SYSTEMATIC REVIEW AND META-ANALYSIS

Horizontal Transmission of *Streptococcus mutans* in Children and its Association with Dental Caries: A Systematic Review and Meta-Analysis

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Abstract: ***Purpose:*** *To systematically evaluate the horizontal transmission of Streptococcus mutans in children and analyze its relationship with dental caries.* ***Methods:*** *Seven databases were searched for observational studies that have determined the transmission of S. mutans among children younger than seven years. Selection of included studies, data extraction, and quality assessment using Downs and Black's (1998) scoring system were performed. The inverse variance random-effect approach was used to pool the results, and statistical heterogeneity was evaluated using I-squared statistics.* ***Results:*** *Fifteen studies were included for qualitative synthesis, five of which were pooled for quantitative analysis. The risk ratio (RR) of sharing only one genotype in caries-free children versus children with caries was found to be 0.60 (95 percent confidence interval [95% CI] equals 0.45 to 0.80; P≤0.001). The RR of sharing more than one genotype was 1.46 (95% CI equals 1.13 to 1.89; P=0.004) in children with caries versus caries-free children. These findings imply that children sharing only one genotype have a 40 percent lesser risk, and children sharing more than one genotype have a 46 percent higher risk of having dental caries.* ***Conclusions:*** *The systematic review provides evidence of the horizontal transmission of S. mutans and its association with dental caries.* *(Pediatr Dent 2021;43(1):E1-E12) Received May 5, 2020 Last Revision September 4, 2020 | Accepted September 11, 2020*

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Dental caries is an infectious and transmissible disease that is caused by cariogenic bacteria present in the oral cavity, among which *Streptococcus mutans* are the principal cariogenic pathogens[1-4] along with *Lactobacilli* aiding in caries progression.[5] Acquisition of these bacteria in the oral cavity occurs during the early years of childhood and has been associated with early childhood caries.[6-8] Early microbial colonization during childhood has been linked to a higher risk of caries in children compared to those who acquire cariogenic microbes later or did not have them in their oral flora.[9] This early colonization and acquisition are associated with increased caries activities in both the primary and permanent dentitions.[10] Developing strategies to prevent early childhood caries necessitates the identification of the source and possible routes of transmission of the causative microorganisms. Various studies have addressed the transmission of cariogenic organisms, with mother-to-child transmission widely accepted as the primary route.[11-13] However, several genotypes not related to the mother's genetic profile have been identified, indicating other possible sources of transmission (i.e., intrafamilial, such as father, siblings, caretakers,[14-17] and extrafamilial, such as nursery and school classmates).[18,19] Vertical transmission occurs between different generations (like from caregivers [mother, father, or caretakers] to the child), while horizontal transmission usually occurs among the same generations (siblings or classmates).[20]

A significant proportion of children attend daycare nurseries and schools globally, where they have a range of contact with each other, providing a favorable environment for the transmission of cariogenic microorganisms, including *S. mutans*.[21] Many studies have reported the genetic similarities of cariogenicmicroorganisms among siblings[17,22] and children attending nursery schools.[18,19]

The purpose of this systematic review was to provide an evidence-based summary regarding the horizontal transmission of oral *S. mutans* among young children.

Methods

Protocol and registration. The present review was performed according to the guidelines suggested by the Cochrane Handbook for Systematic Reviews[23] and is reported per the PRISMA guidelines.[24] The protocol was registered in the International prospective register of Systematic reviews (PROSPERO registration ID: CRD42020164562) and can be freely accessed at *https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=164562*.

Eligibility criteria. Criteria for inclusion of studies in the review were observational studies (cross-sectional or longitudinal) following the PECO strategy, as described below:

- P (participants): siblings or classmates in children younger than seven years;
- E (exposure): presence of *S. mutans* or its different subspecies;
- C (comparison): presence or absence of *S. mutans*;

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- O (outcome): Determination of the presence of transmission of *S. mutans* (sharing of similar genotypes among children) by various molecular laboratory procedures was considered the primary outcome. The sharing of genotypes (one or more than one genotype) among children and its relationship with dental caries was considered the secondary outcome.

Information sources and literature search. A systematic search of the literature was performed on seven electronic databases using broad MeSH terms and keywords on March 23, 2020, by two investigators (MS and SD). The seven databases searched were: Medline (*via* Ovid); Embase (*via* Ovid); Scopus; PubMed; Lilacs; CINAHL; and Web of Science. After removing duplicates, the titles and abstracts of the records were screened against the predetermined eligibility criteria to decide upon the inclusion for further full-text reading. If the reading of the abstract provided an unequivocal interpretation regarding inclusion or exclusion, the record was subjected to full-text reading. The reference lists of the included articles were also checked to identify any potential article which could have been missed in the electronic search. To identify grey literature, www.opengrey.eu and Google Scholar were also searched for any unpublished material.

Study selection. Endnote X 9.2 software for Windows (Clarivate Analytics, Philadelphia, Pa., USA) was used to import the results obtained through the searching of the electronic databases, journals, and grey literature. After the removal of duplicates, the records were scanned by titles and abstracts by two independent investigators (MS and SD) to determine the methodological quality of the trials. A third investigator (YCK) was consulted in case of any discrepancy between the two investigators. Cohen's kappa coefficient (κ) was calculated to establish the level of interrater agreement.

Data collection process. Two investigators (MS and SD) individually extracted the statistical data and characteristics of the studies included in the review on a piloted data extraction form. Opinion from the third investigator (YCK) was sought if any disagreements occurred.

Data items. Extraction of the information and data from each study was conducted as applicable to the following parameters: demographic details of the study; study sample and age; group characteristics; outcomes of interest; methods used; and results.

Risk of bias in individual studies. The quality of the included studies was assessed by a validated index score system by Downs and Black (1998).[25] The scoring system for nonrandomized studies consists of 19 items assigned over four subscales. Only 12 questions relevant for observational studies out of the total 27 were included after discussion by two investigators (details of the questions can be accessed from the original article).[25] These 12 questions were dispersed among the following criteria: reporting (questions one, two, three, five, six, seven, and nine); external validity (questions 11 and 12); internal validity-bias (questions 18 and 20); and internal validity-confounding (selection bias; question 26). The maximum score for question four was 2, while for all others it was 1; therefore, the highest possible score for the system was 13. A score greater than 9 was considered good, while a score of 6 to 8 was moderate, and a score lower than 5 was considered poor quality. Scoring of the articles was performed independently by two investigators (MS and SD), and any inconsistency was resolved through discussion.

Summary measures and quantitative synthesis. The primary outcome measure for the dichotomous variables was risk ratio (**RR**) in children sharing one or more than one type of genotypes of *S. mutans* with siblings or classmates and the relationship with dental caries experience.

