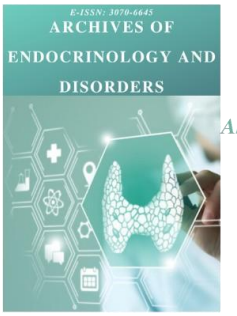

E-ISSN: 3070-6645
Archives of Endocrinology and Disorders

ATHENAEUM SCIENTIFIC PUBLISHERS

Review Article

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The Iron-Sulfide Pathway: Microbiological and Chemical Mechanisms Underlying Extrinsic Black Stain Associated with Iron-Containing Supplements

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Citation: Martinez D, et al. The Iron-Sulfide Pathway: Microbiological and Chemical Mechanisms Underlying Extrinsic Black Stain Associated with Iron-Containing Supplements. Arch Endocrinol Disord. 2026;2(2):1-11.
https://doi.org/10.46889/AED.2026.2204

Received Date: 19-05-2026
Accepted Date: 08-06-2026
Published Date: 15-06-2026

Abstract

Iron-containing supplements are a recognized but mechanistically underexplored cause of Extrinsic Black Stain (EBS) in pediatric patients. This review introduces the iron-sulfide pathway as a unifying framework for understanding how this staining develops: a three-step biochemical sequence in which ionic iron released from oral preparations reacts with hydrogen sulfide produced by chromogenic bacteria within the dental biofilm, precipitating insoluble ferrous sulfide that becomes incorporated into the acquired enamel pellicle and produces the dark pigmentation observed clinically. Each step of this pathway represents a distinct target for clinical intervention. The review examines the pharmaceutical factors that determine oral iron bioavailability, the microbial ecology of black stain biofilms and the role of iron as a selective ecological pressure, the chemistry of ferrous sulfide formation and pellicle incorporation and the clinical consequences of an active pathway, including the paradoxical inverse association between EBS and dental caries. Evidence-based prevention and management strategies are organized around each pathway step and critical gaps in the current evidence base are identified. Extrinsic black stain associated with iron supplementation is not an inevitable side effect of treatment; it is a predictable, comprehensible and potentially interruptible process whose understanding should inform both clinical practice and future research.

Keywords: Extrinsic Black Stain; Iron Supplementation; Salivary Iron Bioavailability; Chromogenic Bacteria; Hydrogen Sulfide; Ferrous Sulfide; Dental Biofilm; Acquired Enamel Pellicle; Pediatric Dentistry

Introduction: Extrinsic Black Stain and the Iron Connection, establishing the Pathway Framework

Extrinsic Black Stain (EBS) is a distinctive form of dental discoloration characterized by dark brown or black deposits appearing along the cervical margins of the teeth, presenting as dotted lines or continuous pigmented bands firmly attached to the enamel surface and observed most frequently on the lingual and proximal surfaces of posterior teeth [1]. Although more commonly

reported in primary dentition and in children, cases in permanent dentition and adults are well documented, confirming that the condition is not restricted to a specific age group [2]. Reported prevalence varies considerably across populations, ranging from approximately 2.4% to 26%, likely reflecting differences in diet, oral microbiota, salivary composition and diagnostic criteria [2,3]. Unlike superficial staining caused by food or beverages, EBS cannot be removed by routine toothbrushing and typically requires professional prophylaxis for complete elimination; even after treatment, recurrence is the rule rather than the exception, making it a persistent clinical and aesthetic concern for patients, caregivers and clinicians alike [1,3].

Among the most clinically relevant yet underrecognized triggers of EBS is the use of iron-containing supplements, particularly liquid ferrous preparations prescribed for iron deficiency anemia, the most prevalent nutritional deficiency worldwide, affecting an estimated 40% of children under five years of age [1,4]. Pediatric dentists and general practitioners increasingly encounter iron-supplemented children presenting with black staining, yet the mechanistic basis for its formation, its determinants and its management options remain insufficiently addressed in the dental literature. Understanding why this staining occurs, in which patients and through which biochemical steps is essential for moving clinical management beyond simple pigment removal toward genuine prevention [3].

