Effect of Antimicrobial Interventions on the Oral Microbiota Associated with Early Childhood Caries

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Abstract: Purpose: The purposes of this systematic literature review were to identify research-based evidence for an effect of antimicrobial therapeutic approaches on the cariogenic microbiota and early childhood caries (ECC) outcomes; and to review methods used to perform microbial assessments in clinical studies of ECC. Methods: Multiple databases were searched; only clinical cohort studies and randomized controlled trials published from 1998 to 2014 were selected. A total of 471 titles and abstracts were identified; 114 studies met the inclusion criteria for a full review, from which 41 studies were included in the meta-analyses. Results: In most of the reviewed studies, moderate reductions in cariogenic bacterial levels, mainly in mutans streptococci (MS), were demonstrated following the use of antimicrobial agents, but bacterial regrowth occurred and new carious lesions developed once the treatment had ceased, particularly in high-risk children. Relatively consistent findings suggested that anti-cariogenic microbial interventions in mothers significantly reduced MS acquisition by children. However, studies of the long-term benefits of ECC prevention are lacking. Conclusion: Based on the meta-analyses, antimicrobial interventions and treatments show temporary reductions in MS colonization levels. However, there is insufficient evidence to indicate that the approaches used produced sustainable effects on cariogenic microbial colonization or ECC reduction and prevention. (Pediatr Dent 2015;37(3):226-44) Received January 23, 2015 Last Revision April 1, 2015 | Accepted April 3, 2015

KEYWORDS: DENTAL CARIES, ORAL MICROBIOTA, TREATMENT EFFECTIVENESS

Despite a continuous decline in caries in the permanent dentition for many children, the prevalence of early childhood caries (ECC) in the United States remains overwhelmingly high among certain low-income or immigrant families, minority populations, and indigenous communities. The overall percentage of ECC children was 17 percent from 1971 to 1975, 16 percent from 1988 to 1994, and 28 percent from 1999 to 2004. Currently, ECC affects more than 25 percent of American preschool-aged children of all races, with rates as high as 46 percent in Hispanic children and 66 percent to 70 percent of American Indian/American Native children. Although ECC is considered preventable, it remains the most frequently experienced and critically important chronic disease of young children because of its tenaciously high prevalence, high treatment costs, and negative effect on the oral health-related quality of life in children.

The pathophysiological etiology of ECC is associated with early colonization and high levels of the cariogenic microorganism (e.g., Streptococcus mutans), an abundance of dental plaque, enamel defects in primary teeth, and childhood diets high in sugar and carbohydrates. Interactions among these primary risk factors produce an acidic environment in dental plaque, resulting in enamel and dentin decalcification. Other bacteria associated with ECC development and severity include Streptococcus sobrinus and Lactobacillus species. A 1998 report by Horowitz noted that “only limited research has been done on chemotherapeutic approaches to prevent or reduce the incidence of ECC” and research on chemotherapeutic interventions should, therefore, “include determining the effectiveness of individual and logical combinations of chemotherapeutic agents for preventing ECC.”

Numerous antimicrobial clinical trials or intervention programs have been conducted worldwide since 1998 with the goal of suppressing cariogenic bacteria and reducing children’s caries experiences. Several antimicrobial agents (e.g., fluoride, chlorhexidine, iodine, xylitol, silver compounds), combined with a range of application methods (e.g., mouthrinse, gel, varnish, cleaning wipe, restorative materials), have been used, resulting in remarkable reductions in S. mutans and S. sobrinus levels. Almost all of the successful results, however, lasted for only a few weeks or months post intervention, and reductions in S. mutans and S. sobrinus colonization were diminished when treatment was suspended. Few chemotherapeutic interventions have targeted the critical link between the pathogenic mechanisms of bacteria in ECC development. A recent search of the Cochrane Library revealed 17 systematic reviews related to fluoride and ECC, four reviews on chlorhexidine plus fluoride and dental caries, three reviews on xylitol, and five reviews on other interventions or treatments of ECC. None of these reviews addressed the microbiological effects of antimicrobial agents on ECC outcomes. Concurrently, high post-treatment caries relapse rates were reported, suggesting that most of the interventions had limited long-term beneficial effects on ECC. Thus, there is a lack of understanding as to the sustainability of bacterial reductions and how antimicrobial interventions can alter the ECC-associated microbial community. As such, the research mission established over a decade ago has not yet been accomplished.

Most microbiology in clinical studies of ECC focuses on mutans streptococci (MS) and lactobacilli (LB), which are routinely detected using selective-culture-based methods. However, the microbiota of caries-associated biofilms have long been recognized to contain a wide diversity of bacteria, including species of Actinomyces, Fusobacterium, Staphylococcus, Bifidobacterium,
Atopobium, Prevotella, Veillonella, and Candida.\textsuperscript{13-17} Advanced clinical study designs and the selection of acid-tolerant bacteria have been explored to distinguish the key contributors to caries progression. The caries-free and ECC microbiotas differ, suggesting that a disturbance of the whole polymicrobial community, and not just the levels of MS and LB, plays a role in caries etiology.\textsuperscript{13,14,19} The review identified several reports of microbial diversity in ECC, some of which linked treatment outcomes with changes in \textit{S. mutans} subtypes or in the microbiota as a whole.

Methods
The systematic review and meta-analysis were conducted according to the methods of the Cochrane Handbook for Systematic Reviews of Interventions.\textsuperscript{20} Multiple searches were performed based on PubMed, Ovid Medline, the Library of Congress, the Web of Science Core Collection, and the Cochrane Database of Systematic Reviews. Our strategy limited keyword searches to: first, clinical trials, randomized controlled trials, systemic reviews, and meta-analysis; next, the 1998 to current database published in English; and finally, three groups of keywords based on the methods and antimicrobial agents used for interventions. These keyword groups were as follows: (1) ECC, dental caries, tooth, deciduous, child, infant, preschool, risk factors; (2) clinical trial, fluoride, chlorhexidine, iodine, xylitol, topical therapeutic use, silver compounds, silver, silver proteins, silver nitrate, silver diamine fluoride; and (3) bacterial infections, anti-bacterial agents, antimicrobial therapy, \textit{Streptococcus}, saliva, sequence analysis, mouth, bacteria, anaerobic, metagenome, oral microbiome, DNA, bacterial proteins, RNA, and ribosomal.

The search strategies, as well as the inclusion and exclusion criteria, are illustrated in Figure 1. Among those excluded were non-clinical trials, cross-sectional studies, case-control studies, studies without microbiological analysis, studies of mixed and permanent dentitions, and animal studies. Randomized controlled trials selected for analysis had to consist of at least four weeks of observation, and prospective cohort studies selected had to include at least three months of observation. The main outcome evaluations for all of the clinical trials were the effects on the cariogenic microbiota and the incidence of new ECC lesions after the antimicrobial treatment. Data were extracted according to study design, number of participants, intervention approach, duration of trials, microbiological assessment methods, outcome measurements, and valid statistical methods used.

The effect size of each antimicrobial intervention on the cariogenic microbiota in preschool-aged children was further examined by a meta-analysis using the Comprehensive Meta-Analysis Program (version 2, Biostate, Englewood, N.J., USA). The variables used for the statistical analysis included estimates of means, variances, proportions, and rates of changes of bacterial measurements, and caries scores, as well as ECC incidence in each experimental, treatment, or control group for a given sample size. For all of the clinical studies, only data at the baseline and at the end of the treatment/intervention period were used for comparisons in the meta-analysis. Statistics for each study and summary effects included odds ratios and 95 percent confidence intervals, which were displayed as forest plots. Cochran’s Q test and the Hinging Index ($I^2$) were used to determine the significance of the heterogeneity among studies.\textsuperscript{21} A fixed-effect model was used to determine the summary results. Heterogeneity tests were employed to validate the fixed-effect model assumption that all studies in the meta-analysis shared a common effect size. A two-sided $P<.05$ was considered significant for all analyses.

