm these findings and identify causal agents for BS formation [Hwang et al., 2020].

Finally, Han et al.'s [2021]comprehensive analysis of oral microbiota in preschool children elucidated the complex interplay between microbial diversity, caries susceptibility, and systemic health factors. Their findings highlight the distinct microbial profiles associated with ECC and black spot formation, underscoring the multifactorial nature of these oral health conditions. Furthermore, the study's predictive models based on microbial biomarkers offer valuable insights for personalised oral health strategies and targeted interventions.

Association of Black Stain with Caries

The reviewed studies collectively provide valuable insights into the intricate relationship between oral microbiota and the development of BS and caries in patients. Bardellini et al. demonstrated the potential of S. salivarius M18 as a preventive intervention against BS formation. Their findings underscore the importance of probiotic supplementation in maintaining oral hygiene and inhibiting the reformation of extrinsic black tooth spots, suggesting a promising avenue for future research in this area [Bandon et al., 2011; Bardellini et al., 2020].

Gultom et al. explored the antibacterial efficacy of NSF against Streptococcus mutans biofilms, a key contributor to caries development. Their results indicate that NSF exhibits comparable antibacterial activity to SDF, offering a potential alternative for inhibiting oral biofilm formation. This study contributes to our understanding of novel antimicrobial agents and their role in preventing caries progression [Gultom et al., 2019]. Contrarily, Carelli et al. [2023] focused on the impact of toothpaste formulations containing sodium fluoride, salivary protein and enzymes on oral microbiota composition and BS prevalence. While the study did not identify any noteworthy variations in oral microbiota composition between the test and control groups, it highlighted the importance of mechanical cleaning in preventing BS. These findings emphasise the need for multifaceted approaches, combining mechanical cleaning with adjunctive treatments, to effectively manage oral health conditions.

Moreover, Borrel Garcia et al. [2021] investigated the oral health benefits of probiotic supplementation in adolescents. While the study did not reach statistical significance, it suggested a potential role for probiotics, specifically L. reuteri strains, in reducing salivary S. mutans levels and improving plaque and gingivitis indices. These findings warrant further investigation into the long-term effects and colonization dynamics of probiotic bacteria in the oral cavity.

Chen et al. [2019], Zhang et al. [2014], and Rui Han et al. [2021] explored the relationship between BS and dental caries. While Chen et al. found no significant differences in microbial diversity between groups, they noted a decrease in Candidate_division_TM7 in BS-positive children, Zhang et al. identified bacterial biomarkers associated with BS formation and observed a lower prevalence of dental caries in children with BS, indicating a potential protective effect. Rui Han et al. further investigated microbial variations associated with early childhood caries, iron deficiency anemia, and BS, highlighting diverse oral flora in IDA ECC versus low diversity in BSCF.

The study of Chen et al. [2019] enrolled 100 primary dentition children from West China Hospital of Stomatology, Sichuan University, Chengdu, China. Dental health was assessed through clinical examination, identifying 10 children with EBS and matching them with EBS-free counterparts. Samples of plaque were gathered and analysed using PCR and high-throughput sequencing. Results showed no significant differences in microbial diversity between groups, but a decrease in Candidate_division_TM7 in EBS-positive children. Actinomyces naeslundii was more prevalent in EBS patients. The study suggests similarities in dental biofilm microbial communities between children with and without EBS, emphasizing further investigation into specific microbial correlations [Chen et al., 2019]. In a study conducted in Shanghai by Zhang et al. [2014], preschool children with black tooth stain (BTS) showed distinct oral microbiome characteristics compared to those without BTS. Microbiome analysis revealed several bacterial biomarkers associated with BTS. These findings suggest a potential link between specific microbial species and BTS formation. Furthermore, children with BTS had reduced dental caries and improved oral hygiene. The study also highlighted the influence of dietary habits and regular fluoride application associated with a reduced likelihood of BTS development. These results provide valuable insights into the microbial basis of BTS and potential preventive strategies [Zhang et al., 2022]. The study of Rui Han et al. [2021] investigates salivary microbiome variations in ECC, exploring associations with iron deficiency anemia (IDA) and extrinsic BS. 136 children (3-6 years) were enrolled, including 93 ECC cases and 43 caries-free (CF) controls. Microbiota analysis revealed diverse oral flora in IDA ECC versus low diversity in BSCF. Biomarker-based random forest classifiers predicted caries, anemia, and BS outcomes with accuracies of 93.35%, 94.62%, and 95.23%, respectively. The findings highlight microbial differences associated with ECC, IDA, and BS, indicating potential clinical implications for disease prediction and management strategies [Han et al., 2021].

