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Literature Review Article

The impact of antibiotic use on dental enamel development defects: a systematic review of studies in animal models

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Abstract

Introduction: Dental enamel development defects (DDEs) affect up to 40% of individuals and result from genetic, epigenetic, systemic, or local factors. DDEs are classified as hypomineralizations (qualitative defects) or hypoplasias (quantitative defects). Prolonged antibiotic use, particularly amoxicillin in early childhood, has been suggested as a potential cause. While in vitro studies indicate an effect on amelogenesis, no systematic reviews have analyzed this association in animal models. This study systematically reviews animal studies on amoxicillin's impact on DDEs. **Objective:** To systematically review animal studies investigating amoxicillin's effects on dental enamel defects. **Material and methods:** This review follows PRISMA guidelines. The PICOS strategy defined eligibility criteria, focusing on animal studies assessing amoxicillin's effects on enamel. Exclusion criteria included: (1) non-animal models, (2) other drug therapies, (3) antibiotics other than amoxicillin, (4) literature reviews or editorials, and (5) in vitro studies. A comprehensive search in PubMed, Embase, and Web of Science was conducted without restrictions. Two independent reviewers screened titles/abstracts, resolving conflicts through a third reviewer. Data extraction included study details (author, year, animal model, dose, and enamel evaluation method). Bias risk was assessed using SYRCLE criteria. **Results:** From 141 articles, 84 duplicates were

removed. After screening, 9 studies (2011–2020) remained for full-text analysis, including 8 case-control studies and 1 randomized clinical trial. Animal models included pigs, mice, and rats, with amoxicillin doses ranging from 50 mg/kg to 3.0 g/kg. Enamel defects were assessed via X-ray microtomography, scanning electron microscopy, and histological staining. Some studies reported reduced enamel mineral density and structural changes in ameloblasts, while others found no significant effects. **Conclusion:** Animal studies suggest a potential link between amoxicillin use and DDEs, particularly enamel hypomineralization. However, inconsistencies in study designs, doses, and evaluation methods highlight the need for further research.

Introduction

Dental enamel development defects (DDEs) are enamel malformations commonly found in the population, with a prevalence of up to 40% [28]. DDEs have etiologies including genetic and/or epigenetic alterations, systemic problems, and local traumatic injuries during the periods of tooth formation and mineralization [16, 17, 32]. They can be classified into two major groups, hypomineralizations (qualitative defects) and hypoplasias (quantitative defects), and according to the etiological factors into DDEs caused by injuries or pulpal infections in deciduous predecessor teeth, dental fluorosis, amelogenesis imperfecta, and molar-incisor hypomineralization [33].

Possible etiological factors studied in the literature relate to the prolonged use of antibiotics during early childhood and the risk of developing DDEs [10, 12, 14]. Amoxicillin is the most commonly prescribed drug of choice in childhood and is described through in vitro studies as a drug that can cause alterations in amelogenesis [13, 22], and increase the risk of developing DDEs [2, 5, 29, 30].

Studies using animal models in laboratory settings are important tools for advancing science, and over the last century have resulted in the publication of major research findings [25, 26]. They are necessary to advance our understanding of the relationships between DDEs and the use of antibiotics [29]. While systematic reviews of animal experimental studies are not yet a common practice, awareness of the merits of conducting such studies is constantly increasing; however, there are currently no articles in the literature that report the impact of antibiotic use on the development of DDEs in animal models.

Therefore, the aim of this study was to conduct a systematic review of animal studies in the main databases, regarding the impact of amoxicillin use on the development of DDEs.

Material and methods

Protocol and registration

This systematic literature review was developed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist.

Eligibility criteria

The PICOS strategy for systematic reviews was used to guide this study and answer the question: "Can the use of amoxicillin lead to the development of dental enamel defects in animals?". The PICOS strategy was: P (animal studies using amoxicillin), I (enamel development defects), C (animals not receiving amoxicillin antibiotic), O (the use of antibiotics in animals causes enamel development defects), S (in vivo animal studies).