Not all black dental staining shares the same origin and recognizing this distinction is central to the purpose of this review. Chromogenic EBS of bacterial origin produces deposits characteristically confined to the cervical third of the crown, arising from the metabolic activity of specific biofilm microorganisms. Iron supplement-induced staining, by contrast, tends to extend beyond the cervical third, involving broader coronal surfaces, because its mechanism is driven by the local availability of ionic iron in the oral environment rather than by microbial pigment production alone [4]. This topographic difference carries diagnostic significance; it reflects a fundamentally different etiological pathway. The most recent narrative review on bacterially driven black dental stains, published in 2025 in the European Journal of Oral Sciences, explicitly excluded iron-medication-associated pigmentation from its scope, defining precisely the gap this paper addresses [5].

The framework proposed to bridge that gap is the iron-sulfide pathway: a stepwise biochemical sequence in which ionic iron from supplements enters the oral environment, encounters hydrogen sulfide produced by chromogenic bacteria within the dental biofilm and precipitates as insoluble Ferrous Sulfide (FeS) that becomes incorporated into the acquired enamel pellicle, producing the dark pigmentation observed clinically [5,6]. This three-step sequence integrates the pharmaceutical, microbiological and chemical dimensions of stain formation into a single coherent model, providing a mechanistic foundation that neither a purely microbiological nor a purely chemical approach has offered in isolation.

Step 1 of the Pathway: Iron Bioavailability in the Oral Environment; From Supplement to Salivary Iron Pool

Iron supplementation remains one of the most commonly prescribed therapies in pediatric medicine given the high global prevalence of iron deficiency anemia, particularly among infants and young children [6]. Oral iron preparations are frequently administered as liquid formulations because they allow easier dose adjustment and improved compliance in pediatric patients [5]. Repeated exposure of the oral cavity to iron-containing supplements has been consistently associated with the development of extrinsic dental pigmentation, especially dark brown or black discoloration affecting primary dentition [1,3]. Unlike classical chromogenic black stain of bacterial origin, iron-associated staining often presents as diffuse deposits extending beyond the cervical third of the crown, suggesting a distinct physicochemical mechanism driven by the local availability of ionic iron in the oral environment [2,4]. Understanding how that ionic iron enters the oral cavity, what chemical forms it adopts and what determines its reactivity represents the first step of the iron-sulfide pathway. The staining potential of oral iron supplementation depends substantially on the pharmaceutical form administered and its behavior within the oral cavity [6]. Ferrous salts, particularly ferrous sulfate (Fe^{2+}) and ferrous fumarate, exhibit high aqueous solubility and rapid ionization under salivary pH conditions, resulting in elevated concentrations of bioavailable ionic iron at the tooth-saliva interface [6,7]. In contrast, ferric polymaltose complexes demonstrate greater molecular stability and slower dissociation under salivary conditions, reducing the immediate release of free iron ions; however, it should be noted that staining has also been reported with polymaltose formulations, suggesting that even slower iron release does not eliminate the risk entirely [9]. These physicochemical differences directly influence the amount of reactive iron available to interact with salivary proteins, dental surfaces and the acquired pellicle [6,7]. Consequently, highly soluble ferrous formulations have been more consistently associated with a greater degree of extrinsic staining than more chemically stable iron complexes [3,7].

Once released into saliva, iron undergoes dynamic speciation that determines its chemical reactivity and oral bioavailability [8]. Salivary iron exists in both free and protein-bound forms, with Fe^{2+} and Fe^{3+} ions associating with transferrin, lactoferrin, mucins and other salivary glycoproteins [8,9]. However, not all salivary iron is chemically available for the subsequent pigment-forming reaction that defines this pathway. The fraction of soluble ionic iron that escapes immediate protein sequestration, the free ionic iron pool, is the most relevant component of Step 1, because it is this fraction that remains capable of interacting directly with enamel surfaces, the acquired pellicle and ultimately with bacterially produced hydrogen sulfide. Salivary pH also plays an important regulatory role in this process; acidic environments favor iron solubility and maintain iron in a more reactive ionic state, whereas neutral or alkaline conditions promote precipitation and reduce chemical availability, shifting the equilibrium away from the reactive free iron pool [6,8]. The acquired pellicle functions as a critical mediator in the first step of the pathway, acting as a concentration site for ionic iron at the enamel surface [8,9]. This protein-rich acellular film forms rapidly on enamel through the selective adsorption of salivary proteins and glycoproteins and current evidence indicates that pellicle proteins possess ion-binding properties capable of retaining metallic ions, including iron, at the tooth surface [9]. This localized concentration of ionic iron within the pellicle is mechanistically significant: it means the substrate for the iron-sulfide reaction is not uniformly distributed throughout the oral cavity but is preferentially enriched at the precise site where the FeS pigment will ultimately form. The pellicle therefore does not merely retain iron passively; it positions iron at the enamel interface where the subsequent microbiological and chemical steps of the pathway will occur. The exact molecular mechanisms governing metallic ion incorporation into the pellicle remain incompletely understood and represent an important area for future investigation [9].