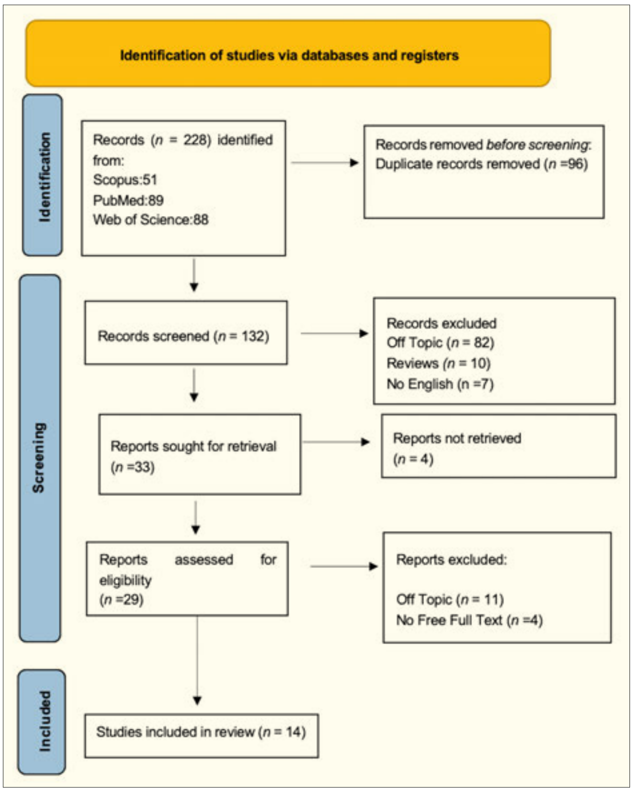

FIG. 1 PRISMA flow-chart used in this review paper.

This study conducted by Lopez Martinez et al. [2016] aimed to investigate the prevalence and factors associated with BS in children aged 10–12 and explore its relationship with dental caries. Following correction for a number of variables, such as dental plaque and brushing practices, the presence of BS was linked to a decreased incidence of caries. This finding aligns with previous studies indicating a protective effect of BS against dental caries. However, further research is needed to clarify the processes that underlie this correlation. The study underscores the importance of large-scale epidemiological investigations to understand the phenomenon of BS and its implications for dental health.

Epidemiological and Clinical Factors

Garcia Martin et al. [2013], Zheng et al. [2023], Lopez Martinez et al. [2016], and Ilgen et al [2023] explored epidemiological and clinical factors associated with BS and its relationship with dental caries. Garcia Martine et al. found an association between iron supplement intake and the presence of BS. A total of 3272 6-year-old children from Oviedo, Spain, were included in the study. Results revealed a prevalence of 3.1% for BS, with a dmft index of 0.35 ± 1.123. A statistically significant association was found between the presence of BS and iron supplement intake. Additionally, there was no significant difference in the prevalence of BS between native Spanish children and immigrants. It was determined that chromogenic microbiota development may be aided by regular consumption of foods high in iron and the use of iron supplements during pregnancy and the early years of life [López Martínez et al., 2016].