The following studies were excluded: (1) studies that did not use animal models; (2) studies that used other drug therapies and evaluated the development of DDEs; (3) studies that evaluated other classes of antibiotics besides amoxicillin; (4) literature reviews, editorials, book chapters, and abstracts; (5) in vitro studies.

Search protocol and keywords

Indexed databases [(PubMed/Medline (National Library of Medicine), Embase (Ovid) and Web of Science (Clarivate)] were searched independently without time and language restrictions using different combinations of free text keywords (table I), by two independent researchers, up to June 2nd, 2022. Manual search of reference lists of eligible and potentially relevant articles that may have been missed in the previous stages was also conducted.

Table I – Electronic search databases and search strategies

PubMed	(amoxicilin) AND (enamel defects); (amoxicillin) AND (molar incisor hypomineralization); (amoxicilin) AND (dental hypomineralization); (amoxicilin) AND (enamel hypomineralization); (antibiotics) AND (hypomineralization)
Embase	'Amoxicillin'/exp OR amoxicillin 'enamel defects' OR (('enamel'/exp OR enamel) AND defects) 'molar incisor hypomineralization'/exp OR 'molar incisor hypomineralization' 'dental hypomineralization' OR (('dental'/exp OR dental) AND ('hypomineralization'/ exp OR hypomineralization))'enamel hypomineralization' OR (('enamel'/exp OR enamel) AND ('hypomineralization'/exp OR hypomineralization))('antibiotics'/exp OR antibiotics) AND ('hypomineralization'/exp OR hypomineralization).
Web of Science	((((((((("amoxicillin"[All Fields])) AND ("hypomineralization"[Topic])) AND ("antibiotics"[Topic])) OR (Enamel[Topic])) AND ("enamel defects"[Topic])) AND ("molar incisor hypomineralization"[Topic])) AND ("wistar"[Topic])) AND ("mice"[Topic])) AND ("piglet"[Topic])

Study selection and data extraction

Two blinded and independent reviewers (ACP and BMM) will duplicate the screening of studies for eligibility in a two-phase screening process, initially screening titles and abstracts, in order to evaluate eligibility based on the full-text articles. Any disagreement that was not resolved by mutual agreement was referred to a third independent reviewer who was consulted in the case (FWGPS). Study selection, including recording of these decisions, was manually recorded in tables created in Excel. All full texts of eligible articles were obtained and examined by three authors (ACP, BMM, and KFM) and processed for data extraction. Data were extracted according to the PRISMA flowchart (figure 1) and entered into a Microsoft Excel spreadsheet by one reviewer (FWGPS) and audited by a second reviewer (FKC). For all studies that met the eligibility criteria, data extraction was composed of the following parameters: (a) author and year, (b) country where the study was conducted, (c) type of study, (d) animals used in the study, (e) gestational intervention protocol/animal culture medium, (f) intervention dose, (g) postnatal intervention protocol, (h) postnatal dose per group.