Results
According to the search criteria, we initially identified 471 titles and abstracts. Examination of these abstracts resulted in 114 publications for detailed review under seven categories of studies about or using: (1) fluoride varnish (FV) topical therapeutic applications; (2) chlorhexidine (CHX) varnish and all other antimicrobial therapies; (3) povidone iodine (PVP-I) applications; (4) full-mouth restorative treatment with or without antimicrobial treatment; (5) xylitol (Xyl)
intervention in MS levels in children; (6) the effect of maternal antimicrobial intervention on MS colonization of children and ECC outcome; and (7) silver and other heavymetal compounds as antimicrobial agents. Only 41 studies met all inclusion criteria (Figure 1) and were selected for meta-analyses under the different review categories. To account for the diversity of the ECC-microbiome, we extended the search to include studies that described some measure of microbial diversity related to the different treatment regimens.

Most clinical studies of ECC that included microbial monitoring limited their bacterial monitoring to MS with or without testing for Lactobacillus species. The microbiological methods consisted of either laboratory cultivation or commercial tests based on selective culture principals. The most frequently used

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age (ys)</th>
<th>Treatment and interventions</th>
<th>Duration (mos)</th>
<th>Microbiological method</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobo et al., 2008</td>
<td>Randomized clinical trial, Brazil</td>
<td>35, ECC (4-8)</td>
<td>Group 1: 1.23% NaF gel Group 2: 1% CHX gel Applied for 10 mins, every 24 hs for 6 consecutive days</td>
<td>1</td>
<td>Selective culture: MSB for MS</td>
<td>At the 6-day treatment, 1% CHX gel was effective in reducing salivary MS. There was a significant MS increase once treatment was suspended. The use of 1.23% NaF under the same regimen was not able to reduce salivary MS levels.</td>
</tr>
<tr>
<td>Plonka et al., 2013</td>
<td>Randomized clinical trial, Australia</td>
<td>622 (0.5-2)</td>
<td>Twice daily tooth brushing with fluoride toothpaste with: Group 1: 10% casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste; Group 2: 0.12% CHX; Group 3: study control (SC)</td>
<td>24</td>
<td>Chairside test: CRT bacteria (Ivoclar Vivadent) for MS and LB</td>
<td>At 12 and 18 months old, MS detection rates were 0% and 5% in CPP-ACP group; 22% and 72% in the CHX group, and 16% and 50% in the SC group. At the 24-month recall, caries incidence rates were 1% in the CPP-ACP group, 2% in the CHX group, and 2% in the SC group. In addition to daily use of fluoride toothpaste, there was insufficient evidence to justify the daily use of CPP-ACP paste or CHX gel to control early childhood caries.</td>
</tr>
<tr>
<td>Plotzitza et al., 2005</td>
<td>Prospective follow-up study, Germany</td>
<td>172 (1)</td>
<td>Fluoride tablets + fluoride salt + fluoride toothpaste Group 1: 1% CHX varnish used 3-month intervals Group 2: No CHX treatment controls</td>
<td>24</td>
<td>Chairside test: CRT bacteria (Ivoclar Vivadent) for MS and LB</td>
<td>Mean dmft value increased from 0.05±0.4 to 0.8±2.9, and mean dmfs value rose from 0.08±0.8 to 1.8±5.9. At 24 months old, 26.2% of 2-year-olds had salivary scores of MS ≥105 CFU/ml in saliva. There were no significant differences in MS scores between the CHX and control groups.</td>
</tr>
<tr>
<td>Pukallus et al., 2013</td>
<td>Randomized clinical trial, Australia</td>
<td>199 (0.5-2)</td>
<td>2x daily tooth brushing using 0.304% w/w fluoride toothpaste alone with: Group 1: 0.12% CHX gel; Group 2: control, low-dose fluoride toothpaste</td>
<td>24</td>
<td>Chairside test: CRT bacteria (Ivoclar Vivadent) for MS and LB</td>
<td>At 24 months, caries prevalence rates were 5% in the CHX group and 7% in the control group. There were no differences in percentages of MS-positive children between the CHX (54%) and control groups (53%). Tooth brushing using low-dose fluoride toothpaste, with or without applying CHX 0.12%, reduced ECC from 23% found in the general community to 5-7%.</td>
</tr>
<tr>
<td>Stecksen-Blicks et al., 2009</td>
<td>Randomized clinical trial, Sweden</td>
<td>248 (1-5)</td>
<td>Group 1: fluoride and probiotic bacteria in skim milk Group 2: skim milk only</td>
<td>21</td>
<td>Selective culture: MSKB (mitis salivarius, kanamyacin, bacitracin) for MS</td>
<td>The proportion of MS compared with the total cultivable microflora was lower in the intervention group vs. the control group after 21 months. The mean MS levels remained unchanged throughout the study period. There was a significant difference in the caries increment after 21 months between the groups with a prevented fraction of 75%.</td>
</tr>
<tr>
<td>Tweetman et al., 1999</td>
<td>Prospective follow-up study, Sweden</td>
<td>37 (1.5)</td>
<td>1% CHX gel 2x daily brush for 14 ds</td>
<td>3</td>
<td>Chairside test: Dentocult SM Strip mutans for MS</td>
<td>A significant reduction of MS was detected after 1 month compared with baseline. After 3 months, the difference from the baseline was diminished.</td>
</tr>
<tr>
<td>Berkowitz et al., 2009</td>
<td>Clinical exploratory study, United States</td>
<td>77 (2-5)</td>
<td>Caries restorative treatment followed by: Group 1: 10% PVP-I solution; Group 2: 1.23% APF foam</td>
<td>3</td>
<td>Selective culture: MSB for MS</td>
<td>Approximately 50% of subjects had a 95% reduction in MS in the saliva at the follow-up visit compared to the MS level at baseline. PVP-I with dental surgery significantly suppressed salivary MS levels for severe ECC for at least 90 days. Treatment with PVP-I may be an important adjunct to dental surgery for SECC.</td>
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Topical application of povidone iodine (PVP-I)

<table>
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<tr>
<th>Author, year</th>
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<th>Sample size and age N (ys)</th>
<th>Treatment and interventions</th>
<th>Duration (mos)</th>
<th>Microbiological method</th>
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<tr>
<td>El-Housseiny et al., 2005&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Randomized clinical trial, Saudi Arabia</td>
<td>54 (4-6)</td>
<td>Group 1: 1.23% APF weekly for 4 wks, then every 3 mos for 1 yr</td>
<td>12</td>
<td>Chairside test: CRT bacteria for MS and LB</td>
<td>There were no significant differences in MS and LB counts between the two groups in all of the evaluation periods, excluding LB at the 3-month evaluation. The number of carious lesions was significantly reduced at the follow-up evaluation compared to baseline, but there were no significant differences between the two groups in the intervening evaluation periods.</td>
</tr>
<tr>
<td>Lopez et al., 2002&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Randomized clinical trial, Puerto Rico, United States</td>
<td>83 (1-1.5)</td>
<td>10% PVP-I</td>
<td>Placebo solution</td>
<td>Selective culture: MSA for MS</td>
<td>Kaplan-Meier survival estimates showed that, among disease-free children, 91% received treatment vs. 54% in the control group. Topical antimicrobial therapy increased disease-free survival in children at a high risk for ECC.</td>
</tr>
</tbody>
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Table 1. Continued