Statistical heterogeneity across the pooled studies was checked using I^2 statistics and chi-square-based Q-statistics at a significance level of P=0.05. The quantitative synthesis was done using Stata 13.1 software (StataCorp, College Station, Texas, USA). The inverse variance random-effect approach was used for quantitative synthesis since more than four studies could be pooled, or a fixed approach (inverse variance for continuous variables and Mantel-Haenszel method for binary variables) was planned if less than four studies were found.

Additional analysis. Different subgroup analyses were performed to check the robustness of the results. Subgroup analyses were performed to identify the relationship between dental caries and the sharing of genotypes according to the: type of molecular technique used for *S. mutans* genotypes isolation (restriction fragment length polymerization [**RFLP**] and multilocus sequence typing [**MLST**]); genotype commonality among siblings (intrafamilial) or classmates (extrafamilial); and types of samples collected for molecular microbiological analysis (plaque, saliva, or tongue scrapings).

Results

Study selection. The PRISMA flow diagram of the study selection process is illustrated in Figure 1. Searching seven databases yielded 2,854 articles after removing duplicates. After screening for titles and abstracts, 29 articles were retrieved for full-text reading. A total of 14 articles[18,19,26-37] were found after full-text reading for qualitative analysis. Manual searching of the references of the included studies provided one additional article,[22] making a total of 15 studies to be included in the review. The

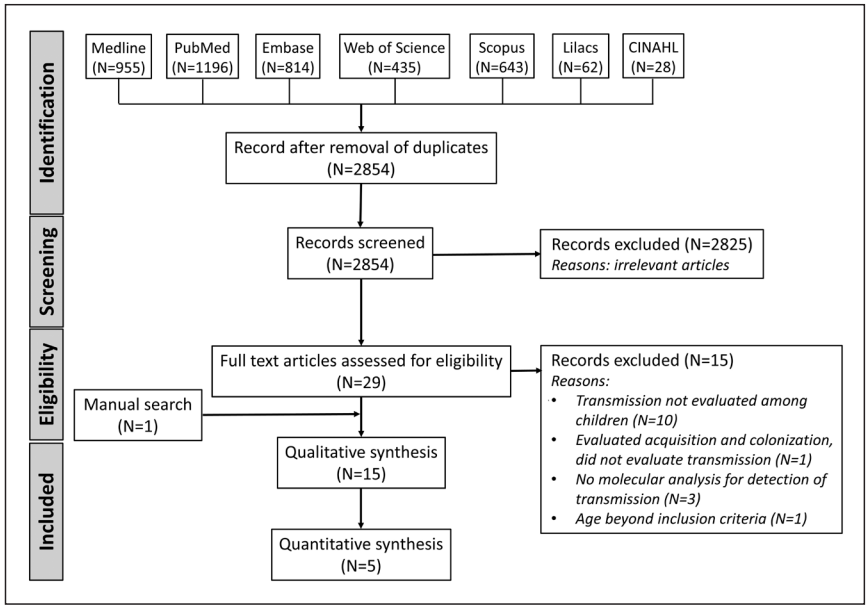

Figure 1. PRISMA flow diagram of the study selection process.

Table 1. SUMMARY OF THE INCLUDED TRIALS

Serial number	Author / year	City, country	Groups	Age range or mean age	Sample	Method used	Outcome of interest	Result
1.	Alves et al. 2009[26]	Sao Paulo, Brazil	160 children were examined at baseline and 6, 12, and 18 months.	5-13 months old	Saliva sample	Analysis of the genetic diversity of *Streptococcus mutans* by AP-PCR. Isolates identified in different subjects but representing the same AP-PCR profile were subjected to chromosomal DNA RFLP analysis to confirm genotypic identity.	*S. mutans* colonization, caries development, and genotypic diversity in children	Overall, 40.3% of children were detectably colonized during the study, and levels of *S. mutans* were significantly associated with the development of caries lesions. Four of these pairs (21.1%) shared identical *S. mutans* RFLP profiles. Identical *S. mutans* genotypes were found in four nursery cohorts. No familial relationship existed in three of these cohorts, indicating horizontal transmission.
2.	Baca et al. 2012[27]	Granada, Spain	42 school-children	6-7 years old	Stimulated whole saliva	Total CFUs with characteristic morphology of *S. mutans* or *Lactobacillus* were counted. *S. mutans* colonies were identified utilizing biochemical tests, and all *S. mutans* strains were genotyped by AP-PCR.	Comparison of mean *S. mutans* and *Lactobacillus* levels and caries index values between children with one and two genotypes	Among 30 *S. mutans*-positive schoolchildren, 11 (36.6%) shared a genotype with at least one other child. Higher *S. mutans* and caries index values were found in those with two genotypes.
3.	Cheon et al. 2013[36†]	Alabama, USA	67 children in an elementary school considered at high caries risk were included and followed for 36 months at 6-month intervals.	5-6 years old	Plaque sample	*S. mutans* isolation and genotyping with rep-PCR were performed using the DiversiLab *Streptococcus* kit followed by DNA fingerprints analysis.	The number of *S. mutans* genotypes were evaluated for association with the CFU and caries examination data. The commonality of genotypes between individuals was evaluated at each visit during the 36 months.	At baseline, 18 distinct genotypes were found among 911 *S. mutans* isolates from 67 children (diversity), and 13 genotypes were shared by at least 2 children (commonality). Further, 62 of 67 children (92.5%) shared at least one genotype with another child.
4.	Doméjean et al. 2010[28]	San Francisco, USA	Out of 96 children enrolled, 47 were infected groups: *S. mutans* only (N=35), *Streptococcus sobrinus* only (N=6), *S. mutans* + *S. sobrinus* (N=6)	5-6 years old	Saliva sample	*S. mutans* were enumerated under a dissecting microscope based on their colony morphology. Differentiation of *S. mutans* and *S. sobrinus* was based on standard fermentation tests with sorbitol, mannitol, melibiose, and raffinose. DNA Extraction and AP-PCR were used to determine the *S. mutans* genotypes.	Salivary *S. mutans* levels and *S. mutans* diversity in children with mixed or single *S. mutans* and *S. sobrinus* infection	Two children (not siblings) in each of the three schools (6%) shared an identical amplitype of *S. mutans*, unique to each pair. The 19 S. *sobrinus* amplitypes were found in 12 children, and all were unique to each child. The suggested horizontal transmission rate in this population was relatively low: 6.3% overall and 13% among the mutans streptococci-infected children.
5.	Hu et al. 2014[29]	Beijing, China	21 children (dmft >6, day nursery class)	4-5 years old	Plaque sample	Plaque samples were cultured under anaerobic conditions for isolating *S. mutans*, which were identified by morphological and biochemical analyses and PCR using primers homologous to the surface protein glucosyltransferase B. The genotypes of the isolated *S. mutans* strains were determined by AP-PCR.	Distribution of genotypes of the isolated *S. mutans* among the 20 participants.	Of the 200 *S. mutans* isolates obtained, 19 were excluded by biochemical analysis, and the remaining 181 isolates were identified as *S. mutans* by PCR with primers of gtfB, showing 37 different genotypes as identified by AP-PCR. Six children were found to carry *S. mutans* of a single genotype, 11 carried 2 genotypes, 2 had 3 genotypes, and 1 had 4 genotypes; 2 children from different classes were found to carry *S. mutans* of the same single genotype. Horizontal transmissions of the strains were not found.