Multiple variables modulate the availability of free ionic iron within the oral environment and their interaction determines the intensity of Step 1 of the pathway [6,8]. Salivary flow rate, protein composition, pH and exposure time all influence iron speciation and retention. Among pharmaceutical variables, the route and form of administration are particularly consequential. Liquid iron supplements produce prolonged direct contact between highly soluble ionic iron and enamel surfaces, generating locally elevated concentrations of reactive Fe^{2+} at the tooth-pellicle interface [6,7]. Capsules and tablets, by contrast, expose the oral cavity more indirectly through salivary diffusion following ingestion, substantially reducing localized iron accumulation on dental surfaces [6]. The viscosity and oral retention time of liquid preparations further extend enamel exposure to ionic iron beyond the moment of administration [9]. These factors collectively explain why liquid ferrous formulations are consistently associated with a greater risk of extrinsic staining in pediatric patients and why modification of the administration route represents a rational first intervention targeting this step of the pathway (Fig. 1).

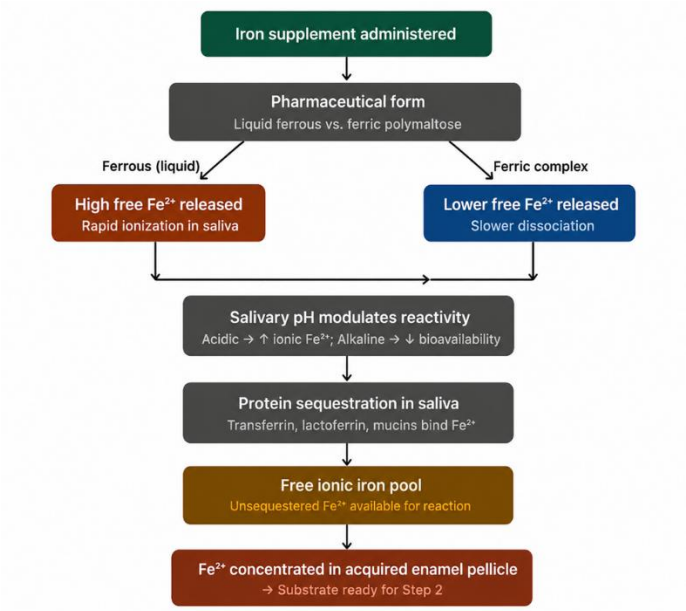

Figure 1: Determinants of free ionic iron bioavailability in the oral environment (Step 1 of the iron-sulfide pathway) [6-8].

The risk of extrinsic black staining associated with iron supplementation therefore depends not on iron exposure alone but on the persistence of bioavailable ionic iron within the salivary environment and, critically, within the acquired pellicle at the enamel surface [8,9]. These early physicochemical events constitute Step 1 of the iron-sulfide pathway and establish the chemical preconditions necessary for the microbiological and reactive steps.

Step 2 of the Pathway: Chromogenic Bacteria and Hydrogen Sulfide Production: The Microbiological Arm

The iron pool established at the enamel surface in Step 1 of the pathway does not react spontaneously to form visible pigment. Its transformation into ferrous sulfide requires a biological intermediary: the production of hydrogen sulfide (H_2S) by chromogenic bacteria within the dental biofilm. Understanding which organisms produce H_2S, through what metabolic mechanisms and how iron availability shapes the microbial community that produces it constitutes the second step of the iron-sulfide pathway [10].

Metagenomic evidence indicates that black-stained dental deposits harbor a microbial community that differs significantly from conventional supragingival plaque, exhibiting greater species richness and diversity rather than the reduced diversity typically associated with dysbiotic or cariogenic biofilms [10]. High-throughput sequencing studies have demonstrated that the microbiota associated with EBS shows increased abundance of genera including Actinomyces, Prevotella, Neisseria, Rothia, Capnocytophaga and Cardiobacterium, supporting the concept that EBS represents an ecologically distinct biofilm condition driven by selective enrichment rather than invasion by a single pathogen [11,12].