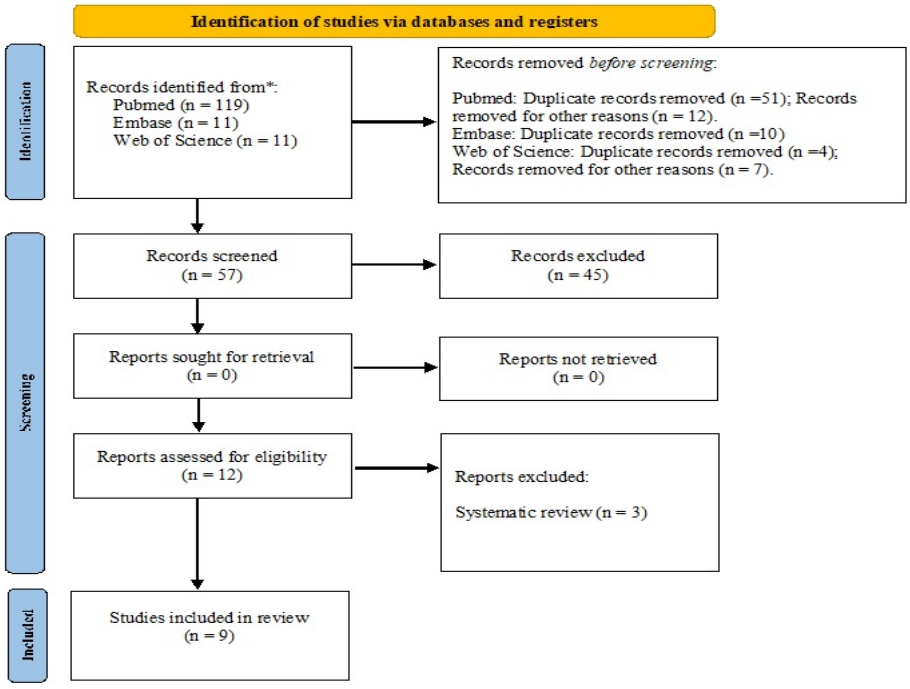

Figure 1 – PRISMA flowchat, selection criteria

Bias risk and study reliability

The studies that met the inclusion criteria were independently assessed for risk of bias using SYRCLE criteria [15] in duplicate by two reviewers (ACP and KFM). Any discrepancies were resolved through discussion involving a third author (FWGPS) and if such discussions did not lead to mutual agreement, the discrepancies were referred to a fourth author (FKC). Attempts were made to contact the authors of articles that presented unclear information. Studies were categorized as: (i) low risk of bias if all criteria were met (adequate randomization and allocation concealment; "yes" response to all questions about data integrity and blinding, and "no" response to selective reporting and other sources of bias); (ii) uncertain risk of bias if one or more criteria were partially met; or (iii) high risk of bias if one or more criteria were not met.

Results

Study selection

Initially, a total of 141 articles were found in the electronic databases. 84 duplicate articles were excluded, and 45 were excluded after reading their titles and abstracts, leaving 12 articles, of which 03 were systematic reviews. Thus, 09 articles were included and read in full (figure 1).

General characteristics of included studies

The characteristics of the included studies are summarized in table II. Studies that evaluated the relationship between the use of amoxicillin and DDEs were included. With respect to the year of publication, the articles ranged from 2011 to 2020, with 8 case-control studies [7, 8, 11, 13, 19, 20, 24, 29], and one randomized clinical trial [21].

Table II – Characteristics of the included studies

Authors	Year studied	Population	Amoxicillin doses	Evaluated teeth	Data analysis	Results
Kusco *et al.* [21]	2013	Pigs	50 and 90 mg/kg	Molars	X-ray microtomography	The prevalence of MIH was 0%, however, X-ray microtomography analysis suggested a reduction in enamel mineral density.
Mihalaş *et al.* [24]	2016	Mice	50, 100 and 150 mg/kg	Incisors	Photographic records with ambient light	Irregular enamel pattern, changes in color and translucency. Yellow and whitish pigmentation in some areas. Slight roughness present. Histological results revealed less organized ameloblasts and increased amounts of vacuoles in the cytoplasm.
Kameli *et al.* [19]	2019	Wistar rats	50 and 100 mg/kg	Molars	H&E staining	Hypomineralization was observed in both the enamel and dentin layers of the groups treated with amoxicillin (50 and 100 mg/kg). Additionally, abnormalities (the presence of multiple vacuoles in this layer) are clearly evident, especially in the odontoblastic layer in the groups treated with amoxicillin (50 and 100 mg/kg).
Gao *et al.* [11]	2020	Mice	50 and 100 mg/kg	Incisors and molars	Scanning electron microscopy	Amoxicillin induces hypomineralization of the enamel in the incisors and molars of rats.
Gottberg *et al.* [13]	2014	Rats	50 and 100 mg/kg	Molars	Optically examined and histologically evaluated using the Down technique	There were no alterations in dental development observed.

To be continued...