Table 1. CONTINUED

Serial number	Author / year	City, country	Groups	Age range or mean age	Sample	Method used	Outcome of interest	Result
6.	Köhler et al. 2003[30]	Göteborg, Sweden	16 families (16 mother-child pairs, seven fathers, and four siblings). All mothers and children were monitored at 4-month intervals, starting at the age of 15 months until the age of 3 years, and were recalled for further follow-ups at 4, 7, 11, 15, and 19 years of age.	15 months old	Saliva sample	A total of 284 strains from 16 mother-child pairs, seven fathers, and four siblings were available for analysis in the present study. Genomic DNA was digested by the restriction endonuclease HindIII, followed by gel electrophoresis, southern blotting, and hybridization with a digoxigenin-labeled 16S rDNA probe, and hybrid detection by enhanced chemiluminescence.	Intrafamilial distribution of identified ribotypes among the strains of *S. mutans* and *S. sobrinus* analyzed	In 2 of the 3 families from which strains from siblings were available, a common ribotype occurred in all family members. Two siblings in one family harbored an identical *S. mutans* ribotype (2/4=50%).
7.	Liu et al. 2007[31]	Chengdu, China	56 children (28 males and 28 females). According to caries status: 1. No manifest lesions (N=35) 2. Manifest lesions (N=21) According to class 1. Day and night nursery class (N=24) 2. Day nursery class (N=32)	3 and 4 years old	Plaque sample	Chromosomal DNA isolation and DNA fingerprinting: AP-PCR fingerprinting profiles were examined by chromosomal DNA RFLP analysis.	Comparisons of the distribution of 41 children with one or more *S. mutans* amplitypes	*S. mutans* was isolated from 41 of the 56 children. A total of 140 *S. mutans* isolates from 41 children were analyzed by AP-PCR, and 88 different amplitypes were identified. Of the 41 children with *S. mutans* isolates, 82.9% carried two or more genotypes. In our study, 13 children who attended the day and night nursery class carried two identical strains. Another two children in the day nursery class also carried one identical strain (15/41=36.6%).
8.	Mattos-Graner et al. 2001[18]	Sao Paulo, Brazil	1. According to age group (months): 12-18 (N=4) 19-24 (N=8) 25-30 (N=12) 2. According to the number of erupted teeth: 1-19 (N=16) 20 (N=8); 3. According to caries status: No manifest lesions (N=13 Manifest lesions (N=11) 4. According to oral levels of *S. mutans* (CFUs): 1-20 (N=9) 21-99 (N=4) ≥ 100 (N=11)	12 and 30 months old	Saliva sample	Genotyped by AP-PCR and RFLP analysis	Comparisons of the distribution of 24 children with one or more *S. mutans* amplitypes	A total of 76 *S. mutans* isolates from 35 children were analyzed by AP-PCR; 6 children (25%) were found to carry 2 distinct amplitypes of *S. mutans* and 1 child (4.2%) carried three different amplitypes. Only children carrying 2 or more isolates of *S. mutans* species (N=24) were included in the statistical analysis for comparisons of genotypic diversity regarding the other variables analyzed. Two children (2/24=8.3%) attending the same nursery carried the same *S. mutans* genotype.

Table 1. CONTINUED

Serial number	Author / year	City, country	Groups	Age range or mean age	Sample	Method used	Outcome of interest	Result
9.	Momeni et al. 2016[32†]	Alabama, USA	CH1: 91 school-children and their household family members recruited from an elementary school. CH2: 90 infants and their household family members recruited from a local community center.	CH1: Age 5-6 years CH2: Age 6-18 months	Plaque, saliva, and tongue scraped samples	Rep-PCR was performed using the Diversilab system with the *Strep-tococcus* DNA finger-printing kit. MLST was performed on 13 newly identified rep-PCR genotype representative library strains.	Comparison of child-to-child and intrafamilial transmission among children evaluated by rep-PCR genotype	Six genotypes (G01b, G05, G14, G15, G22, and G26) appeared to be predominately shared among the children (child-to-child transmission). Overall, children had 1-9 genotypes and those with multiple genotypes were 2.3 times more likely to have caries experience (dmft/s >0). Among 73 cases that did not share the genotype with the mother, 37 (51%) shared the genotype only with sibling(s).
10.	Momeni et al. 2018[33†]	Alabama, USA	CH1 consisted of 51 school-children; CH2 consisted of 57 infants.	CH1: Age 5-6 years CH2: Age 12-18 months	Whole saliva, plaque, and/or tongue samples	A total of 200 isolates (19 to 20 children per genotype) were evaluated in the 10 rep-PCR dendrograms representing 108 children. From the 200 isolates in the rep-PCR dendrograms, 97 were selected for MLST analysis to determine ST match no-match.	Using rep-PCR to predict matching and non-matching MLST sequence types; the commonality of MLST sequence types for children only and parent-child pairs with rep-PCR genotype G12	Overall, rep-PCR was 75% effective at determining MLST ST match/no-match and 90% effective when applied to related individuals. Six children shared an MLST sequence type with another family member, of which five children (5/24=20.8%) shared a sequence type with another child in the family (sibling, cousin, other).
11.	Spolidorio et al. 2003[22]	Sao Paulo, Brazil	22 families comprising father, mother, and infant (firstborn child)	Infant 5-18 months old	Plaque and saliva samples	*S. mutans* isolates were obtained from all family members and AP-PCR typed separately with a random primer (OPA-13). Bacterial cell lysates were used as a template in PCR reactions, and the amplified DNA fragments obtained were compared by agarose gel electrophoresis.	Detection of degrees of polymorphism among *S. mutans* isolates, and establishment of the level of intrafamilial similarity between isolated strains, by AP-PCR.	A greater similarity (54.5%) occurred between children and the mother, followed respectively between siblings (31.8%) and between children and the father (13.7%).
12.	Shang et al. 2006[34]	Chengdu, China	32 children (day nursery class)	3-4 years old	Plaque sample	Dental plaque samples were cultured, and individual *S. mutans* colonies representative of the colonial morphologies was subcultured on TPY plates. These strains were biochemically identified to species level. Chromosomal DNA isolation and DNA fingerprinting: AP-PCR fingerprinting profiles were examined by chromosomal DNA RFLP analysis.	Comparison of child-to-child amplitypes and genotype similarity between isolated strains.	*S. mutans* was isolated from 78.1% of children. A total of 57 genotypes were identified by AP-PCR. More than one amplitypes were identified in 88% of 25 children with *S. mutans* colonization. Two pairs of children (4/25=16%) shared more common genotypic *S. mutans.*

* AP-PCR=arbitrarily primed polymerase chain reaction; CFUs=colony-forming units; CH1=cohort group 1; CH2=cohort group 2; DNA=deoxyribonucleic acid; gtfb=glucosyltransferase B; MLST=multilocus sequence typing; PCR=polymerase chain reaction; rep-PCR=repetitive extragenic palindromic-PCR; RFLP=restriction fragment length polymorphism.