Among these organisms, species within the genus Actinomyces occupy a central mechanistic role in Step 2 of the pathway. Actinomyces israelii and Actinomyces naeslundii have been specifically identified as producers of hydrogen sulfide through anaerobic degradation of sulfur-containing amino acids, particularly cysteine and methionine, present within the biofilm environment [13]. This metabolic capacity, known as sulfur amino acid catabolism, generates H_2S as a byproduct of protein breakdown under anaerobic conditions. Additional species including *Actinomyces odontolyticus*, *A. graevenitzii* and *A. radicidentis* have been associated with the production of pigments ranging from brown to black and the relative proportions of Actinomyces species within an individual's biofilm may help explain why some patients develop more intense pigmentation than others [11]. Prevotella species contribute a parallel route to H_2S generation through their proteolytic metabolism of sulfur-containing substrates, reinforcing the sulfide-producing capacity of the black stain biofilm [12].

It is important to recognize that chromogenic capacity and H_2S production do not fully overlap among oral microorganisms. Some anaerobic proteolytic species contribute primarily to sulfur metabolism and H_2S generation without producing visible pigment independently; others accumulate iron-sulfide pigment within the biofilm matrix through direct interaction with ionic iron. The clinical stain represents the combined output of both populations operating within the same biofilm [13,14].

The availability of iron in the oral cavity, established in Step 1, acts as an ecological selective pressure within the dental biofilm that favors anaerobic and proteolytic bacterial populations [10,13]. Iron is an essential cofactor for the metabolic activity of many oral bacteria and its elevated availability in the biofilm environment of patients receiving iron supplementation shifts the competitive balance toward iron-utilizing species. This ecological shift promotes biofilm maturation, enhances anaerobic metabolism and increases the collective capacity of the community to generate H_2S, thereby intensifying the conditions necessary for Step 3 of the pathway. Two competing hypotheses have been proposed regarding the directionality of the relationship between iron and the chromogenic microbiome and both deserve critical consideration [5]. The first proposes that the black pigment is the direct metabolic byproduct of iron deposition by specific microorganisms; that is, certain bacteria actively sequester iron ions from the oral environment and deposit them as part of their metabolic activity and the dark color results from this biological iron deposition. Under this model, the microbiome is the primary driver and the iron merely the substrate. The second hypothesis inverts the causality: environmental factors, specifically elevated ionic iron from supplements, create the chemical conditions for FeS precipitation independently of microbial activity and this altered chemical environment subsequently selects for and stabilizes the chromogenic microbiome. Under this model, the chemistry drives the ecology [5-9]. Current evidence does not definitively resolve this debate and the most plausible interpretation is that both mechanisms operate simultaneously and reinforce each other within a self-sustaining biofilm system, which would explain both the selectivity and the persistence of the stain. Individual susceptibility to EBS development under iron supplementation cannot be explained by iron exposure alone, as only a subset of children receiving comparable supplementation develop visible staining. This variability likely reflects differences in baseline oral microbiome composition, particularly the presence and relative abundance of H_2S-producing Actinomyces and Prevotella species, as well as differences in salivary chemistry, biofilm maturation dynamics and pellicle protein composition [11,12]. These host-level factors interact with the environmental iron load to determine whether the conditions for Step 3 of the pathway are met.

Step 3 of the Pathway: The Iron-Sulfide Reaction; Chemistry of Pigment Formation and Pellicle Incorporation

The third step of the iron-sulfide pathway is the chemical reaction itself: the meeting of bacterially produced hydrogen sulfide with bioavailable ferrous iron at the enamel surface and the precipitation of the insoluble pigment that gives EBS its characteristic appearance [14]. This is the mechanistic core of the paper and understanding it in chemical terms is essential for interpreting both the clinical behavior of the stain and the rationale for any intervention.