Children with BS had a lower prevalence of caries, according to Zheng et al. [2023]. They looked into the BS epidemiology and possible connections between the oral microbiome (OM) and gut microbiomes (GM) and the development of BS and the

Authors	Type of the study	Age and numbers of participants	Aim of the study	Materials and Clinical data	Results and percentages
Li et al., [2015]	Clinical study	10 children, aged four to five, with BS and 15 without it.	Examination of the BS microbiota in the primary dentition.	Samples of BS, dental plaque, and saliva were taken. With the aid of the pipeline tool MOTHUR, data were examined. The Mann-Whitney U test was utilised to evaluate the relative abundances of the microbial taxa, and the Student's t-test was employed to assess alpha diversities.	The development of BS may be linked to changes in oral flora.
Heinrich-Weltzien et al. [2014]	Clinical study	47 children with non-discolored plaque and 46 with BS, aged 7.9 ± 1.3 years, were compared.	To evaluate the microbiome and caries experience in children in systemically healthy families with non-discolored plaque and BS.	Dental plaque samples from BS patients were obtained, and the samples' DNA was extracted and examined to ascertain the overall quantity of distinct bacterial species.	It is possible that the microbial makeup of BS differs from that of afflicted people, which accounts for the lower caries experience.
Garcia Martin et al. [2013]	Clinical study	3272 children aged 6 years old.	To find out the risk factors and frequency of BS in preschool-aged Spanish children.	In the entire group, the frequency of BS was 3.1%.	Regularly consuming iron-rich foods and taking iron supplements in pregnancy and early childhood may promote the growth of chromogenic bacteria.
Han et al. [2021]	Clinical study	136 children aged 3-6 years.	Analysis of salivary microbiome variations in early childhood caries (ECC), exploring associations with iron deficiency anemia (IDA) and extrinsic BS.	Analysis of saliva of the children included in the sample.	The findings highlight microbial differences associated with ECC, IDA, and BS, indicating potential clinical implications for disease prediction and management strategies.
Chen et al. [2019]	Clinical study	20 children, 10 with positive extrinsic black stain (EBS) and 10 without.	To look into the structure and diversity of the microbiota in the supragingival plaque.	The nonparametric Mann-Whitney U test, Pearson Chi-Square test, Fisher's Exact test, and one-way ANOVA tests were used to analyse the data.	Variations in the microbiota of dental plaque may have an impact on the primary dentition's EBS process.
Zhang et al. [2022]	Cross-sectional study	250 children aged 3-4 years.	To shed light on the microbiological and epidemiologic characteristics of BTS in preschoolers.	Dental plaque and saliva samples were taken from caries-free individuals in the BTS group (n = 21) and the control group (n = 48) who did not receive BTS.	Numerous microbial biomarkers that are associated with BTS's microbiome can be used as indicators for the diagnosis and prognosis of the disease.
Zheng et al. [2023]	Clinical study	38 children aged 3–6 years.	16S RNA sequencing and collection of samples of supragingival plaque and feces.	Collected plaque samples were divided into four groups: BCP (EBS+, caries+), BP (EBS+, caries–), CP (EBS–, caries+), and P (EBS–, caries–). Additionally, four sets of fecal samples were created: F (EBS–, caries–), BF (EBS+, caries–), CF (EBS–, caries+), and BCF (EBS+, caries+).	In the BCP group, Actinomyces and Cardiobacterium were more prevalent, while Lautropia and Pseudopropionibacterium were prevalent in the BP group ($p<0.05$).
López Martínez et al. [2016]	Cross-sectional study	Multistage sampling of children (1211) aged 10-12 years.	To determine the frequency of BS and the variables that contribute to their occurrence, as well as to look into the relationship between BS and caries experience after correcting for other risk factors.	Children's dental caries and BS were assessed using the DMFT/dmft index, and a questionnaire was used to gather socioeconomic data. The variables connected to the frequency of BS and the experience of caries were evaluated using univariate and multivariate Poisson regressions.	Lower caries experience was linked to BS prevalence (RR 0.67; 95%CI 0.49–0.92).
İlgen et al. [2023]	Cross-sectional study	From a group of 1000 children, were considered 88 children aged 3-12 years, 44 with BS and 44 without BS.	To evaluate the prevalence of black tooth stain among children aged 3-12 years and to investigate the colonisation of dental plaque samples with or without BS by Actinomyces, Lactobacillus, Capnocytophaga, and Streptococcus mutans.	Dental plaque samples were obtained from children in the study group (BS) and control group (no BS) in order to make an isolation and identification of microorganisms.	Capnocytophaga spp. levels in children with BS were significantly higher when compared to the control group. Higher levels of Capnocytophaga directly or indirectly may prevent caries formation.