† The authors were contacted to clarify the data and obtain additional information. According to the authors, the cohorts of these three studies (Momeni et al. 2016,[32] Momeni et al. 2018,[33] and Cheon et al. 2013[36]) overlapped significantly, but they advised that one study (Momeni et al. 2016[32]) might be considered to be a comprehensive paper. Out of these three papers, only paper (Momeni et al. 2016[32]) could be included for meta-analysis in the present review.

Table 1. CONTINUED

Serial number	Author / year	City, country	Groups	Age range or mean age	Sample	Method used	Outcome of interest	Result
13.	Tedjosasongko and Kozai 2002[19]	Hiroshima, Japan	39 children in a day nursery (23 boys and 16 girls) containing 10 siblings	2-25 months old	Plaque sample	The occurrence of dental caries, the number of erupted teeth, and the presence of interdental space of maxillary incisors in children. The isolates of *S. mutans* of the subjects were examined by a DNA fingerprinting method to determine the source of infection.	Cumulative probability of the initial acquisition of *S. mutans* in relation to the number of erupted teeth in the children and as a function of the child's age in months.	The initial acquisition of MS occurred in 21 of 39 children within the range of age 8 months to 52 months, with a mean age of 24 months. Homologies of strain types were found between child and mother in 33.3%, child and father in 8.3%, and child and others in 58.4%. Transmission among the children was also recognized, since 6 children (6/21= 28.5%) shared the same strain type of *S. mutans*.
14.	Zhou et al. 2005[35]	Chengdu, China	24 children from day nursery class (12 with caries; 12 no caries)	3-4 years old	Plaque sample	Dental plaque samples were cultured, and individual *S. mutans* colonies representative of the colonial morphologies was subcultured on TPY plates. These strains were biochemically identified to species level. Chromosomal DNA isolation and DNA fingerprinting: AP-PCR fingerprinting profiles were examined by chromosomal DNA RFLP analysis.	Comparison of child-to-child *S. mutans* amplitypes and genotype similarity between isolated strains.	*S. mutans* was isolated from 66.7% (16) of the 24 children, 58.3% in the caries-free group, and 75% in the caries group. There was a total of 46 *S. mutans* isolates from the 24 children; 29 different amplitypes were identified and 45.8% carried two genotypes. There were 2 genotypes of *S. mutans* repeatedly isolated among 12 nursery children (12/16=75%).
15.	Zou et al. 2006[37]	Chengdu, China	44 children (N = 20-day nursery class; N=24-day and night nursery class); 20 mothers of children in the day nursery class	3-4 years old	Plaque sample	Dental plaque samples were cultured, and individual *S. mutans* colonies representative of the colonial morphologies was subcultured on TPY plates. These strains were biochemically identified to the species level. Chromosomal DNA isolation and DNA fingerprinting: AP-PCR fingerprinting profiles were examined by chromosomal DNA RFLP analysis.	Comparison of child-to-child and child-to-mother *S. mutans* amplitypes and genotype similarity between isolated strains	*S. mutans* was isolated from 65.9% (N=29) of the 44 children and 50% of the 20 mother-child pairs; 29 genotypes of *S. mutans* were identified from the 24 children in the day and night nursery class, and there were 2 genotypes of *S. mutans* repeatedly isolated among 13 children (13/29=44.8%).

* AP-PCR=arbitrarily primed polymerase chain reaction; CFUs=colony-forming units; CH1=cohort group 1; CH2=cohort group 2; DNA=deoxyribonucleic acid; gtfb=glucosyltransferase B; MLST=multilocus sequence typing; PCR=polymerase chain reaction; rep-PCR=repetitive extragenic palindromic-PCR; RFLP=restriction fragment length polymorphism.

† The authors were contacted to clarify the data and obtain additional information. According to the authors, the cohorts of these three studies (Momeni et al. 2016,[32] Momeni et al. 2018,[33] and Cheon et al. 2013[36]) overlapped significantly, but they advised that one study (Momeni et al. 2016[32]) might be considered to be a comprehensive paper. Out of these three papers, only paper (Momeni et al. 2016[32]) could be included for meta-analysis in the present review.

complete search strategy of the electronic databases searched with the yields (number of hits) is presented in the Appendix. The κ value for the selection of the studies from 29 full-text articles was 0.86, indicating a substantial level of agreement.

Characteristics of the included studies. Table 1 provides a summary of the studies included in the current review. The 15 included studies were all observational (cross-sectional or longitudinal) in nature, in which different types of molecular laboratory techniques were used to identify the presence or absence of transmission of *S. mutans* among children. Only three studies[26,30,36] among the 15 included studies were longitudinal, with one[36] having a maximum follow-up of 36 months with an examination every six months. Another longitudinal study[26] had a follow-up of 18 months with examinations every

Table 2. RISK OF BIAS ASSESSMENT (DOWNS AND BLACK's SCORING SYSTEM, 1998)[25]*

Authors (year)	Reporting (maximum score=8)	External validity (maximum score=2)	Internal validity (bias) (maximum score=2)	Internal validity (confounding/selection bias; maximum score=1)	Overall score (maximum score=13)
Alves et al. 2009[26]	7	0	2	1	10
Baca et al. 2012[27]†	8	2	2	1	13
Cheon et al. 2013[36]	6	0	2	1	9
Domejean et al. 2010[28]†	7	0	2	1	10
Hu et al. 2014[29]†	7	0	2	1	10
Köhler et al. 2003[30]	5	0	2	0	7
Liu et al. 2007[31]†	6	0	2	1	9
Mattos-Graner et al. 2001[18]†	6	0	1	1	8
Momeni et al. 2016[32]†	6	0	2	1	9
Momeni et al. 2018[33]†	4	0	2	1	7
Spolidorio et al. 2003[22] †	5	0	2	1	8
Shang et al. 2006[34]†	7	0	2	1	10
Tedjosasongko and Kozai 2002[19]†	4	0	2	1	7
Zhou et al. 2005[35]†	6	0	2	1	9
Zou et al. 2006[37]†	6	0	2	1	9

* Overall score: 9-13=good quality; 6-8=moderate quality; 0-5=poor quality.
† Cross-sectional studies, loss to follow-up not considered.

six months; the third study[30] followed the children at 15 months and three, four, seven, 11, 15, and 19 years of age. Four studies[22,30,32,33] evaluated the intrafamilial transmission among siblings, whereas the other 11 studies[18,19,26-29,31,34-37] evaluated extrafamilial sharing of *S. mutans* genotypes among unrelated children in nurseries or schools. Among these 11 studies conducted on school or nursery children, nine studies[18,19,26-29,34-36] recruited children who attended schools or nurseries during the daytime; the other two studies[31,37] had two different cohorts from daycare as well as from the day and night care nursery schools. Four studies[22,27-29] used arbitrarily primed polymerase chain reaction (**AP-PCR**) to analyze the *S. mutans* strains, two studies[32,33] used MLST, three studies[19,30,36] used DNA fingerprinting, and six studies[18,26,31,34,35,37] used the chromosomal DNA RFLP technique.