Continuation of table II

Authors	Year studied	Population	Amoxicillin doses	Evaluated teeth	Data analysis	Results
Kumazawa *et al.* [20]	2011	Rats	3,0 g/kg	Incisors	Scanning electron microscopy	No morphological changes or hypomineralization were observed in the amelogenesis phase. However, contact microradiography revealed a clear increase in the area of interglobular dentin, indicating altered mineralization that occupied a significant portion of the incisal dentin during the initial phase of dentinogenesis.
Souza *et al.* [30]	2016	Rats	100 and 500 mg/kg	Molars	H&E staining	Amoxicillin interfered with the early stages of amelogenesis, causing a reduction in enamel matrix and morphological alterations in ameloblasts.
Serna Muñoz *et al.* [29]	2018	Mice	50 mg/kg	Molars	H&E staining	Amoxicillin reduces the amount of enzyme present in the enamel organ during the maturation period, but not during its activity.
Souza *et al.*	2012	Rats	100 and 500 mg/kg	Incisors and molars	X-ray microtomography	The electron density in molars was similar among the groups. In incisors, the higher dose of amoxicillin significantly decreased electron density in some rats, but the difference between the groups was not statistically significant.

The studied population consisted of piglets [21], mice [11, 24, 29], and male and female rats [7, 8, 13, 19, 20]. The doses of amoxicillin varied from 50 mg/kg to 500 mg/kg for daily applications over 12 to 60 days or in a single dose. In pregnant rats, the gestational intervention protocol varied from the 13th to the 22nd day of gestation. All studies evaluated molars and/or molars and incisors, with the exception of Mihalaş *et al.* [24] and Kumazawa *et al.* [20], which evaluated only incisor teeth.

The presentation forms of study results varied from X-ray microtomography [21], optical microscopy [13, 20], microradiography [20], X-ray microtomography [8], photographic records with ambient light [24], scanning electron microscopy (SEM) [11, 20, 24, 29], energy-dispersive X-ray spectroscopy (EDX) [11, 24, 29], and histological evaluations [7, 11, 13, 19, 20, 24, 29]. The clinical defects varied between changes in color, cracks, and hypomineralization, while the laboratory defects consisted of mineralization alterations, decreased mineral content, or even no alterations present.

Risk of bias

The methodological quality of the studies included in this review was evaluated using the SYRCLE tool [15], which assesses the risk of bias in animal studies. This tool assesses the risk of selection, performance, detection, attrition, reporting, and other sources of bias in the studies. Ten questions are applied to the included articles, with responses being "YES", indicating low risk of bias, "NO", indicating high risk of bias, and "UNCLEAR", indicating uncertain risk of bias.

In this context, the SYRCLE evaluation showed that the 9 studies had reasonably good quality. Of these, it is important to note that 100% of the included studies reported sequence generation and baseline characteristics, 80% reported allocation and randomization, only 20% presented blinding of researchers regarding performance, and 50% regarding the detection of risk. Of the studies, 40% presented outcome randomization. None of the studies made it clear whether there were incomplete outcome data, 90% showed low risk of bias in selective reporting of results, and none of the included studies reported other sources of bias (figure 2).

