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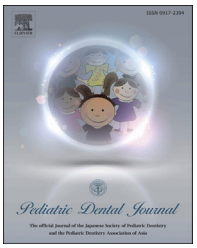

Original Article

Mutans streptococci colonization in early childhood caries in Ibadan, Nigeria

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ABSTRACT

Purpose: This study aimed to assess mutans streptococci (MS) colonization in plaque samples of children and its relationship with early childhood caries in Ibadan, Nigeria.
Method: Children from eight nursery schools in Ibadan were examined for caries and 120 children were randomly selected into two groups: caries-free (decayed, missing, or filled primary teeth *dmft*) = 0) and caries present groups (*dmft* > 0). Plaque samples were collected with wooden toothpicks and cultured for MS using Dentocult® SM strips (Orion Diagnostica, Espoo, Finland) at 37 °C for 48 h. The presence of mutans colonization was confirmed by the detection of raised dark blue to light blue colonies on the inoculated strips. Inspection of the growth for raised colonies was done with a magnifying glass (10×). The result from each child was evaluated as follows: Class 0: $<10^4$ cfu/ml, Class 1: $<10^5$ cfu/ml, Class 2: 10^5–10^6 cfu/ml, and Class 3: $>10^6$ cfu/ml.
Results: There was MS bacterial growth on all plaque samples. About 67% of children with caries had high bacterial score ($>10^6$ cfu/ml), while 55.2% of caries-free children had low bacterial score ($p = 0.033$). When bacterial levels were compared with mean *dmft*, a statistically significant relationship was also observed ($p = 0.033$). Three-year-old children were observed to be 4.2 times less likely to develop caries (odds ratio (OR) = 0.237, confidence interval (CI) = 0.086–0.651) and children with MS colonization level $>10^6$ cfu/ml being 3.6 times more likely to develop caries (OR = 3.63, CI = 1.414–9.34).
Conclusion: MS prevalence was 100% among children studied and significantly higher levels of MS colonization ($>10^6$ cfu/ml) were present in children with caries.

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1. Introduction

Early childhood caries (ECC) is a chronic and infectious oral disease affecting young children. This term was defined by the American Academy of Pediatric Dentistry (AAPD) as the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled surfaces in any primary tooth in a child at 71 months of age or younger [1]. Despite the fact that it is preventable, this disease remains one of the commonest causes of pain and infection that mostly result in dental extractions in these children. ECC is a disease produced by an interaction between microorganisms, substrate (sucrose), and susceptible host (tooth, saliva) [1]. These factors interact over a certain period of time, causing an imbalance in the demineralization and remineralization between tooth surface and the adjacent plaque (biofilm) [2].

Sucrose is the most widely consumed and regarded as the most important cariogenic food [3]. It serves as a specific substrate for the synthesis of extracellular polysaccharides (dextran and levan) favoring mutans streptococci (MS) adherence to tooth surfaces and subsequent acid production by the plaque bacteria [4]. The microbiota of the dental plaque is known to consist of a variety of acidogenic, non-acidogenic, and base-producing organisms and to differ in composition in different dentition sites [5]. The main cariogenic microorganisms are the mutans streptococci (MS). ECC is closely associated with MS, especially *Streptococcus mutans* and *Streptococcus sobrinus*, which are known to be crucial in the initial phase of caries development [6]. These microorganisms have the ability to metabolize sucrose, which can function as fermentable disaccharide and serve as a substrate for intracellular polysaccharide synthesis with subsequent production of organic acid responsible for the caries process. More importantly, sucrose is the substrate for glucosyltransferase-mediated glucan production, which promotes the adhesion of MS to the tooth surface [7].

Early acquisition of MS is a key event in the natural history of early childhood caries and this may occur via vertical or horizontal transmission [8]. Vertical transmission is from the mother or primary caregiver, while horizontal transmission is from other members of the family or community to whom an infant belongs [9]. It has been shown that many children acquire MS in early childhood mostly transmitted from their mothers and that the bacterial levels are closely related to dental caries prevalence in preschool children regardless of the extent of caries [10]. Monitoring the colonization of these microorganisms in this group of children can be an alternative method of evaluating current caries activity and predicting future caries risk apart from the traditional visual inspection and probing.

In Nigeria, there is paucity of information in the literature investigating mutans streptococci colonization in dental caries generally and particularly in early childhood caries. This study was hereby designed to assess MS colonization in preschool-age children and investigate its relationship with early childhood caries.

2. Materials and methods

Ethical approval was obtained from the joint University of Ibadan/University College Hospital (UI/UCH) Ethical Review Committee. Children were examined for caries in eight nursery schools within Ibadan city using World Health Organization (WHO) standard method and criteria after informed consents were obtained from the parents/guardians. Ibadan is the largest metropolitan geographical city in Nigeria, located in southwestern region of the country. A total of 120 children were selected randomly into two equal groups: group A (decayed, missing, or filled primary teeth (*dmft*) = 0) and group B (*dmft* > 0). The socioeconomic status (SES) of the children was determined from a modified version of socioeconomic index score developed in Nigeria [11]. Plaque samples were collected with wooden toothpicks from any two interproximal and any two buccal/labial surfaces of the maxillary primary central incisors and the mandibular primary molars [12]. Four different sites were simultaneously sampled for each child. The plaque-covered toothpicks were spread thoroughly and gently on the rough surface of Dentocult® SM strips (Orion Diagnostica, Espoo, Finland).

The plaque-covered strips were then immersed in a liquid broth medium, one strip per vial. The typical formulation of the broth medium consisted of tryptose (10 g/l), peptone (10 g/l), glucose (1 g/l), saccharose (300 g/l), K_2HPO_4 (5 g/l), trypan blue (12 mg/l), and 1 ml of 1% K-Tellurite. A bacitracin disc was placed in each selective culture vial at least 15 min before sampling for plaque in order to suppress other bacteria in the vial. The sampled strips were placed in the broth (a strip per vial) with the smooth surfaces clipped and attached to the cap of the vial. The cap of the vial was turned one quarter of a turn open to allow for oxygen (Orion Diagnostica, Espoo, Finland).

The vials were placed in an upright position in an incubator (Gallenkamp incubator, model IH-100) under an aerobic condition at 37 °C for 48 h in the Department of Microbiology, U.C.H. Ibadan. After incubation, the presence of MS was confirmed by the detection of raised dark blue to light blue colonies on the inoculated strips.

Inspection of the growth for raised colonies was done with a (10×) magnifying glass [13]. The inoculation, incubation, and microbial analysis were according to the manufacturer's instructions.

The result from each of the four inoculated sites for each child was evaluated according to the following manufacturers' chart:

Class 0: $<10^4$ cfu/ml
Class 1: $<10^5$ cfu/ml
Class 2: 10^5–10^6 cfu/ml
Class 3: $>10^6$ cfu/ml

The highest score of the four tested surfaces was chosen as the score for each child.

All data generated were entered into a personal computer. Computer analysis was done using Statistical Package for Social Sciences (SPSS) version 16.0. Frequency distribution of variables was generated and measures of central tendency were calculated to summarize the numerical data. The Chi-