Topical application of povidone iodine (PVP-I)

El-Housseiny et al., 2005<sup>115</sup> | Randomized clinical trial, Saudi Arabia | 54 (4-6) | Group 1: 1.23% APF weekly for 4 wks, then every 3 mos for 1 yr | 12 | Chairside test: CRT bacteria for MS and LB | There were no significant differences in MS and LB counts between the two groups in all of the evaluation periods, excluding LB at the 3-month evaluation. The number of carious lesions was significantly reduced at the follow-up evaluation compared to baseline, but there were no significant differences between the two groups in the intervening evaluation periods. |
| Lopez et al., 2002<sup>22</sup> | Randomized clinical trial, Puerto Rico, United States | 83 (1-1.5) | 10% PVP-I | Placebo solution | Selective culture: MSA for MS | Kaplan-Meier survival estimates showed that, among disease-free children, 91% received treatment vs. 54% in the control group. Topical antimicrobial therapy increased disease-free survival in children at a high risk for ECC. |

Table 1. Continued

The role of fluoride as an antimicrobial agent is supported by many epidemiological investigations. The mechanism by which fluoride inhibits carbohydrate metabolism by acidogenic microorganisms has been demonstrated based on *in vitro* studies. Currently, the most frequently used agents are: five percent sodium FV (22,500 ppm F); 1.23 percent acidulated phosphate fluoride gel (12,300 ppm F); 0.2 percent sodium fluoride (NaF) mouthrinse (900 ppm F); and 1.1 percent NaF (5,000 ppm F) brush-on pastes/gels. FV has been shown to be a safe and effective chemopreventive agent and is increasingly incorporated into dental and medical clinical practices and in community-based interventions for ECC. Although administering FV treatment at least twice a year is highly recommended by the American Dental Association and the American Academy of Pediatric Dentistry for children with an increased caries risk, very few studies have described FV antimicrobial efficacy in ECC children.

Our initial literature search revealed 338 articles on topical fluoride application in children, among which 178 were clinical trials with differing designs. None of the 178 studies incorporated microbiological evaluations of fluoride as a single agent for intervention. We found that only five studies used different fluoride applications combined with other interventions that met the selection criteria and were included in the meta-analysis (Table 1). The meta-analysis indicated that combining NaF application with other antimicrobials showed some degree of MS and LB reduction. The odds ratio for the summary effect was 1.11, with a 95 percent confidence interval of 0.87 to 1.42 and a *P*-value of 0.386, indicating that the overall reduction was not statistically significant (Figure 2).

Effect of CHX varnish on reducing oral microbiota. CHX has a long history of use in caries prevention trials. A previous meta-analysis of eight studies published between 1975 and 1994 reported that the caries-inhibiting effect of CHX treatment was approximately 46 percent. More recent findings, however, has been inconclusive regarding the use of CHX varnishes for caries prevention, mostly for permanent dentitions, in high-risk groups. It has been suggested that the observed inconsistencies might not be simply due to the agent itself but to a combination of factors, such as the concentration used, nature of delivery, frequency, and duration of the application. Although there are a number of clinical trials using CHX varnish or CHX gel for young children, very few of these
studies included microbial assessments after CHX application. Using the search strategy, we identified 50 studies of CHX and dental caries. As listed in Table 1, four studies reported combined treatment with various CHX agents and fluoride or other anti-microbial applications. We found only one prospective observational study that evaluated the effect of one percent CHX varnish as an ECC intervention agent on MS colonization.\(^43\) In a comparison study, Lobo et al. observed that CHX treatment demonstrated a significantly higher efficacy in MS reduction versus NaF.\(^44\) A study led by Klinke et al. demonstrated that daily brushing with a 0.2 percent CHX gel for two weeks was effective in reducing salivary MS, LB, and Candida species.\(^45\) However, because all of the children in the study received comprehensive restorative treatment after the CHX regimen, either the CHX or the restorative treatment could have contributed to the microbial reductions. Using only CHX as a preventive agent, Twetman et al. reported a significant reduction in MS at an early stage of the intervention; however, after three months, the significance of the reduction was diminished.\(^43\) Results from the current meta-analysis indicated that there is insufficient evidence to conclude that the daily use of CHX alone or in combination with fluoride application for an extensive period would reduce MS or LB levels in young children (Figure 3).

**Effect of PVP-I treatment on reducing oral microbiota.** PVP-I solutions are stable chemical complexes used as effective broad-spectrum topical antimicrobial agents with less toxicity toward mammalian cells than other commonly used agents.\(^46\) PVP-I has been used for decades as a topical antimicrobial therapy in the treatment and prevention of dental caries in clinical studies.\(^47\) Several studies have found that PVP-I temporarily reduced MS and LB counts in young children\(^48,49\) and was associated with decreased ECC risk in high-risk children. A combination of PVP-I and FV led to a greater reduction in caries incidence than the use of FV alone.\(^50,51\) However, most of the studies were performed on permanent or mixed dentitions. Additionally, very few studies incorporated detailed microbiological evaluations to test the efficacy of PVP-I applications.

Our literature search identified 14 clinical trials of iodine or povidone iodine and ECC intervention. We examined 11 studies; eight were excluded due to a lack of microbiological analyses, leaving only three studies for the meta-analysis (Table 1). Two studies (by Berkowitz and El-Housseiny, respectively) reported significant reductions of MS and LB lasting at least three months in the experimental groups treated with 10 percent PVP-I; however, adding these studies in the meta-analysis model did not improve the overall effects on the cariogenic bacterial reduction (Figure 3). Despite ambiguity in the long-term effects of PVP-I on bacterial and ECC reductions, the meta-analysis of ECC outcomes revealed that biweekly topical application of PVP-I for 12 months (Lopez's study) significantly increased...
Effect of a full-mouth comprehensive restoration on reducing oral microbiota. Full-mouth restorative treatment under general anesthesia is used for children with severe ECC, particularly children from low socioeconomic families. The regimen generally includes surgical removal of carious lesions, extraction of unrestorable teeth, and restoration of cavities. Significant reductions in cariogenic bacterial counts in saliva have been reported after comprehensive treatment. Clinicians frequently add an antimicrobial application to the treatment procedure to further reduce the risk of caries recurrence. Nevertheless, questions remain regarding the beneficial effects of either full-mouth treatment under general anesthesia alone or in combination with antimicrobial approaches to curb the total cariogenic microbiota as well as the outcome of caries incidence in children.

We identified eight studies that incorporated microbiological evaluations after comprehensive restorative treatment under general anesthesia (Table 2). Two of the eight studies were observational and did not include antimicrobial therapy. There were three observational follow-up studies and three randomized clinical trials in which children were given single or combined antimicrobial therapies before or after extensive restorations. The meta-analysis clearly showed a significant overall effect on the reduction of MS levels. Interestingly, three reports showed that the extensive treatment was more effective at reducing LB levels compared with MS levels (Figure 5). It is not clear whether the bacterial reductions were the result of the surgical procedures or the antimicrobial treatments. The combined comprehensive restoration and PVP-I treatment decreased the total bacterial counts, but the reduction was not significant. The meta-analysis further showed that the odds ratio was 0.30, with a 95 percent confidence interval of 0.22 to 0.40, and the summary effect was significant when comparing different treatments (P < .001; Figure 5). These findings suggest that full-mouth comprehensive treatment under general anesthesia is an effective approach for dramatically reducing MS and LB levels immediately after treatment. In most cases, however, the bacterial levels in the saliva and plaque increased significantly six to 12 months after the treatment and 20 to 60 percent of the treated children developed new carious lesions. The meta-analysis also suggests that pretreatment with CHX, PVP-I, or FV has only a limited effect on bacterial reduction and caries relapse rates (Table 2).