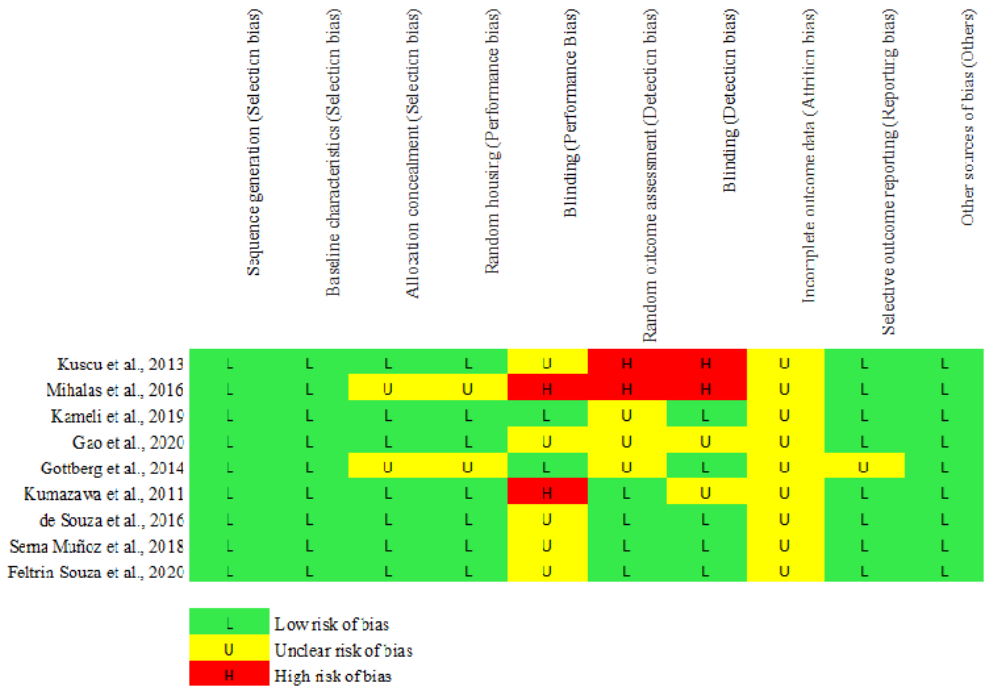

Figure 2 – SYRCLE evaluation

Discussion

The development of teeth can be influenced by both genetic and environmental factors. Factors that interfere with dental development may not only impair the number of teeth developed, but also affect the structure and quality of enamel, leading to the emergence of Developmental Dental Defects (DDEs). As hard dental tissues cannot be remodeled, these effects can be observed in the secretion and deposition of enamel [28, 32]. Enamel formation, known as amelogenesis, is strictly controlled by genes. However, if enamel organ cells are exposed to environmental stress for an extended period during critical periods of amelogenesis, DDEs such as Amelogenesis Imperfecta, Dental Fluorosis, Enamel Hypoplasia, and Molar Incisor Hypomineralization (MIH) can occur [17, 30].

Amoxicillin is a broad-spectrum semi-synthetic antibiotic with bactericidal action against both gram-positive and gram-negative bacteria. It is the first choice of antibiotics for respiratory, gastrointestinal, neural, and skin infections, and is most commonly prescribed in early childhood [6]. In dentistry, amoxicillin is administered to combat complex microorganisms associated with dentoalveolar and soft tissue abscesses, complex maxillary sinus infections [4], and endocarditis [27]. The most frequently reported side effects of amoxicillin include gastrointestinal problems and increased sensitivity [6].

The prescribed dosage of amoxicillin for children is ≥25 mg/kg [9, 18]. According to the drug conversion guideline from humans to mice provided by the US Food and Drug Administration [9], a dose of 25 mg/kg in humans is approximately equivalent to 310 mg/kg in mice. However, some studies have shown that pregnant rats and adult mice exposed to 50 and 100 mg/kg of amoxicillin exhibit enamel defects [13, 24], indicating that enamel is quite sensitive to amoxicillin.

De Souza *et al.* [7] observed structures similar to vacuoles in the ameloblastic layer in rats treated with amoxicillin at doses of 100 mg/kg and 500 mg/kg/day and concluded that these structures may have been formed by interference of amoxicillin with the transmitter molecule medium, which ultimately reduced protein secretion and transmission. These cytoplasmic alterations may be responsible for the reduction in enamel secretion, resulting in decreased enamel thickness. Similarly, Mihalas *et al.* [24], in order to evaluate disturbances in amelogenesis in lower incisors of mice at doses of 50, 100, and 150 mg/kg of amoxicillin/clavulanic acid, found that there was an association between the use of amoxicillin/clavulanic acid and changes in amelogenesis. These changes were dose-dependent, and during

the maturation phase, there was a disturbance in the functions of ameloblasts. Histologically, there were increased amounts of clear vacuoles in the cytoplasm and a slightly elongated and less condensed nucleus, which could be detached from the enamel matrix. However, this study did not evaluate molars. On the other hand, Serna Muñoz *et al.* [29], evaluating the effect of antibiotics and nonsteroidal anti-inflammatory drugs on COX2, found that amoxicillin, either alone or supplemented with clavulanic acid, did not produce changes in enamel or in the Ca/P ratio, which are the main components of hydroxyapatite in all hard tissues.