The central reaction of the pathway is the combination of Hydrogen Sulfide (H_2S), produced by chromogenic bacteria as described in Section 3, with ferrous ions (Fe^{2+}) concentrated at the enamel-pellicle interface as described in Section 2. This reaction produces ferrous sulfide (FeS), an insoluble dark compound responsible for the characteristic pigmentation of EBS:

$$Fe^{2+} + H_2S \rightarrow FeS\downarrow + 2H^+$$

An investigation into the nature of the black pigment in EBS confirmed that the material is a ferric salt, most probably ferrous sulfide, formed by the reaction between hydrogen sulfide produced by bacterial action and iron present in saliva or gingival exudate [15]. This reaction proceeds most readily under mildly acidic to neutral pH conditions, consistent with the salivary environment of the oral cavity and is favored by reduced oxygen tension within mature biofilms, where anaerobic conditions promote both H_2S production by bacteria and the maintenance of Fe^{2+} in its reduced, reactive form [15,16]. Under more alkaline conditions, iron tends to precipitate as hydroxide rather than sulfide, reducing the efficiency of FeS formation; conversely, highly acidic conditions, while increasing iron solubility, can inhibit the anaerobic bacterial activity required for H_2S generation, creating a narrow optimal pH window for the reaction to proceed efficiently [1,15]. Alongside FeS, related ferric sulfide compounds with similarly dark optical properties may also form through secondary oxidation reactions, contributing to the full spectrum of pigmentation observed clinically [15,17].

The chemical stability of FeS is the primary reason EBS is so clinically persistent. Ferrous sulfide is highly insoluble under the neutral to mildly alkaline conditions of healthy saliva, rendering it resistant to dissolution by salivary flow, conventional oral hygiene products and most over-the-counter whitening agents. Its strong surface adhesion to the acquired pellicle means that mechanical disruption, rather than chemical dissolution, is required for its removal, which explains why professional prophylaxis is necessary and why the stain returns rapidly once the pellicle reforms [1,15].

The incorporation of FeS into the acquired pellicle is not a passive process of simple surface deposition. The pellicle proteins, including mucins, proline-rich proteins and glycoproteins, possess metal-binding domains that interact with iron ions and create anchoring points for FeS nucleation directly at the enamel surface [17,18]. Once initial FeS crystals form at these binding sites, they serve as nucleation foci for further precipitation, progressively building the pigmented deposit within the pellicle matrix. This process is accompanied by the co-precipitation of calcium and phosphate, which contribute to the partial mineralization of the deposit. Biochemical analysis of black stain gingival debris has confirmed significantly higher concentrations of calcium and phosphate in affected individuals compared with controls, supporting the concept that EBS represents a partially mineralized biofilm rather than a simple surface pigment [16]. Copper has also been identified as a cofactor in some forms of dark dental staining; iron-copper-sulfide complexes can form in the presence of salivary copper ions, producing a darker and more adherent deposit than FeS alone and may account for some of the variability in stain intensity observed between patients [15,17].

The topographic distribution of the pigment provides a chemically interpretable clinical clue that distinguishes bacterially-driven EBS from iron supplement-induced staining. In bacterially-driven EBS, the stain is characteristically limited to the cervical third of the crown because this is where biofilm accumulates most densely under normal salivary flow and where anaerobic conditions are most easily established within the plaque. The FeS reaction occurs within this mature biofilm zone and the resulting pigment deposits in the same location [1]. In iron supplement-induced staining, however, the distribution is broader, extending beyond the cervical third to involve larger coronal surface areas. This occurs because liquid iron formulations deliver ionic Fe^{2+} directly and diffusely across all accessible tooth surfaces during administration, not only to the subgingival biofilm zone. The reaction therefore occurs wherever ionic iron and H_2S coexist, which in supplement-exposed teeth is a considerably wider territory than in the bacterially-driven form (Table 1) [4].

Feature	Bacterially Driven EBS	Iron Supplement-Induced EBS
Topographic distribution	Restricted to cervical third of the crown	Extends beyond cervical third; broader coronal surfaces involved
Primary driver	Microbial metabolic activity within mature biofilm	Local availability of ionic Fe^{2+} from supplement administration
Location of FeS reaction	Within anaerobic biofilm zone at cervical margin	Wherever ionic Fe^{2+} and H_2S coexist across accessible tooth surfaces
Iron source	Salivary iron and gingival exudate	Direct enamel contact with ionic iron from liquid preparation
Oxygen conditions	Strictly anaerobic; limited to mature subgingival biofilm zone	Less dependent on strict anaerobiosis; reaction occurs across broader surfaces
Clinical appearance	Discrete dark band or dotted line at cervical margin	Diffuse dark film extending coronally
Diagnostic significance	Suggests chromogenic biofilm activity	Suggests active iron-sulfide pathway driven by supplementation

Table 1: Systematic comparison of pigment distribution patterns in extrinsic black stain (Section 4 Step 3 of the iron-sulfide pathway) [15,16].