Effect of children’s xylitol trials on reducing MS colonization. We identified 23 observational studies and clinical trials, but only five studies included microbial evaluations and, therefore, met the inclusion criteria (Table 3). Several xylitol delivery vehicles were used, including chewing gums, tablets, and wipes, and combined treatment with NaF. The age of the children studied ranged from six months to five years old. The meta-analysis of xylitol-based interventions indicated an overall significant reduction of MS colonization in young children (Figure 6). A high degree of heterogeneity was observed in caries outcomes among the five studies (I^2 statistic = 93 percent; P < .001; Figure 6). Although two of the five studies reported development of significantly fewer new carious lesions in the experimental group, with an overall significant caries reduction, the results should be interpreted cautiously, given the: (1) inconsistent effect size (odds ratios ranged from 0.02 to 1.03); (2) limited number of studies included in the analysis; and (3) lack of true comparative control groups in the clinical studies. Although there is strong evidence supporting the use of xylitol-containing chewing gum to reduce dental caries in adolescent and adult populations, one should not automatically assume that the gum will be as effective for preschool-aged children. Better-designed, placebo-controlled, randomized clinical trials are needed to independently test the antimicrobial properties of xylitol and confirm the caries-preventing effect of xylitol in young children.

Effect of maternal xylitol trials on acquiring MS in children. We identified 214 studies using the search keywords clinical trial, xylitol, mother/maternal, antimicrobial, and Streptococcus. Nineteen studies with at least a three-month follow-up evaluation were analyzed (Table 4). Based on an average of 39 months of observation, most studies reported positive correlations between maternal exposure to xylitol or other antimicrobial agents and a delay in MS colonization in young children. Despite some controversy regarding the xylitol dosage needed and the mode of delivery, the meta-analysis indicated that anticariogenic-microbe interventions in mothers can significantly affect MS acquisition in children (Figure 7) and subsequently lower children’s caries outcomes (Figure 8). Xylitol-based interventions show a better caries-protective effect (odds ratio...
counts were in saliva and plaques increased significantly. Selective culture: an agar plate with bacitracin.

- **Restorations without antimicrobial treatment**

  **Litsas, 2010**
  - Prospective observational follow-up study, United States
  - 39, ECC (2-5)
  - Full-mouth restoration under general anesthesia
  - Duration (mos): 3
  - Microbiological evaluation method: Selective culture
  - Evidence: The operative procedures under general anesthesia significantly decreased *S. mutans* for at least 3 months. By 6 months, *S. mutans* in saliva and plaques increased significantly.

  **T wetman et al., 1999**
  - Prospective observational follow-up study, Sweden
  - 108, ECC (2.5-6)
  - Full-mouth restoration under general anesthesia
  - Duration (mos): 6
  - Microbiological evaluation method: Chairside test
  - Evidence: MS but not LB levels were strongly correlated with caries prevalence, immigrant background, and frequency of night-time meals. MS and LB post-treatment levels were significantly reduced at the 1- and 6-month recalls. LB levels were more dramatically reduced compared to MS, but the reduction was not significantly related to the type of treatment. No difference was found in the saliva buffer capacity between pre- and post-treatment.

- **Restorations with additional antimicrobial treatment**

  **Amin et al., 2004**
  - Randomized clinical trial, Canada
  - 25, ECC (2-7)
  - Full-mouth restoration under general anesthesia; 10% PVP-I 3x at 2-month intervals
  - Duration (mos): 12
  - Microbiological evaluation method: Selective culture
  - Evidence: There was a 49% reduction in *S. mutans* and a 17% reduction in total bacterial counts at 6 months after the combined treatment. However, the difference between the 2 groups was not significant. At the 1-year recall, 63% of the children in the control group and 18% in the experimental group had new caries.

  **Chase et al., 2004**
  - Prospective observational follow-up study, Canada
  - 79, ECC (2.3-7.3)
  - Full-mouth restoration under general anesthesia; topical fluoride application
  - Duration (mos): 6
  - Microbiological evaluation method: Selective culture
  - Evidence: Comprehensive restorative therapy resulted in a statistically significant reduction in salivary MS reservoirs in children treated for ECC; 37% of the children who returned for follow-up visits had new smooth surface carious lesions. There were no statistically significant differences in MS levels between the caries relapse and non-relapse groups.

  **Hughes et al., 2012**
  - Prospective observational follow-up study, United States
  - 117 (2-6)
  - Full mouth restoration under general anesthesia; prophylaxis, fluoride varnish (Duraphat)
  - Duration (mos): 12
  - Microbiological evaluation method: Selective and non-selective culture
  - Evidence: At baseline, *S. mutans* and *S. sobrinus* counts were significantly higher in severe ECC children than in caries-free children. After treatment, *S. mutans* counts were decreased, particularly in children without caries recurrence. *S. sobrinus* counts before treatment, but not *S. mutans* counts, were correlated with recurrent caries. Over 70% of the acid-tolerant and 90% of the total microbiota found in severe ECC children were not *S. mutans*.

  **Klinke et al., 2014**
  - Prospective follow-up study, Germany
  - 50, ECC (1-5)
  - 0.2% CHX gel; parents instructed to apply the gel when brushing their children’s teeth 2x a day for 2 weeks followed by full-mouth restoration under general anesthesia
  - Duration (mos): 12
  - Microbiological evaluation method: Chairside test
  - Evidence: Numbers of MS, LB, and *Candida albicans* were significantly reduced after restorative treatment. The decrease remained significant for 12 months. At the 12-month visit, pretreatment with CHX had a limited antimicrobial effect for MS and LB, all of the micro-organisms showed regrowth, and 34% of the children developed new dentinal lesions. High scores for LB before treatment predicted caries relapse. Satisfactory and sustainable success could not be achieved in MB, LB, or *C. albicans* colonization or in caries relapse rates.

  **Simratvir et al., 2010**
  - Randomized clinical trial, Ludhiana, India
  - 30 (4.2)
  - Full-mouth restoration under general anesthesia; Group 1: 10% PVP-I at 3-mo interval for 12 mos; Group 2: placebo control
  - Duration (mos): 12
  - Microbiological evaluation method: Selective culture
  - Evidence: The application of 10% PVP-I resulted in a significant reduction in the rise of *S. mutans* levels from baseline and a decrease in the relapse of caries. Oral rehabilitation coupled with regular application of 10% povidone iodine application can be a good alternative to control caries in ECC children.
* CHX=chlorhexidine; MS=mutans streptococci; LB=lactobacilli; CRT=Caries Risk Tests; PVP-I=povidone iodine; APF=acidulated phosphate fluoride; MSB=mitis salivarius bacitracin; TYCSB=trypticase yeast-extract cystine sucrose bacitracin.