Hwang et al. [2020]	Clinical study	There were four children (mean 8.3 years) without BS and six (mean 8.1 years) with BS.	to look at the relationship between the production of BS and the makeup of the oral microbiota of supragingival plaque.	Pyrosequencing was used to acquire the bacterial metagenome from supragingival dental plaque.	The oral microbiome's composition must shift, and a number of species in the genera Neisseria, Porphyromonas, and Streptococcus may play a significant role in the development of BS.
Bardellini et al. [2020]	Interventional Study	58 children aged 4-10.	To evaluate the efficacy of S. salivarius M18 in preventing the reformation of extrinsic black tooth spots (BSs)	Treatment (Group A) or control (Group B). Group A received daily tablet containing S. salivarius M18; Group B received no treatment. Clinical evaluation using simplified oral hygiene index (OHI-s).	Lower prevalence of BSs in Group A at T1 and T2, indicating preventive effect of S. salivarius M18.
Gultom et al. [2019]	Experimental Clinical Trials	Silver Diamine Fluoride (SDF) 38%; Nano Silver Fluoride (NSF).	To evaluate the efficacy of NSF against Streptococcus mutans ATCC 31987 biofilms	Culturing S. mutans biofilms with various concentrations of NSF; evaluating their viability at different stages of biofilm formation	Demonstrating efficacy of NSF in inhibiting S. mutans biofilm formation; increased sensitivity of S. mutans to NSF exposure compared to Enterococcus faecalis.
Carelli et al. [2023]	Randomised Controlled Trial	26 subjects (10 without black spots, 16 with BS) aged 6 to 13 years.	To evaluate the effectiveness of toothpaste containing sodium fluoride, enzymes, and salivary protein in reducing pathogenic bacteria and preventing black spots compared to toothpaste with amine fluoride.	The test group used toothpaste with sodium fluoride, enzymes, and salivary protein, control group used toothpaste with amine fluoride.	Toothpaste with salivary enzymes and proteins reduced some pathogenic bacteria in saliva but did not significantly affect oral microbiota or oral health compared to control toothpaste.
Borrell García et al.[2021]	Randomised, Double-blind, Placebo-controlled Trial	Adolescents aged 12 to 18 years.	To assess the presence of probiotics in the oral cavity after 45 days and their potential oral health benefits.	Experimental and control groups received probiotic tablets or placebo; saliva samples collected at intervals to assess microbial levels and salivary pH; assessment of plaque, gingivitis, and bleeding indices, dietary, and oral hygiene habits; statistical analysis conducted.	Probiotics showed a beneficial effect on salivary S. mutans levels and plaque and gingivitis indices, although statistical significance was not reached.