The 15 included studies were carried out on a maximum of 969 children (there was significant overlapping of the subjects in three studies,[32,33,36] as mentioned by the corresponding author contacted for additional information), with age varying among the included studies ranging from birth up to seven years. In five studies,[18,19,22,26,30] children were younger than three years; in four studies,[31,34,35,37] children were three to four years old; in one study,[29] children were four to five years old; in two studies,[28,36] children were five to six years of age, and one study[27] had six to seven year olds. Two studies had two different age group cohorts, among which one study[32] had a cohort with six to eighteen months old children and another study[33] had a cohort with twelve to eighteen months old children, both with the second cohort of five to six year olds. The analysis of *S. mutans* genotypes and transmission were performed through one or more of the aforementioned molecular laboratory procedures.

A comparison of the distribution of *S. mutans* genotypes among children of similar age groups in nurseries, schools, or at home was analyzed in all the included studies. The presence of similar genotypes of *S. mutans* (or its different subspecies) among children was reported in all the included studies except one,[29] which could not detect the similarity of genotypes of *S. mutans* among children.

The presence of matching or common strains of *S. mutans* genotypes among the children suggests that horizontal transmission may exist for the isolated strains. Five studies[18,26,31,32,35] compared the distribution of children with one or more *S. mutans* genotypes with the caries status of the children. Four studies[27-29,36] analyzed the relationship between the number of isolated genotypes with the decayed, missing, and filled primary teeth/surfaces (dmft/dmfs) scores of the children. One study[19] evaluated the cumulative probability of the initial acquisition of *S. mutans* with the number of erupted teeth in the children.

Risk of bias within studies and results of individual studies. Table 2 shows the quality and bias assessment (Downs and Black's scoring system)[25] of the included studies. Five studies[18,19,22,30,33] were considered moderate quality, whereas the other 10 studies[26-29,31,32,34-37] were assigned as high quality based on the four criteria evaluated through 12 questions in the index score system. Fourteen out of 15 studies were assigned a zero score for the external validity criteria, which try to direct the representativeness of the findings of the studies to the population from which the participants were derived. As 12 out of the 15 included studies were cross-sectional, being lost to follow-up was not considered, and a score of one was given for confounding or selection bias criteria. Two studies[26,36] among the three longitudinal studies included in the review reported the loss to follow-up. Thus, only one study[30] out of 15 was assigned a score of zero for confounding or selection bias. imilarly, only one study[18] was given a score of one out of a maximum score of two for internal validity or bias criteria.

Synthesis of results. Fourteen studies have reported the percentage of horizontal transmission (commonality of at least one *S. mutans* genotype) among siblings/classmates ranging from 8.3 to 92.5 percent (Table 1). However, one study[29] concluded that no horizontal transmission was found as no common strains of genotypes of *S. mutans* were detected.

Five studies[18,26,31,32,35] were pooled to assess the caries status (as a dichotomous variable [i.e., presence or absence of caries]) of the children sharing one or more genotypes of *S. mutans* with their siblings or classmates. The RR of sharing only one genotype in caries-free children *versus* children with caries was

found to be 0.60 (95 percent confidence interval [**95% CI**] equals 0.45 to 0.80; $P \leq 0.001$), which implies that children sharing only one genotype were 40 percent (95% CI equals 20 percent to 55 percent) less likely of developing caries (Figure 2). Similarly, the RR for sharing more than one genotype in children with caries *versus* caries-free children was 1.46 (95% CI equals 1.13 to 1.89; $P=0.004$), which can be interpreted as children sharing more than one genotype were 46 percent (95% CI equals 13 percent to 89 percent) more likely to have caries (Figure 3).

Additional analysis. The subgroup analysis (among siblings or classmates) for genotype commonality in relation to caries showed significant results in siblings but not in classmates (Figures 2 and 3). However, the significant results in siblings are based on a single study[32] and should be interpreted with caution. Sensitivity analysis using the technique (MLST or RFLP) showed significant results in the MLST technique but not in the RFLP technique (Figures 4 and 5). Similarly, a subgroup analysis was performed for either plaque or saliva samples or using either (saliva/plaque/tongue scrapings according to the convenience), but the results were significant only in a single study[32] that used either plaque, saliva, or tongue scrapings according to the convenience (Figures 6 and 7).

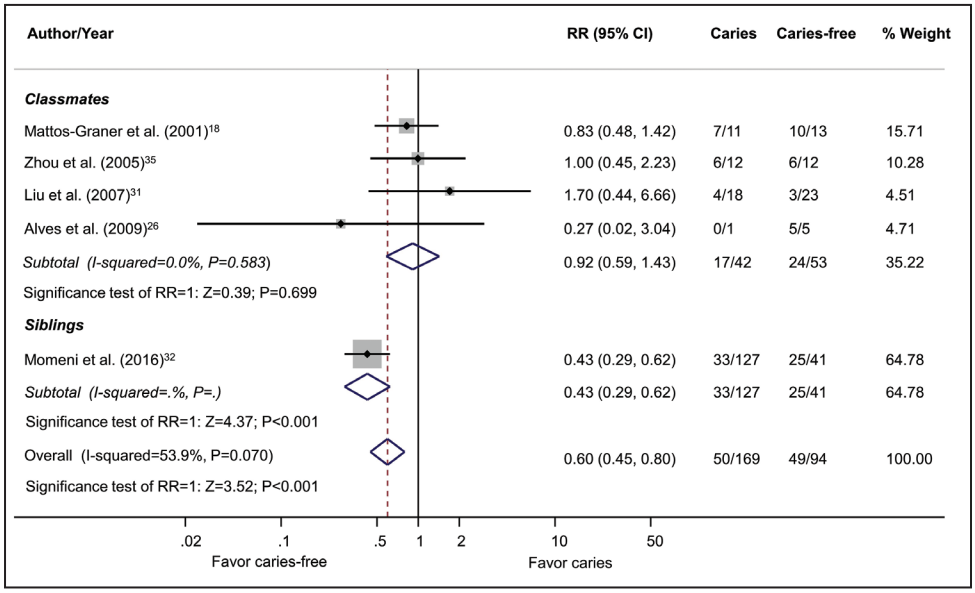

Figure 2. Forest plot showing the risk ratio (RR) of sharing only one genotype in caries-free children versus children with caries (subgrouped according to the classmates and siblings). The 95 percent confidence interval (95% CI) is depicted through the horizontal line, whereas a meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is indicated by the dotted vertical line.