### Table 2. Continued*

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age n (ys)</th>
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<tr>
<td>Zhan et al., 2006</td>
<td>Randomized clinical trial, United States</td>
<td>22, ECC (2-6)</td>
<td>Full-mouth restoration under general anesthesia; both groups: prophylaxis and 1.23% APF gel application (2 mins) prior to restoration; after restoration: intervention of 10% PVP-I for 2 mins; control: phosphate saline</td>
<td>12</td>
<td>Selective and nonselective culture: MSB agar for MS; Rogosa-tomato juice for LB; BHI-blood agar for total counts</td>
<td>MS and LB levels in the PVP-I group were significantly reduced at 1 hour, 3 weeks, and 3 months; 60% of the children had new carious lesions. Complete surgical treatment of caries plus prophylaxis and fluoride gel application at baseline were insufficient to prevent new caries in more than 60% of the children who had a high risk of caries.</td>
</tr>
</tbody>
</table>

### Table 3. EFFECTS OF XYLITOL USAGE ON MUTANS STREPTOCOCCI (MS) LEVELS AND CARIES IN CHILDREN WITH EARLY CHILDHOOD CARIES (ECC)

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age n (ys)</th>
<th>Treatment and interventions</th>
<th>Duration (mos)</th>
<th>Microbiological evaluation method</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaltonen et al., 2000</td>
<td>Prospective cohort study, Finland</td>
<td>122 (1-1.3)</td>
<td>Fludent tablet containing NaF (0.25 mg F), xylitol (159 mg), sorbitol (153 mg) Group T: Fludent in pacifier; Group C: Fludent in food</td>
<td>12 mos</td>
<td>Chairside test: Dentocult SM Strip for MS</td>
<td>Children in group T developed significantly fewer new lesions than children in group C when children were between 2 and 3 years old. Significantly fewer children in group T were MS positive compared to group C. The administration of a NaF-xylitol-sorbitol preparation with a slow-release pacifier proved effective in reducing the incidence of caries in children 2 to 5 years old.</td>
</tr>
<tr>
<td>Autio, 2002</td>
<td>Randomized clinical trial, United States</td>
<td>61 (3-5)</td>
<td>Group 1: xylitol gum 3x for 3 wks; Group 2: control</td>
<td>3 wks</td>
<td>Chairside test: Dentocult SM Strip for MS</td>
<td>The shift from higher MS scores to lower scores was greater in the xylitol group than in the control group; therefore, chewing xylitol gum may reduce salivary MS and provide a feasible caries prevention method for preschool children.</td>
</tr>
<tr>
<td>Oscarson et al., 2006</td>
<td>Randomized clinical trial, Sweden</td>
<td>132 (2)</td>
<td>Group 1: xylitol tablet 0.48 g 1x/day bedtime; Group 2: control</td>
<td>24 mos</td>
<td>Chairside test: Dentocult SM Strip for MS</td>
<td>No statistically significant differences in MS levels were detected between the 2 groups at any of the follow-up visits. Caries prevalence was low in the xylitol group, but the difference was not statistically significant. The findings do not support a low-dose xylitol tablet program for caries prevention in preschool children.</td>
</tr>
<tr>
<td>Seki et al., 2011</td>
<td>Randomized clinical trial, Japan</td>
<td>161 (3-4)</td>
<td>Experimental group: xylitol gum, 1.8 g (66% xylitol by weight), 3x/day for 3 mos; control-fluoride varnish (5% NaF) every 6 mos</td>
<td>12 mos</td>
<td>Chairside test: Dentocult SM Strip for MS</td>
<td>Xylitol gum consumption showed a significant negative association with MS levels. Xylitol gum is effective in avoiding increased plaque MS in young children. Over 10% of the xylitol group children experienced diarrhea.</td>
</tr>
<tr>
<td>Zhan et al., 2012</td>
<td>Randomized clinical trial, United States</td>
<td>44 (0.5-3)</td>
<td>Xylitol: wipe; placebo: wipe</td>
<td>12 mos</td>
<td>Selective culture: MSB agar for MS; Rogosa-tomato juice for LB</td>
<td>No significant differences between the 2 groups were observed in levels of MS and LB at all time points. Significantly fewer children in the xylitol-wipe group had new carious lesions at 1 year vs. those in the placebo-wipe group.</td>
</tr>
</tbody>
</table>
with MS-colonized children.\textsuperscript{68,69} It was hypothesized that the maternal use of xylitol chewing gum can prevent dental caries in children by delaying or prohibiting MS transmission from mother to child.

Another 10-year mother-child oral health longitudinal follow-up study by Thorild et al. reached a similar conclusion: the children of mothers who used high-content xylitol gums had lower MS counts at 18 months old and were more likely to have less caries at 10 years old.\textsuperscript{70-72} As the few reports in the literature have variable findings, clearly, more clinical studies will be needed to validate the long-term benefits of maternal xylitol gum exposure on children’s dental health, since only marginal differences in caries prevalence were observed between the experimental groups, and the sample sizes of those studies were limited.

Effect of silver compounds on the oral microbiota in ECC. For centuries, silver has been known to exhibit antimicrobial effects due to its properties as a heavy metal.\textsuperscript{73} A recent study suggested that silver ions inhibit microorganism growth by inactivating bacterial DNA replication ability and protein formation.\textsuperscript{74} Through the use of in vitro bacterial models, silver ions were found to enhance antimicrobial activity against multi-species cariogenic biofilm formation on carious dentin and reduce demineralization.\textsuperscript{75-77} Clinically, topical therapeutic application of silver diamine fluoride (SDF), silver fluoride, nano-silver fluoride (NSF), and silver nitrate are highly effective for inhibiting carious lesion progression.\textsuperscript{75,78} Although the mechanisms by which silver compounds inhibit bacterial growth and arrest carious lesions have not been fully explored, the caries-treatment effects have been reported in a number of epidemiology and clinical studies worldwide.\textsuperscript{78} We found very few clinical microbiology investigations that adequately examined the antibacterial efficacy of SDF and other silver compounds on ECC treatment outcomes. After an extensive search, we identified 12 ECC-related clinical studies published after 1997, only seven of which were well-designed randomized controlled clinical trials using SDF (30 to approximately 38 percent, or 44,800 ppm) or NSF (33,990 ppm) as an intervention agent for ECC. However, none of the studies included a microbiological evaluation; therefore, no study was selected for the meta-analysis.

Several additional antimicrobial approaches, other than fluoride, PVP-I, CHX, and xylitol, have been evaluated for managing ECC. Gudipaneni et al. showed that brushing with toothpaste containing lactoferrin, lysozyme, and lactoperoxidase significantly reduced salivary levels of MS and \textit{Lactobacillus acidophilus} in children with severe ECC.\textsuperscript{79} Lobo et al. suggested that clinical trials were needed to test the
efficacy of a novel essential oil of *Lippia sidoides* Cham. (LSO) against ECC. A few studies reported the clinical efficacy of different glass ionomers and dental resin adhesive materials with fluoride/xylitol slow-release functions or antibacterial activity. Yet, none of these studies met the inclusion criteria for the current meta-analysis.

**Effect of ECC on oral microbiota.** We identified 15 reports that investigated the potential correlation between ECC and oral microbial diversity (Table 5). Most of the studies show differences in the oral microbiota between children with and without ECC. The diversity was either decreased or increased in ECC compared with caries-free status, which depended in part on the microbiological assay used. A high degree of similarity between the oral microbiota of mother and child was observed, suggesting that the mother, as a primary caregiver, is a major source of the bacteria that colonize the oral cavity of young children. Results differed between studies in the microbial composition before and after treatment. For example, Fontana et al. reported that the maternal use of xylitol gum had no effect on microbial composition in children. Tanner et al. showed significant microbial changes in children before and after extensive restorative treatment under general anesthesia using microbiological analyses of a microarray containing 300 oral bacterial probes. Tanner's study also demonstrated the feasibility of using this assay that incorporates sufficient bacterial probes to detect differences in the caries microbiome and evaluate successful treatment.