TABLE 2 Examined articles.

prevention of dental caries. 12.10% of 2,675 kids aged 3 to 6 years had EBS. A decreased incidence of caries and decayed, missing, filled teeth (DMFT) was linked to EBS. The control group's dental plaque had the most intricate microbiome. There was less evenness in the plaque but a higher operational taxonomic unit (OTU) richness in the children with caries and EBS. In EBS with caries, Actinomyces and Cardiobacterium were more common, whereas Pseudopropionibacterium and Lautropia were more common in EBS without caries. Agathobacter and Ruminococcus were found in caries-free groups, while Veillonella species were more prevalent in caries groups. The results suggest a potential protective role of EBS against caries and highlight microbiome biomarkers associated with EBS formation. Further research with larger sample sizes and consideration of environmental factors is warranted to understand the complex interactions involved. Lopez Martinez et al. [2016] reported a protective effect of BS against dental caries, emphasising the need for further research to understand underlying mechanisms. The study aimed to investigate the prevalence and factors associated BS in children aged 10–12 and explore its relationship with dental caries. After adjusting for various factors, including dental plaque and tooth brushing habits, BS presence was associated with lower caries experience (RR 0.67; 95%CI 0.49–0.92). This finding aligns with previous studies indicating a protective effect of BS against dental caries. However, further research is needed to elucidate the mechanisms underlying this association. The study underscores the importance of large-scale epidemiological investigations to understand the phenomenon of BS and its implications for dental health. Ilgen et al. investigated demographic, medical, dental, and dietary factors associated with BS and found no significant associations but confirmed a lower dental caries score in BS children. They studied the relationship between BS presence, dental caries, and oral microbiota in children aged 3-12 years. Among 1,000 children, 44 with BS and 44 controls were selected. No significant associations were found between BS and demographic, medical, dental, or dietary factors. However, BS was significantly associated with lower dental caries scores (DMFT/DMFS) compared to controls. Microbiological analysis revealed higher levels of Capnocytophaga spp. in BS children, potentially contributing to reduced caries risk. While Actinomyces spp. predominance in BS has been suggested to lower caries risk, the role of Capnocytophaga spp. in caries prevention warrants further investigation. Limitations include the small BS prevalence and the need for larger studies to confirm findings. Overall, the study sheds light on potential microbiological mechanisms underlying the protective effect of BS against dental caries. Further research, particularly on Capnocytophaga spp., is warranted to elucidate this relationship.

Microbiological Mechanisms and Future Directions

Collectively, these studies provide insights into the microbiological aspects of BS formation and its association with dental caries. Key bacterial genera, such as Porphyromonas, Streptococcus, and Actinomyces, have been implicated in BS formation, highlighting the need for further research to elucidate

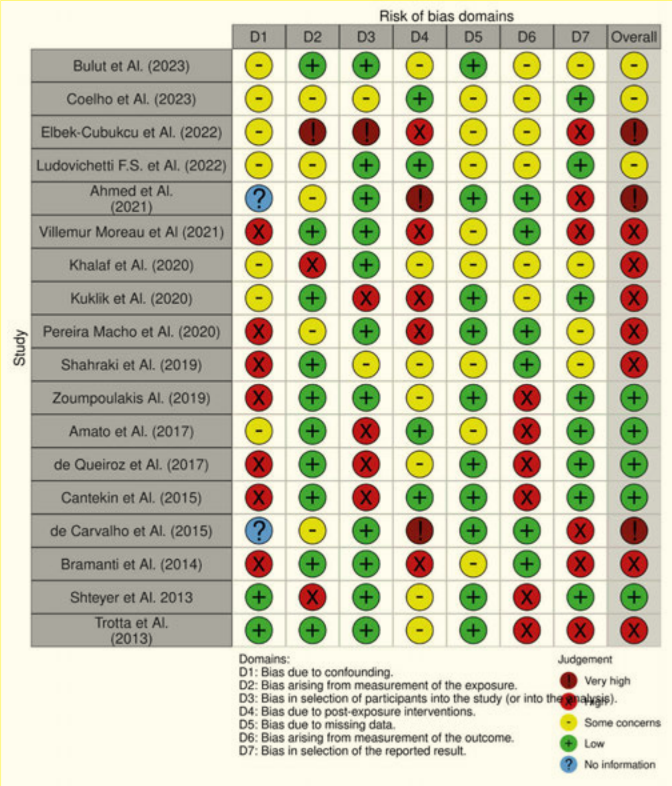

FIG. 2 Bias assessment

underlying mechanisms and potential preventive strategies. Larger sample sizes and consideration of environmental factors are warranted to better understand the complex interactions involved in BS formation and its impact on oral health.