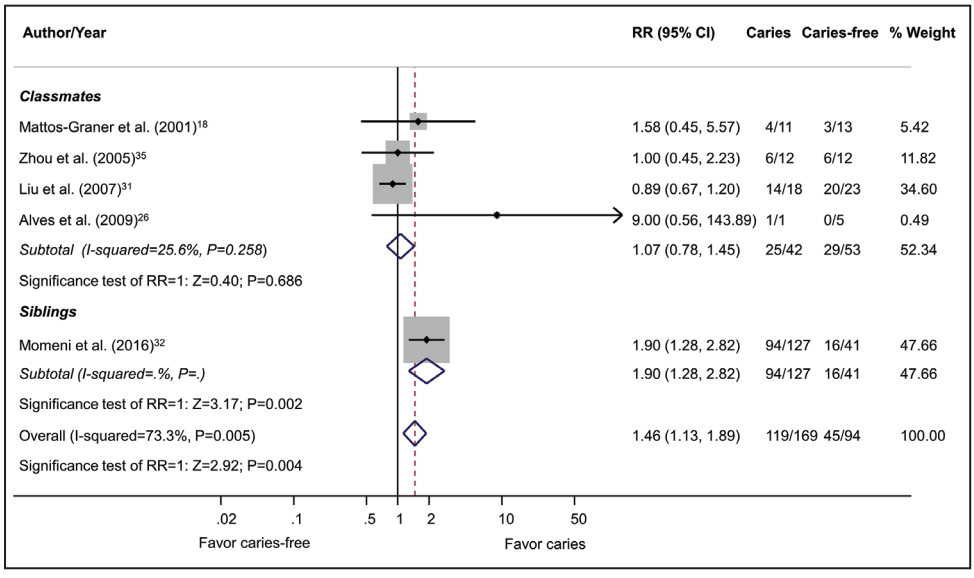

Figure 3. Forest plot showing the risk ratio (RR) of sharing only more than one genotype in children with caries versus caries-free children (subgrouped according to the classmates and siblings). The 95 percent confidence interval (95% CI) is depicted through the horizontal line, whereas the meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is indicated by the dotted vertical line.

Discussion

The current systematic review was performed to establish the evidence of transmission of *S. mutans* among children using various laboratory molecular techniques to confirm the presence of identical strains of *S. mutans* and understand its association with dental caries. The present review has considered the definition of transmission of microorganisms, as stated by Berkowitz (2006),[20] which defines vertical transmission as: *"transmission of microbes from caregiver to the child and horizontal transmission as the transmission of microorganisms among children of similar age groups (siblings or in school/ nursery)."*

For decades, the transmission of oral microorganisms has been attributed to the mother as the primary source, as she is considered the principal caretaker of the child. A systematic review[38] has demonstrated the vertical transmission of *S. mutans* strains from the mother to the child through various genetic analysis techniques. As social trends are changing, mothers are often working full-time, leaving their kids to alternate caregivers which may be fathers, grandparents, and any other unrelated care provider. These alternate caretakers spend considerable intimate time with the child, which reflects the possibility of sharing similar genotypes. These alternate routes of transmission have been confirmed by various studies[14,15,18,22] that investigated other possible routes (e.g., intrafamilial, child-to-child) for the transmission of oral microorganisms. Children are also in contact with other children in nurseries or schools or at home for a significant amount of time, which may be another route for the horizontal transmission of microorganisms.

Horizontal transmission has been summarized in this review by including those studies that have analyzed *S. mutans* commonality through molecular genetic techniques (chromosomal DNA fingerprinting, ribotyping, arbitrarily primed PCR, MLST, or chromosomal DNA RFLP) among siblings or classmates. The results of all the studies confirm the commonality of the genotypes among children and demonstrate the transmission of *S. mutans*, irrespective of the sample size and technique used, except for one study[29] which could not identify similar genotypes among children of the same class. This might be due to proper hygiene care and awareness at school while sharing toys, foods, or utensils.

Various genetic analysis techniques used to confirm the similarity of genotypes among children included microbial repetitions and showed a considerable variation in the percentage of transmission of microorganisms. In the present review, after performing the subgroup analysis for the type of molecular technique (MLST or RFLP) used for the genotype isolation, the results of both the techniques individually favored caries-free as compared to caries when sharing one genotype of *S. mutans* (Figure 4). Similarly, both techniques favored caries *versus* caries-free when sharing more than one genotype of *S. mutans* (Figure 5). The result was significant in only the MLST technique, but it was based on a single study.[32] *AP*-PCR and rep-PCR (repetitive extragenic palindromic-PCR) techniques have been well-accepted and widely used in epidemiological studies of microbial analysis, as they are relatively easy and fast to perform. However, two studies[32,33] used MLST as an alternate approach to validate the strain's uniqueness after analysis through rep-PCR. Similarly, in six studies,[18,26,31,34,35,37] MS isolates obtained after AP-PCR from children with similar fingerprinting profiles were reexamined by RFLP analysis to confirm the genotypic identity.