Determining which bacteria to target is discussed next, but we propose that the general strategy to achieve a healthy, caries-free, compatible microbiota is to reverse the microbial community that led the alteration from health to disease.

**ECC-associated microbiome.** The wide diversity of bacteria in dental caries has been revealed using both culture and molecular microbial methods. Most of the species detected make up a core microbiome, whereas other species in the climax community may be associated with disease. It is likely that several species interact with each other to produce the acidic conditions that promote dental caries. Cultured bacteria formed the basis of the ecological plaque hypothesis applied to dental caries and its modification. Under these models, the biofilm composition changes with the development of caries lesions. As lesions progress, the proportions of acid-producing *Streptococcus* and *Actinomyces* species increase, followed by acid-tolerant bacteria such as *S. mutans* and *Lactobacillus* species.

The bacterial diversity of ECC-associated biofilms is supported by molecular studies as well as parallel observations of biofilms in periodontal, endodontic, and other oral sites. The major bacterial genera detected in ECC include *Streptococcus, Lactobacillus, Actinomyces, Bifidobacterium, Propionibacterium,* and *Scardovia,* all of which are Gram-positive bacteria. Many

### Table 4. EFFECTS OF MATERNAL ANTIMICROBIAL INTERVENTION ON CARIOGENIC MICROBIAL REDUCTIONS AND EARLY CHILDHOOD CARIES (ECC) OUTCOMES IN CHILDREN

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age N (mos)</th>
<th>Treatment and interventions</th>
<th>Duration (mos)</th>
<th>Microbiological evaluation method</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alamoudi et al., 2014</td>
<td>Randomized clinical trial, Saudi Arabia</td>
<td>60 mother-child dyads (10-36)</td>
<td>Group 1: chewing xylitol gum after 3 meals for 3 mos; Group 2: fluoride varnish (5% NaF) every 6 mos</td>
<td>24</td>
<td>Chairside test: Dentocult SM Strip methods for MS</td>
<td>Children with high MS counts: no significant difference was found between the two groups.</td>
</tr>
<tr>
<td>Brambilla et al., 1998</td>
<td>Prospective observational study, Italy</td>
<td>60 mother-child dyads (0-24)</td>
<td>Group 1, F tablet daily + rinsed daily with 0.05% NaF and 0.12% CHX for 6 mos; Group 2: F tablet daily for 6 mos only</td>
<td>30 (started at 6 mos of pregnancy)</td>
<td>Selective culture: MSB agar for MS levels</td>
<td>Over the 30-month study period, the NaF and CHX treatment regimens significantly reduced the salivary MS levels in children. Fewer children in the experimental group were colonized by MS in saliva compared to those in the control group. The treatment significantly reduced salivary MS levels in mothers and delayed bacterial colonization in their children for approximately 4 months.</td>
</tr>
<tr>
<td>Dasanayake et al., 2002</td>
<td>Randomized clinical trial, United States</td>
<td>75 mother-child dyads (6-48)</td>
<td>Group 1: 10% CHX varnish (Chlorozol); Group 2: varnish contained 1% hydroxypropyl cellulose, 0.2% quinine hydrochloride</td>
<td>24</td>
<td>Selective culture: MSB agar for MS level</td>
<td>Mothers in the CHX group exhibited a significant reduction in <em>S. mutans</em> levels in the saliva compared to the control group for up to 12 months. There were no significant differences in the percentage of children with detectable levels of <em>S. mutans</em> in plaque during the study period. There were no significant differences in caries increment either among mothers or among children.</td>
</tr>
</tbody>
</table>