Our findings offer fresh perspectives on the management of caries and EBS. The study sample size ought to be increased in the future. Saliva's pH and other factors should be taken into account to investigate the components of EBS and the predominant bacteria present, it should be graded. Following the in vitro culture of the dominant bacteria, animal experiments should be carried out to confirm the oral and intestinal dominant bacteria of EBS in order to derive the appropriate preventative and therapeutic measures. In conclusion, the reviewed studies collectively contribute to our understanding of the role of oral microbiota in BS formation and caries risk. While promising interventions such as probiotics and antimicrobial agents show potential, further research is warranted to validate their efficacy and explore novel preventive strategies for improving oral health outcomes in patients.

Limitations

Despite the valuable insights provided by the studies reviewed, several limitations warrant consideration. Firstly, the sample sizes in some of the studies were relatively small, which may limit the generalisability of the findings. For instance, the study conducted by Ilgen et al. included only 44 children with BS and 44 controls, indicating the need for larger studies to confirm their results. Additionally, the diversity of factors influencing BS formation and its association with caries risk is extensive and multifaceted. While efforts were made to account for various demographic, medical, dental, and dietary factors, there may still be unmeasured confounders impacting the observed outcomes. Furthermore, the methodologies employed across the studies varied, making direct comparisons challenging. For instance, different sequencing techniques and analytical approaches were used to characterise the oral microbiota, potentially influencing the identification of microbial biomarkers. Addressing these limitations through larger, well-designed studies with standardised methodologies will be essential for advancing our understanding of the complex interactions between BS, microbiota, and caries risk.

Conclusions

In conclusion, the studies reviewed collectively highlight the intricate relationship between BS formation, oral microbiota composition, and dental caries risk. While BS appears to confer a protective effect against caries development in several studies, the underlying mechanisms remain incompletely understood. Future research endeavors should focus on elucidating the specific microbial taxa and metabolic pathways involved in BS formation and its association with caries risk. Moreover, larger-scale epidemiological investigations incorporating diverse populations and environmental factors are needed to validate and extend these findings. Furthermore, prospective longitudinal studies are warranted to assess the temporal dynamics of BS formation and its impact on oral health outcomes over time. Integration of advanced omics technologies, such as metagenomics and metabolomics, holds promise for unraveling the complex microbiological mechanisms underpinning BS and informing targeted preventive strategies. By addressing these knowledge gaps, we can develop more effective interventions aimed at preserving oral health and reducing the burden of dental caries in affected populations.

Author Contributions

Conceptualisation, R.S., A.P., I.T., and G.D.; methodology, F.I., G.L.; software, I.T. and A.P; validation, R.S., G.D. and F.I.; formal analysis, A.M.I., A.D.I, A.P., I.T. and R.S..; investigation, A.P., R.S., G.L., A.D.I. and A.M.I.; resources, A.P. and G.L.; data curation, G.D.,F.I., G.D., I.T., G.L.; writing—original draft preparation, A.M.I., I.T., R.S., and C.P.; writing—review and editing, R.S., I.T. and G.D.; visualisation, A.M.I., G.L.; supervision, A.D.I., A.P. and F.I; project administration, F.I., G.D. and I.T.; funding acquisition, A.M. and F.I. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

BS: Black Stain
EBS: Extrinsic Black Stain
BTS: Black Tooth Stains
DMFT: Decayed Missing Filled Teeth in permanent teeth

IDA: Iron Deficiency Anemia
ECC: Early Childhood caries
SDF: Silver Diamine Fluoride
NSF: Nano Silver Fluoride
OM: Oral Microbiome
GM: Gut Microbiome

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