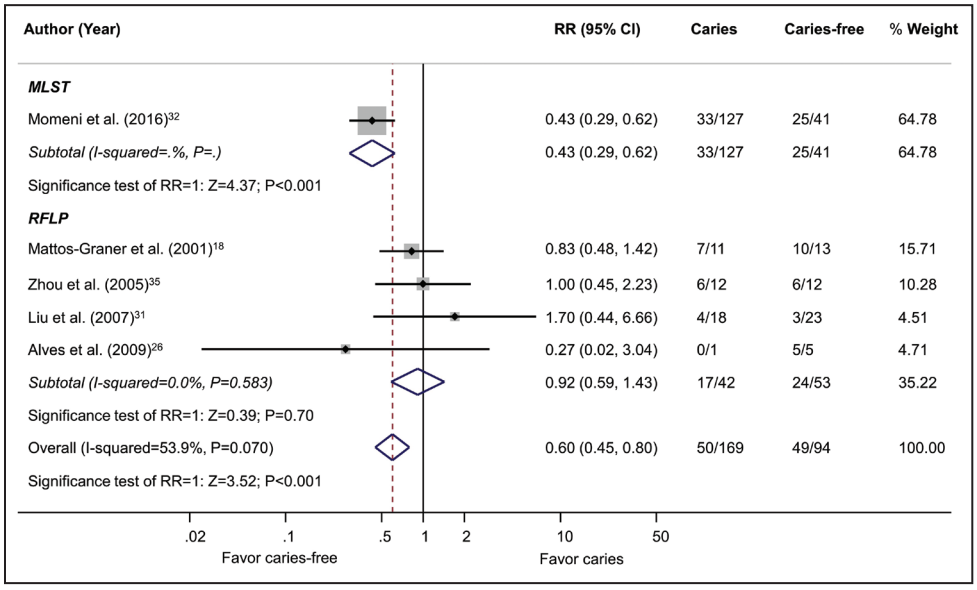

Figure 4. Forest plot showing the risk ratio (RR) of sharing only one genotype in caries-free children versus children with caries subgrouped according to the two techniques used: MLST (multilocus sequence typing) and RFLP (chromosomal DNA restriction fragment length polymorphism). The 95 percent confidence interval (95% CI CI) is depicted through the horizontal line, whereas the meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is indicated by the dotted vertical line.

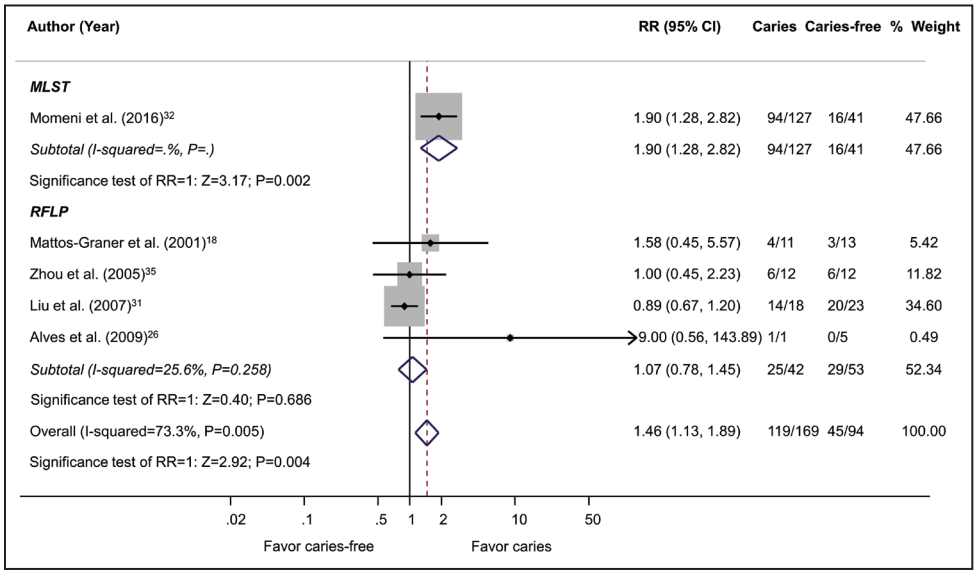

Figure 5. Forest plot showing the risk ratio (RR) of sharing only more than one genotype in children with caries versus caries-free children subgrouped according to the two techniques used: MLST (multilocus sequence typing) and RFLP (chromosomal DNA restriction fragment length polymorphism). The 95 percent confidence interval (95% CI CI) is depicted through the horizontal line, whereas the meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is shown by the dotted vertical line.

Variations were observed in the studies for the selection of the samples for genetic analysis, as some used saliva[18,26-28,30] or plaque[19,29,31,34,35,37]; some studies used both.[22] Also, two studies[32,33] used any among plaque/saliva/tongue scrapings. It is important to consider that *S. mutans* level of adherence and quantity differs in different parts of the oral cavity. A subgroup analysis was conducted for the type of sample (saliva/plaque/tongue scrapings) used for microbiological analysis in the present review. The result showed that children who shared one genotype of *S. mutans* were more likely to be caries-free when saliva samples were used for analysis. Also, children who shared more than one genotype were more likely to have caries when saliva samples were used. By contrast, children were more likely to have caries when plaque samples were used for analysis when children shared only one genotype whereas were more likely to be caries free when they shared more than one genotype. However, all the values were nonsignificant (Figures 6 and 7). Tooth eruption contributes to an increased surface area for the adherence of *S. mutans,* which in turn is related to the

age of the child.[19] Also, the age of the child significantly influences the transmission of microorganisms because children are in contact with multiple people for different time periods. However, the age of the children included in the current review shows a wide variation, with children without teeth and with teeth analyzed together.[22]

Dental caries is a multifactorial disease, and detection of *S. mutans* alone does not indicate the presence of caries in the child. It is necessary to understand the different pathways of acquisition and transmission of cariogenic microorganisms and their relation with other factors for proper risk assessment and to develop more effective preventive strategies that can be applied not only to the family but also to the people with whom the child is in contact during his or her early childhood years. Other confounding factors to be considered in future studies are the different number of hours per day a child attends the nursery/school, diet, caries status, contact with teachers, and socioeconomic and environmental factors that might influence the transmission of organisms. The included studies differ in the presentation and correlation of the current caries status of the children with the number of similar genotypes of *S. mutans* found after genetic analysis. Few studies[18,26,31,32,35] have correlated the presence or absence of caries in children with one or more common genotypes among them. However, three studies[27-29] presented the caries status as dmft/dmfs and compared it to the commonality of the number of genotypes among children.

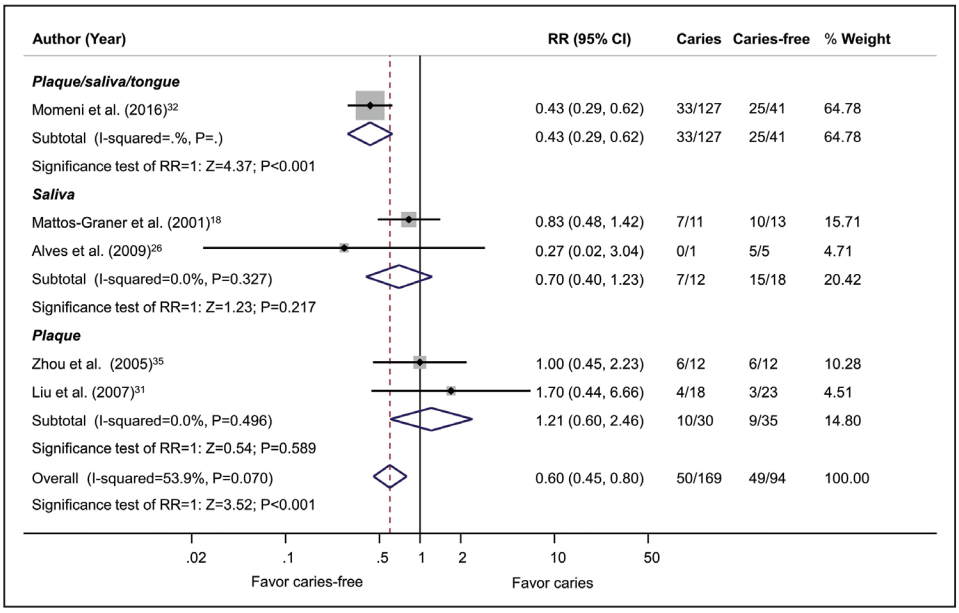

Figure 6. Forest plot showing the risk ratio (RR) of sharing only one genotype in caries-free children versus children with caries subgrouped according to the types of samples (plaque/saliva/tongue scrapping) used for the microbiological analysis. The 95 percent confidence interval (95% CI) is depicted through the horizontal line, whereas the meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is indicated by the dotted vertical line.