The recurrence of EBS following professional prophylaxis is chemically predictable from the pathway itself. Mechanical removal of the stain eliminates the FeS deposit but does not remove any of the three components responsible for its formation. The acquired pellicle reforms within minutes to hours of prophylaxis, re-establishing the iron-binding protein scaffold at the enamel surface. Salivary iron derived from ongoing supplementation replenishes the free ionic iron pool [1]. The chromogenic microbiome, undisturbed by prophylaxis alone, rapidly recolonizes the pellicle and resumes H_2S production. Within this environment, FeS precipitation begins again and the clinical stain re-emerges, often within days to weeks [15]. Recurrence is therefore not a treatment failure; it is the biologically inevitable consequence of leaving all three pathway components intact.

Clinical and Microbiological Consequences: Caries Risk Paradox, Enamel Impact and Individual Susceptibility

A paradoxical finding consistently reported in the literature on extrinsic black stain is its association with a lower incidence of caries in primary dentition, despite representing a mature and visually prominent biofilm. Systematic reviews and meta-analyses have demonstrated lower caries prevalence and severity in children with EBS compared with unaffected controls, challenging the conventional assumption that visible plaque accumulation directly increases cariogenic risk [19]. Mechanistically, this observation has been linked to a less acidogenic oral ecosystem associated with EBS, characterized by reduced dominance of *Streptococcus mutans*, enrichment of non-cariogenic taxa and salivary profiles with greater buffering capacity and higher concentrations of calcium and phosphate [20]. Iron compounds within the biofilm have also been proposed to exert bacteriostatic effects on cariogenic species, potentially contributing to the reduced caries activity observed in affected individuals [20]. At the enamel level, the consequences of the iron-sulfide pathway must be distinguished into two mechanistically separate processes that may occur simultaneously in children receiving liquid iron supplements. The first is iron-mediated pigmentation, in which Fe^{2+} ions interact with the acquired pellicle and bacterially produced H_2S to form FeS deposits, as described in Section 4. This process produces discoloration without directly damaging the enamel crystalline structure. The second is acid-mediated demineralization, in which the low pH of liquid iron formulations, particularly ferrous sulfate drops, promotes enamel dissolution and reduces surface microhardness independently of the FeS reaction [21,22]. In vitro evidence demonstrates measurable reductions in primary enamel microhardness following exposure to iron drops, with scanning electron microscopy revealing surface irregularities consistent with early erosive change [21]. These two mechanisms have different clinical implications: esthetic staining alone does not compromise enamel integrity, but concurrent acid exposure may introduce a genuine structural risk that warrants separate clinical attention and, where possible, remineralization strategies (Table 2) [23].

Feature	Iron-Mediated Pigmentation	Acid-Mediated Demineralization
Mechanism	Fe^{2+} reacts with bacterially produced H_2S within the acquired pellicle to form insoluble FeS	Low pH of liquid ferrous preparations promotes enamel dissolution independently of the FeS reaction
Enamel impact	Surface discoloration; crystalline enamel structure remains intact	Reduced surface microhardness; surface irregularities on SEM consistent with early erosive change
Evidence source	Biochemical and metagenomic studies of black stain composition	In vitro microhardness and SEM studies of primary enamel exposed to iron drops.
Clinical risk	Aesthetic; no structural compromise	Structural; genuine demineralization risk requiring separate clinical attention
Management	Professional prophylaxis; pathway interruption strategies	Remineralization protocols; pH-neutral formulations where available; minimize oral contact time
Reversibility	Stain removable by professional prophylaxis; recurs if pathway intact	Enamel loss irreversible; prevention is the priority

Table 2: Parallel enamel consequences of liquid iron supplementation in pediatric patients (Section 5-Clinical consequences). [15-17,20-22].

The clinical manifestation of EBS does not occur uniformly among children exposed to iron supplementation and this variability is best understood through a tripartite susceptibility model. Three components must converge for the pathway to produce visible staining: bioavailable ionic iron in the oral environment, a chromogenic microbial community with H_2S-producing capacity and a susceptible acquired pellicle with sufficient iron-binding protein content to concentrate and anchor the FeS precipitate. The absence of inadequacy of any one of these three components may prevent clinical staining even in the presence of the other two, explaining why only a subset of children receiving comparable iron supplementation develops visible EBS [11]. Microbiological studies have described a consistent bacterial profile associated with EBS, notably including Actinomyces, Rothia and Neisseria and differences in salivary protein composition may further influence whether the pellicle provides a suitable scaffold for FeS nucleation [8]. EBS should therefore be understood as the expression of a particular oral ecosystem rather than a uniform pharmacological consequence of iron supplementation.