*Table continued on next page*
### Table 4. Continued

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age n (mos)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Fontana et al., 2009&lt;sup&gt;21&lt;/sup&gt;</td>
<td>Randomized clinical trial, United States</td>
<td>97 mother-child dyads (9-14)</td>
<td>Group 1: xylitol gum (3x/day for 9 mos); Group 2: xylitol gum (3x/day for 3 mos); Group 3: sorbitol gum 3x/day for 9 mos; Group 4: no gum</td>
<td>9-10</td>
<td>Selective culture: MSB for MS counts; MSA for total streptococci counts</td>
<td>MS could be recovered from one third of the pre dentate infants. There were no statistically significant differences in the effects of maternal use of xylitol-containing chewing gum for 3 or 9 months on MS colonization and total bacterial counts in 9- to 14-month-old infants.</td>
</tr>
<tr>
<td>Gripp et al., 2002&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Randomized clinical trial, Germany</td>
<td>44 mother-child dyads (6-24)</td>
<td>Group 1: high MS score, received 40% CHX varnish at 3-mo intervals; Group 2: high MS score, no CHX varnish; Group 3: low MS score, received CHX varnish at 6-mo intervals</td>
<td>24</td>
<td>Mothers—chairside test: Dentocult SM Strip methods for MS counts; children—selective culture: MSB for MS counts</td>
<td>For mothers, a significant decrease in high MS values was observed in the CHX group compared to baseline. For children at 24 months, 19% were MS positive in the CHX group: 40% in Group 2 high MS score and 20% in Group 3 low MS score. The differences were significant.</td>
</tr>
<tr>
<td>Gunay et al., 1998&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Prospective observational study, Germany</td>
<td>86 mother-child dyads (0-72)</td>
<td>Group 1: recalled every 6 mos and intervention (oral hygiene instructions; professional tooth cleaning; topical fluoride varnish application; CHX mouth rinsing; dietary counseling); Group 2: no intervention</td>
<td>48 (started in the 3rd trimester of pregnancy)</td>
<td>Chairside test: Dentocult SM Strip methods for MS counts</td>
<td>There were significant reductions in MS score and percentage of MS positivity in saliva for both mothers and children. Pre- and postnatal preventive programs may significantly improve the oral health of mothers and their children. The study prophylaxis concept is recommended for incorporation into the routine (dental) care of mothers and their young children.</td>
</tr>
<tr>
<td>Hanno et al., 2011&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Randomized clinical trial, Saudi Arabia</td>
<td>60 mother-child dyads (24-60)</td>
<td>Group 1: mother (xylitol chewing gums); children (xylitol chewing tablets); Group 2: NaF varnish</td>
<td>3</td>
<td>Chairside test: CRT kit (Vivadent-Ivoclar, Lichenstein) for MS counts</td>
<td>At the 3-month examination, the number of mother-child pairs with high MS levels in the experimental group significantly decreased but not in the control group. No difference was observed in the caries scores of the children.</td>
</tr>
<tr>
<td>Isokangas et al., 2000&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Randomized clinical trial, Finland</td>
<td>169 mother-child dyads (0-120)</td>
<td>Group 1: xylitol gum 2-3x/day; Group 2: CHX varnish at 6, 12, 18 mos; Group 3: FV at 6, 12, 18 mos</td>
<td>120</td>
<td>Selective culture: MSB agar for MS counts</td>
<td>At 2 years old, the differences in MS levels were not significant between the FV and CHX groups. At 3 years old, compared with the xylitol group, the risk of MS colonization was 2.3-fold higher in the F group. The differences between the FV and CHX groups were significant. At 5 years old, dentinal caries (dmft) in the xylitol group were reduced by 71% vs. the FV group and 74% vs. the CHX group. The difference between the CHX and FV group was not statistically significant. At 6 years old, 51.6% of the children in the xylitol group, 83.9% in the CHX group, and 86.4% in the FV group were colonized by MS. The difference was significant between the xylitol and FV groups. At 10 years old, the children who were not colonized by MS at the age of 2 years old had a longer caries-free survival time and a lower caries experience vs. MS-colonized children. Conclusions: Maternal use of xylitol chewing gum can prevent dental caries in children by suppressing transmission of MS from mother to child.</td>
</tr>
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Table continued on next page
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<tbody>
<tr>
<td>Nakai et al., 2010&lt;sup&gt;128&lt;/sup&gt;</td>
<td>Randomized clinical trial, Japan</td>
<td>107 mother-child dyads (0-24)</td>
<td>Group 1: xylitol gum chewed 5 mins, 4x/day; Group 2: no xylitol control</td>
<td>24 (started at 3-5 mos of pregnancy)</td>
<td>Chairside test: Dentocult SM Strip methods for MS counts</td>
<td>Children in the xylitol group were significantly less likely to be MS-positive than those in the control group. Children in the control group acquired MS 8.8 months earlier than those in the xylitol group. Maternal xylitol gum chewing in Japan shows beneficial effects.</td>
</tr>
<tr>
<td>Olak et al., 2012&lt;sup&gt;219&lt;/sup&gt;</td>
<td>Randomized clinical trial, Estonia</td>
<td>90 mother-child dyads (24-36)</td>
<td>Groups 1-2: xylitol gum chew 4x/day for 33 mos; Group 3: no xylitol control</td>
<td>36</td>
<td>Chairside test: Dentocult SM Strip methods for MS counts</td>
<td>The numbers and proportions of caries-free children were 80% at 2 years and 64% at 3 years old. The number of caries-free children was significantly higher in the intervention group than in the control group at both 2 and 3 years old.</td>
</tr>
<tr>
<td>Plonka et al., 2013&lt;sup&gt;110&lt;/sup&gt;</td>
<td>Randomized clinical trial, Australia</td>
<td>622 mother-child dyads (6-18)</td>
<td>Group 1: 0.12% CHX gel; Group 2: 10% CPP-ACP cream; Group 3: control</td>
<td>24</td>
<td>Selective culture: BHI agar for MS counts</td>
<td>MS-positive at 24 months: 72% in the CHX group; 5% in the CPP-ACP group; 50% in the control group. LB-positive at 24 months: 63% in the CHX group; 63% in the CPP-ACP group; 65% in the control group. Caries incidence at 24 months: 2% in the CHX group; 1% in the CPP-ACP group; 2% in the control group. There is insufficient evidence to justify the daily use of CPP-ACP or CHX gel to control early childhood caries.</td>
</tr>
<tr>
<td>Ramos-Gomes et al., 2012&lt;sup&gt;119&lt;/sup&gt;</td>
<td>Randomized clinical trial, United States (Mexican American)</td>
<td>361 mother-child dyads (12-36)</td>
<td>Intervention: mother received CHX (0.12% mouthrinse) 2x/day for 3 mos; children received FV (5% NaF) every 6 months from 12-36 mos old; control children received FV only if precavitated lesions developed</td>
<td>36 (started at 4 mos postpartum for all mothers)</td>
<td>Maternal MS levels declined during CHX use but increased following discontinuation. At 36 months old, 34% of the children in each group developed caries. There were no significant differences in the incidence of caries between the 2 groups. Approximately half of the control group developed precavitated lesions and received therapeutic FV. Maternal postpartum CHX regimens, oral health counseling, and preventive child FV applications were not more efficacious than maternal counseling with child therapeutic FV for precavitated lesions for ECC prevention.</td>
<td></td>
</tr>
<tr>
<td>Thorid et al., 2004, 2006, and 2012&lt;sup&gt;79-72&lt;/sup&gt;</td>
<td>Randomized clinical trial, Sweden</td>
<td>173 mother-child dyads (36-120)</td>
<td>Mothers with high counts of salivary MS were randomly assigned into 3 groups: Group 1: xylitol (n=61); Group 2: CHX/xylitol/sorbitol (n=55); Group 3: NaF/xylitol/sorbitol (n=57)</td>
<td>120</td>
<td>Chairside test: Dentocult SM Strip methods for MS counts</td>
<td>At 3 years old, lower but non-significant levels of salivary MS and dental decay were observed in 3-year-old children of mothers who used high-content xylitol gums. At 4 years old, the difference between the xylitol and NaF/xylitol/sorbitol groups was significant. Thus, fewer caries were observed in children of xylitol gum mothers vs. non-xylitol gum groups. At 10 years old, the overall caries prevalence in the combined groups was 31%. There were no significant differences between the 3 experimental groups. Conclusions: No long-term beneficial effects from maternal xylitol gum exposure on their children's dental health were demonstrated when compared with gums exposed to CHX and fluoride. The study demonstrated a significant positive effect on the reduction of salivary MS colonization at 18 months old and lower caries experience at 10 years old as a result of xylitol usage in a Swedish population.</td>
</tr>
</tbody>
</table>

* CHX=chlorhexidine; MS=mutans streptococci; CRT=Caries Risk Tests; dmft=decayed, missing, and filled primary teeth; FV=fluoride varnish; CPP-ACP=casein phosphopeptide-amorphous calcium phosphate; MSB=mitis salivarius bacitracin; MSA=mitis salivarius agar without bacitracin; BHI=brain heart infusion.
The microbial diversity and complexity of the microbial biota in dental plaque was highly similar within an individual over a 2-year period. Repeated observations of the same children indicated that the salivary bacterial communities were remarkably stable over time. A combination of lifestyle factors, such as dietary habits, oral hygiene practices, and systemic health conditions, could influence the composition of the oral microbiota. Longitudinal studies have shown that a variety of bacterial species can be detected in the oral cavity, and that the diversity of these species can change over time. This suggests that the oral microbiota may be responsive to external factors and that changes in the composition of the microbiota may be associated with changes in oral health. Overall, the oral microbiota can be considered a dynamic system that is influenced by a variety of factors. Future research should focus on identifying the factors that influence the composition of the oral microbiota and how these factors may impact oral health.
species of Gram-negative bacteria have also been detected, including Campylobacter, Haemophilus, Aggregatibacter, Fusobacterium, Prevotella, Porphyromonas, Capnocytophaga, and Treponema (Spirochetes) species. However, based on molecular methods, the traditional S. mutans, Lactobacillus Actinomyces, and Bifidobacterium species appeared to be less important or missing, which suggests that additional species other than S. mutans and Lactobacillus may also be responsible for ECC. In fact, some of these discrepancies resulted from technical differences between methods, resulting in Actinomyces, Bifidobacterium, and Scardovia species being underestimated in molecular studies.\(^{96,97}\)

Hence, understanding the microbial diversity of ECC requires information from both culture-based and molecular studies.

**Cariogenic pathogens in the bacterial microbiome.**

Several approaches have been used to isolate potential caries pathogens from the microbial complex. Culture studies for ECC have used acidic (low-pH) isolation media to select aciduric bacteria.\(^93\) Acidic agar (pH of five to 5.2) suppressed 90 percent of the microbiota but enhanced the growth of MS, Bifidobacterium, and Lactobacillus species, suggesting the successful enrichment of putative caries pathogens. ECC-associated, acid-tolerant, and acidogenic bacteria cultured from a low-pH broth included S. mutans, Actinomyces israelii, and Lactobacillus species.\(^94\) The non-MS Streptococcus oralis and Streptococcus intermedius were acid tolerant but associated with caries-free children rather than ECC children, indicating that acid-tolerance per se is not sufficient to describe a caries pathogen. Using acid agar with anaerobic incubation, the major ECC-associated species were found to be S. mutans, S. sobrinus, Parvotabulovia denticolen, and the new species Scardovia wiggsiae.\(^95\) S. wiggsiae was associated with ECC in S. mutans-negative samples, suggesting that this new species may be important in ECC that is not associated with MS. S. wiggsiae and P. denticolen belong to the family or phylum Bifidobacteriaceae that includes Bifidobacterium species. The latter were cultured from occlusal lesions of children at similar proportions to those of S. mutans.\(^96\)

Based on selective isolation, the dominant species in childhood caries were Bifidobacterium dentium and P. denticolen.