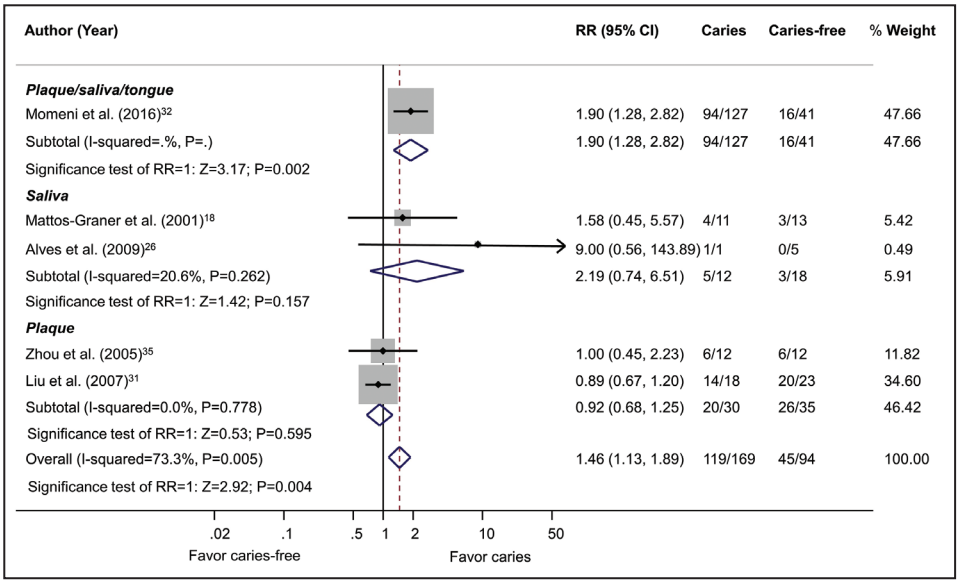

Figure 7. Forest plot showing the risk ratio (RR) of sharing only more than one genotype in children with caries versus caries-free children subgrouped according to the types of samples (plaque/saliva/tongue scrapping) used for the microbiological analysis. The 95 percent confidence interval (95% CI) is depicted through the horizontal line, whereas the meta-analysis pooled estimate is shown as a diamond with its CI. The location of the meta-analysis pooled effect estimate is indicated by the dotted vertical line.

The present systematic review has few limitations; for instance, it has only taken *S. mutans* (or its subspecies) into consideration as a potential and primary causative microorganism in the dental caries process, which is not true as there are many other microorganisms involved in caries initiation and progression. Various studies[39,40] have reported that many microorganisms (e.g., *Lactobacilli* and *Candida albicans*) other than *S. mutans* are involved in the dental caries process. A study[41] reported that *C. albicans* plays an important role in caries progression and is associated with early childhood caries. A systematic review[42] indicated that children with a higher level of oral *C. albicans* have a higher chance of having early childhood caries compared to those without *C. albicans*. Thus, future studies could consider the horizontal transmission of other cariogenic microorganisms to better understand the dental caries microbiology involved in early childhood caries and attempt to analyze the correlation between the children's caries status and the rate of transmission. Besides, most of the included studies in the current review were cross-sectional, and only a few longitudinal studies were found. It is recommended that more longitudinal studies be conducted in the future to determine the transmission pattern of other cariogenic microorganisms and among all people who are in close contact with children during their early years of childhood to evaluate all the possible routes of transmission. This would be helpful in early caries risk assessment and the development of appropriate preventive strategies for early childhood caries.

Conclusions

Based on the results of this systematic review and meta-analysis, the following conclusions can be made:

1. There is evidence of horizontal transmission of *S. mutans* genotypes (as sharing of similar genotypes) among the children at home or in schools/day nurseries.
2. Children who shared one genotype had a lesser risk, and those who shared more than one genotype had a higher risk of having dental caries.
3. Evaluation of possible routes of transmission of other cariogenic microorganisms through more longitudinal studies is needed so that complete transmission patterns can be assessed.

Acknowledgments

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Appendix. Search Strategy in the databases with the yield (number of hits per keyword) performed on 23rd March 2020

Database searched	Keywords used	Number of yields
Scopus	TITLE-ABS-KEY ((caries OR streptococcus OR bacteria OR microbiota) AND (transmission OR acquisition) AND (pediatric OR child) AND (oral OR dental))	643
Web of Science	((caries OR streptococcus OR bacteria OR microbiota) AND (transmission OR acquisition) AND (pediatric OR child) AND (oral OR dental))	435
PubMed	(caries OR streptococcus OR bacteria OR microbiota) AND (transmission OR acquisition) AND (pediatric OR child) AND (oral OR dental)	1,196
CINAHL	(caries OR streptococcus OR bacteria OR microbiota) AND (transmission OR acquisition) AND (pediatric OR child) AND (oral OR dental)	28
Lilacs	(caries OR streptococcus OR bacteria OR microbiota) AND (transmission OR acquisition) AND (pediatric OR child) AND (oral OR dental)	62
Medline (via Ovid)	1. Dental caries.mp. or exp dental caries/	48,997
	2. Streptococcus mutan.mp. or exp streptococcus mutans/	10,474
	3. Bacteria/ or bacteria.mp.	444,357
	4. Transmission.mp. or exp disease transmission, infectious/	455,982
	5. Acquisition.mp.	124,734
	6. Exp child, preschool/ or exp pediatrics/ or pediatric.mp.	973,744
	7. Children.mp. or exp child/	2,060,907
	8. 1 or 2 or 3	495,009
	9. 4 or 5	573,661
	10. 6 or 7	2,090,843
	11. 8 and 9 and 10	955
Embase (via Ovid)	1. Streptococcus mutan.mp. or exp streptococcus mutans/	13,298
	2. Dental caries.mp. or exp dental caries/	59,629
	3. Acquisition.mp.	194,713
	4. Transmission.mp.	616,906
	5. 1 or 2	676,751
	6. 3 or 4	801,555
	7. 5 and 6	814