The recurrence of EBS after professional cleaning is clinically predictable from the persistence of all three pathway components. Prophylaxis removes the visible FeS deposit but leaves the chromogenic microbiome, the iron-binding pellicle proteins and the ongoing iron supply from supplementation entirely intact [1]. The pellicle reforms within minutes to hours, iron reaccumulates at the enamel interface and the chromogenic biofilm recolonizes, resuming H_2S production. Recurrence is therefore not a failure of treatment but a biologically inevitable consequence of treating the product of the pathway without addressing its inputs [20]. Although EBS is generally considered biologically benign from a caries perspective, its psychosocial consequences in pediatric populations deserve clinical recognition. Because the stain is visually prominent, recurrent and resistant to removal by the child's own oral hygiene efforts, it can negatively influence self-esteem, self-confidence and personality development, particularly during a period when children are acutely sensitive to peer perception of their appearance [2,24]. Aesthetic concerns about dental appearance in childhood are well documented as sources of social discomfort and parental anxiety and the burden of repeated professional cleanings may itself contribute to dental anxiety in young patients. These outcomes support the inclusion of psychosocial assessment as part of the clinical management of EBS in pediatric patients, alongside its physical treatment.

Synthesizing the Pathway Framework

The iron-sulfide pathway described throughout this review, Fe^{2+} from supplements combined with bacterially produced H_2S within the acquired pellicle to form insoluble FeS pigment, is not a pharmacological side effect that must simply be accepted as

the price of treating iron deficiency anemia. It is a predictable, mechanistically comprehensible biochemical sequence with three distinct components, each of which represents a rational target for clinical intervention. This section organizes prevention and management strategies according to the step of the pathway they address and closes with a frank assessment of the evidence gaps that remain.

Strategies Targeting Step 1: Reducing Iron Bioavailability in the Oral Environment

The most upstream intervention available to clinicians is modification of the iron source itself. Pharmaceutical form has a measurable impact on oral iron bioavailability; ferrous sulfate and ferrous fumarate in liquid form produce the highest concentrations of free ionic Fe^{2+} at the enamel surface, while ferric polymaltose complexes release iron more slowly and at lower free ionic concentrations [6,7]. When staining is a clinical concern, discussing a formulation change with the prescribing pediatrician represents a rational first step, provided that the therapeutic efficacy for iron deficiency anemia is not compromised. This conversation requires interdisciplinary coordination, since the decision to change iron preparation must balance hematological effectiveness against the oral consequences of the current regimen. Administration technique is the second Step 1 strategy and the most immediately actionable for caregivers. Liquid iron supplements administered through a straw bypass much of the tooth surface contact that enables ionic iron to accumulate in the pellicle, reducing the local concentration of Fe^{2+} available for the FeS reaction. Rinsing the mouth with water immediately after administration dilutes salivary iron and shortens the contact time between ionic iron and enamel surfaces. Mixing liquid iron with food or juice, while sometimes recommended for palatability, may reduce the pH at the oral surface and paradoxically increase enamel acid exposure without meaningfully reducing ionic iron contact and should therefore be recommended with caution [22].

Strategies Targeting Step 2: Modulating the Chromogenic Microbiome

Mechanical biofilm control remains the cornerstone of Step 2 intervention. Effective toothbrushing twice daily with a fluoride toothpaste reduces total bacterial load and disrupts biofilm maturation, limiting the anaerobic conditions under which H_2S-producing species such as Actinomyces and *Prevotella thrive* [24]. Professional reinforcement of oral hygiene technique at every recall visit is particularly important in children receiving long-term iron supplementation. Oxidizing agents incorporated into toothpastes have shown favorable results in reducing extrinsic staining, likely through oxidation of sulfide-containing pigments at the biofilm surface; enzymatic compounds derived from fungal proteolytic activity incorporated into specialist dentifrices may also reduce extrinsic stains by disrupting the protein matrix that anchors the biofilm to the pellicle [25]. Both represent adjunctive strategies rather than replacements for mechanical biofilm control.