To differentiate bacteria associated with caries progression, several molecular-based studies have compared lesions at different stages. Based on this design, open-ended cloning and sequencing studies compared three sites in ECC children: (1) caries-free; (2) white spot lesions (initial caries); and (3) cavities.\(^13,100-102\) These studies were instrumental in revealing the wide diversity of bacterial species in both ECC and caries-free children. A recent study utilizing cloning and sequencing strategies reported that S. mutans, S. sobrinus, Streptococcus parasanguinis, Streptococcus vestibularis/salivarius, and Veillonella atypica/dispar/parvula increased from healthy regions to cavitated lesions.\(^13\) The authors suggested that S. sobrinus, S. salivarius, and S. parasanguinis could be alternate ECC pathogens, in addition to S. mutans, based on their presence in progressing ECC sites that lacked S. mutans. Taken together, these findings indicate a major role for S. mutans in ECC but also suggest that additional species of importance in ECC include S. sobrinus and S. wiggsiae.

**Rapid detection of species and microbial communities in plaque biofilms.**

Molecular methods have been developed to rapidly detect individual species and multiple species simultaneously and have great potential for use in clinical studies of ECC. A DNA probe checkerboard study found that Lactobacillus gasseri, Lactobacillus fermentum, Lactobacillus vaginalis, and S. mutans with S. sobrinus were associated with ECC, but not L. acidophilus, a probiotic species.\(^96\) This suggested specificity among Lactobacillus species with respect to ECC. Probes based on the 16S rRNA have been used in the checkerboard format\(^100-102\) and in its successor, the human microbe identification microarray (HOMIM)\(^103\) which contains more than 300 different probes. The HOMIM assay was used in a treatment study of severe ECC. While the microbiota did not change in children with new lesions (relapse) after therapy, there were changes in the children without disease progression.\(^98\) This suggested that major changes had occurred in the biofilm composition, which would require an assay capable of detecting multiple species. The polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis has been used to examine bacterial profiles in ECC\(^108,104\) and demonstrate differences in the microbial community between children with and without ECC\(^19\) as well as bacterial differences before and after treatment.\(^105\)

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Table 5. Continued*

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design, country</th>
<th>Sample size and age (N mos)</th>
<th>Microbiology evaluation method</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al., 2014(^{98})</td>
<td>Exploratory study, China</td>
<td>19: 10 ECC, 9 CF (12-24)</td>
<td>16S rRNA gene pyrosequencing</td>
<td>A high bacterial diversity was noted in the plaques of ECC children but was not significant compared to caries-free children. Principal component analysis (PCA) showed that caries-related genera included Streptococcus and Veillonella, whereas Leptotrichia, Selenomonas, Fusobacterium, Capnocytophaga, and Porphyromonas were more related to the caries-free samples. Neisseria and Prevotella presented numbers that were approximately in between.</td>
</tr>
<tr>
<td>Zhan et al., 2012(^{22})</td>
<td>Randomized clinical trial, United States</td>
<td>22: 11 xylitol wipe, 11 placebo wipe (6-35)</td>
<td>AP-PCR for MS genotyping</td>
<td>No significant differences in the prevalence of xylitol-resistant genotypes or in the biofilm-formation capacity of MS were observed between the two groups.</td>
</tr>
</tbody>
</table>

* HOMD=Human Oral Microbiome Database; HOMIM=Human Oral Microbiome Identification Microarray; ECC=early childhood caries; CF=caries free.  
† PCR-DGGE=polymerase chain reaction-based denaturing gel gradient electrophoresis.  
‡ AP-PCR=arbitrarily primed-polymerase chain reaction.
PCR can rapidly detect bacterial species, while quantitative PCR (qPCR) can measure bacterial levels and, therefore, determine DNA amounts and bacterial count equivalents. Genetic assays can be more sensitive than culture methods and improve the detection of S. sobrinus compared with a culture. \(^{56}\) Studies using PCR-based methods revealed that detection of S. mutans with S. sobrinus improved predictions of ECC and ECC progression versus detection of the individual species. \(^{58,59,108}\) PCR and qPCR assays have also been developed and used to detect many Lactobacillus species in deep dental lesions, \(^{108,109}\) as well as oral Bifidobacterium species \(^{59}\) and S. wiggsiae in dental plaque. \(^{108,109}\) Using PCR-based assays, S. mutans, S. sobrinus, S. wiggsiae, and Bifidobacterium species were shown to be significantly associated with severe ECC. \(^{58}\)

**Summary**

In this systematic review, we identified 41 clinical studies that incorporated microbiological evaluations of ECC treatments or other interventions. In many studies, reductions in salivary MS or Lactobacillus species were observed following the topical application of antimicrobial agents. Perhaps the most significantly effective anticaries and antimicrobial regimens involved interventions in mothers to influence outcomes in children. Although antimicrobial therapeutic approaches show reductions in MS colonization, bacterial regrowth occurred in most of the studies, with a concomitant high incidence of ECC once the intervention had ceased. These results raise questions regarding the sustainability of the bacterial reductions and whether the antimicrobial interventions and treatments used to date produce sustainable reductions in ECC development, caries relapse rates, cariogenic microbial transmission and acquisition, or other microbiological parameters. The meta-analysis highlighted the paucity of high-quality randomized controlled clinical trials that demonstrated the efficacy of commonly used antimicrobial agents and procedures. Many of the tested agents have been evaluated in adult populations and were highly recommended by dental professional organizations; thus, it was assumed that the same agents would provide preventive benefits for young children.

The overall limitations of the studies evaluated included: (1) paucity of good clinical trials evaluating caries outcomes with microbial reductions; (2) inability of agents to elicit long-term reductions in caries or the cariogenic microbiota; (3) wide variation in the study designs used, some of which were reflected in the Higgins index ($I^2$ statistics analysis); and (4) lack of adequate control groups, including (in most of the studies) the fact that control children were exposed to various forms of fluoride. Thus, the results of those studies should be interpreted with caution. This review also suggests that additional well-designed, placebo-controlled, randomized clinical trials are needed to individually test specific antimicrobial treatments, particularly to elucidate the critical link between antipathogenic mechanisms and caries prevention in young children.

Despite the potential limitations and the risk of bias, this literature review, which combines information from clinical studies for multiple meta-analyses, provides updated evidence on the effectiveness of antimicrobial approaches on the ECC-associated microbiota and ECC management. This information will provide a basis for designing future research studies and clinical interventions.

**Conclusions**

Based on this study’s results and meta-analyses, the following conclusions can be made:

1. Antimicrobial interventions and treatments show temporary reductions in mutans streptococci colonization levels.
2. There is insufficient evidence to indicate that the antimicrobial therapeutic approaches currently used produced sustainable effects on cariogenic microbiota or early childhood caries reduction and prevention.

**Acknowledgments**

The authors wish to thank the Office of Continuing Dental Education of the University of Maryland School of Dentistry, Baltimore, Md; the American Academy of Pediatric Dentistry, Chicago, Ill.; DentaQuest Foundation, Boston; the William Bingham 2nd Trust, for their support; and, in part, research grants DE015706, DE019455, DE016937 supported by the National Institute of Dental and Craniofacial Research, Bethesda, Md., USA.

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