Gao *et al.* [11] aimed to evaluate the effects of amoxicillin on enamel mineralization, ameloblast morphology, as well as the expression of calicrein-related peptidase 4 (KLK4) and tight junction proteins, claudin 1 (CLDN1), claudin 4 (CLDN4) and occludin (OCLN), in juvenile mouse ameloblasts. The studied concentrations were 50 mg/kg and 100 mg/kg. In this study, amoxicillin decreased the Ca/P ratio in the enamel of lower incisors and molars. There was a change in enamel color and translucency, and more intercellular spaces between maturation-stage ameloblasts were observed in the amoxicillin-treated groups. Amoxicillin decreased the expression of mature ameloblast proteins and induced enamel hypomineralization. Similarly, Kameli *et al.* [19] evaluated tetracycline and amoxicillin on DDEs and dentin of pregnant Wistar rats. The doses of tetracycline were 130 mg/kg and amoxicillin was 50 mg/kg and 100 mg/kg, every 8 hours, from the 13th day until the end of gestation. After birth, the offspring received the same treatment from the 1st to the 10th day of life. The findings suggested that amoxicillin caused a reduction in ameloblasts and enamel thickness, which may have been due to the interference of amoxicillin with the transmitter molecule environment and the consequent impairment of ameloblasts and reduction in enamel secretion.

Likewise, Gottberg *et al.* [13] also related the administration of doses of 100 mg/kg of amoxicillin with hypomineralization in 100% of the tested groups. For the dose of 50mg/kg, half of the groups presented this histological alteration. These studies demonstrate that the incorporation of amoxicillin into developing tooth enamel may be favored by its liposolubility (superior to other penicillins), the wide range of blood vessels found in the intermediate layer in the intercuspidic regions of molars, and the low affinity this medication has due to plasma proteins [31].

Kuscu *et al.* [21], evaluating the association between doses of 50 mg/kg/day and 90 mg/kg/day of amoxicillin and molar incisor hypomineralization (MIH), in a study in pigs with 10 months of evolution, did not find clinically visible association. However, X-ray microtomography suggested a reduction in mineral density at the microscopic level. Since dentin secretion precedes enamel formation and is essential for ameloblastic differentiation, amoxicillin may cause delays in the differentiation of this dental epithelium, resulting in a reduction in enamel matrix secretion. Also, in the study by Feltrin-Souza *et al.* [8], amoxicillin did not significantly alter enamel mineralization and thickness in rats, and there were no statistically significant differences in the Ca/P content, although the higher dose of amoxicillin may affect the enamel in some animals. In addition, impaired dentin formation has been reported in the incisors of rats treated with amoxicillin [20]. A single dose of amoxicillin significantly increased interlobular dentin, and amoxicillin reduced ameloblastic activity, but no morphological alteration or hypomineralization was observed in the enamel, which can be explained by the experimental design used in this study, where a single intraperitoneal application was made instead of daily doses, as in the other studies.

Thus, with the widespread use of amoxicillin in pregnant women and children, as well as the lack of conclusive evidence on its effect on dental enamel development, it suggests the need to establish whether there is a relationship between the prescription of this medication and dental disorders. This study found that these disorders can occur with the use of amoxicillin and would be dose-dependent, possibly affecting the Ca/P levels of the hydroxyapatite layer or during the early stages of amelogenesis. However, other studies have not found an association between amoxicillin and DDEs.

The studies reviewed here were mostly case-control studies, with low risk of bias, but with issues regarding blinding and randomization, which shows limitations and does not allow for conclusions about the etiology or association of amoxicillin and DDEs, only suggesting that there may be an association. Ideally, systematic reviews should include blinded randomized clinical trials, which were not found in the literature. Therefore, it is suggested that robust randomized and blinded clinical studies be conducted. Additionally, to clarify the mechanism of the effect of amoxicillin on ameloblasts and odontoblasts, studies including ultrastructural analysis of in vivo studies in humans are necessary to address clinical doubts. However, based on these findings, it is possible to suggest that amoxicillin interferes with the development of dental enamel in animals.

Conclusion

Amoxicillin may lead to enamel development defects in animals, mainly due to its action in amelogenesis. However, further studies need to be conducted to confirm this issue.

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