Probiotics represent the most clinically promising emerging strategy for Step 2 intervention. A randomized controlled trial in 58 children with black stains demonstrated that oral administration of *Streptococcus salivarius* M18 for three months significantly reduced the reformation of black stains following professional cleaning, compared with an untreated control group [26,27]. The proposed mechanism is competitive displacement of chromogenic and H_2S-producing species through bacteriocin production and ecological rebalancing of the oral microbiome. This is, to date, the only available RCT directly addressing microbiome modulation as a strategy for EBS recurrence prevention in children and its findings support further investigation of probiotic approaches in iron-supplemented pediatric patients specifically.

Strategies Targeting Step 3: Managing the Pellicle and the Pigment

Once FeS has formed and become incorporated into the pellicle, mechanical removal through professional prophylaxis is the primary intervention. Air polishing with glycine powder has demonstrated efficacy in removing extrinsic stain deposits with significantly lower enamel surface abrasion than sodium bicarbonate or conventional prophylaxis paste, making it particularly appropriate for use in primary dentition and in children requiring repeated prophylaxis due to frequent EBS recurrence [28]. The recommended recall interval for children with active EBS under iron supplementation should be individualized based on stain recurrence rate, but intervals of three to four months are frequently necessary given the rapid pellicle reformation and microbiome recolonization described throughout this paper [29].

Antimicrobial Photodynamic Therapy (aPDT) represents an emerging strategy with dual potential in this context. In vitro evidence has demonstrated that aPDT using indocyanine green with 808 nm laser irradiation and methylene blue with 660 nm laser irradiation significantly reduced colony counts of *Actinomyces naeslundii* and *Aggregatibacter actinomycetemcomitans*, the two chromogenic bacterial species most directly implicated in dental black staining, with indocyanine green demonstrating the

greater antibacterial effect of the two irradiation protocols [30]. By simultaneously targeting the chromogenic microbiome and potentially oxidizing FeS pigments, aPDT addresses both Step 2 and Step 3 of the pathway in a single clinical session. The evidence base currently remains limited to in vitro and early clinical case data and its routine implementation in pediatric practice requires further evaluation before a formal recommendation can be made [29].

Biomimetic hydroxyapatite formulations have been investigated for their capacity to reduce plaque accumulation and improve enamel surface smoothness, which may indirectly reduce the efficiency of FeS nucleation by creating a less adhesive pellicle surface [26]. While promising in adults with extrinsic staining, direct evidence for their efficacy in iron supplement-induced EBS in children is not yet available and caution is warranted in extrapolating findings from those populations.

Evidence Gaps

Despite the mechanistic framework proposed in this review, the evidentiary foundation for most specific interventions targeting iron supplement-induced EBS remains limited. No randomized controlled trials have been specifically designed to evaluate the prevention of iron supplement-induced staining through administration technique modifications or formulation changes in pediatric patients [27]. The iron speciation studies available are predominantly conducted in vitro and do not account for the full complexity of the salivary environment, the individual microbiome or the interaction between pellicle protein composition and iron-binding dynamics. Longitudinal studies tracking the natural history of EBS in iron-supplemented children from supplementation initiation through treatment completion remain absent from the literature [12,29]. Studies that simultaneously measure salivary iron speciation, microbiome composition and clinical staining outcomes as linked variables have not been conducted; without such data, the three-step pathway proposed in this review remains a well-supported conceptual model rather than a fully validated mechanistic sequence [8,28].

Conclusion

Extrinsic black stain associated with iron supplementation is not an inevitable pharmacological side effect. It is the predictable clinical output of a three-step biochemical pathway in which iron bioavailability, chromogenic microbial activity and pellicle incorporation interact to produce a chemically stable pigment that resists conventional oral hygiene. Each step of this pathway is a legitimate target for clinical intervention and the most effective management will address all three simultaneously rather than treating only the visible deposit. For the prescribing pediatrician, awareness of formulation and administration-technique choices can meaningfully reduce the iron load available to the pathway. For the dentist, understanding the pathway reframes recurrence not as treatment failure but as a biological inevitability in the absence of upstream intervention. For the researcher, the pathway provides a testable mechanistic model around which rigorous trials can be designed. Extrinsic black stain associated with iron supplementation is a condition we understand well enough to prevent better than we currently do.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Acknowledgement

The authors have no acknowledgments to declare.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement

Informed consent was obtained from all participants included in the study.

Authors' Contributions

All authors contributed equally to this paper.

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