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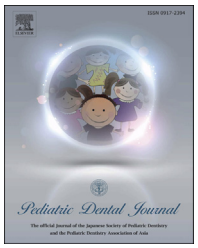

Original Article

Isolation of amoxicillin-resistant oral streptococci from children and their mothers

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ABSTRACT

Background and objective: Infective endocarditis has been reported to be induced by invasive dental treatments in individuals with certain underlying heart disorders. Although oral amoxicillin (AMPC) is widely used for prophylaxis in the dental field, scant information is available regarding transmission of AMPC-resistant strains. In the present study, AMPC-resistant strains harbored in the oral cavities of children and their mothers were examined to consider the possibility of transmission between them.

Design: AMPC-resistant strains were isolated from 320 saliva specimens taken from 150 healthy Japanese mother–child pairs using selective medium for streptococci containing AMPC. The minimum inhibitory concentrations of these strains were evaluated using a macrodilution broth method. Randomly amplified polymorphic DNA analysis was performed to compare the fingerprinting patterns of the strains.

Results: AMPC-resistant streptococcal strains were isolated from 11 children and seven mothers, which included four mother–child pairs, although the fingerprinting patterns were consistent in only three. The proportion of children harboring AMPC-resistant strains with mothers who also harbored them was significantly higher than that of children whose mothers did not.

Conclusion: Our results suggest that AMPC-resistant strains can be transmitted between mothers and their children.

1. Introduction

Infective endocarditis (IE) is a life-threatening disease caused by bacterial vegetation formed on impaired endocardium or heart valves in individuals with certain types of underlying heart disorders [1–3]. In order to prevent the onset of IE, antibiotic prophylaxis is generally recommended when invasive dental procedures, such as tooth extraction or periodontal surgery, which can induce bacteremia, are performed for patients at risk of IE [4]. Oral amoxicillin (AMPC) is widely used as prophylaxis in general dental practice [5]. However, several IE cases caused by penicillin-resistant strains have been reported in children even though standard AMPC prophylaxis was performed [6,7].

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Our previous study showed that approximately 5% of healthy Japanese children harbored AMPC-resistant strains in the oral cavity [8]. As for Japanese children at risk of IE, the distribution frequency of those who harbored AMPC-resistant strains was shown to be elevated to approximately 20%, although those strains appeared to not be indigenous but rather transient [9]. Although evidence of mother-to-child transmission of oral streptococci and periodontitis-related species has been accumulating [10–16], transmission of AMPC-resistant strains between mothers and their children remains to be demonstrated.

In the present study, we investigated the distribution of AMPC-resistant oral streptococcal strains in 320 saliva specimens collected from 150 mother–child pairs to examine the possibility of mother-to-child transmission.

2. Methods

2.1. *Participants and clinical specimens*

The protocols used in this study were approved by the Ethics Committee of Osaka University Graduate School of Dentistry, Osaka, Japan. Prior to collecting specimens, the participants were informed of the study contents and gave approval for their participation, with that for children and adolescents provided by their guardians. The participants, 170 children aged 4–13 years and their mothers ($n = 150$) aged 26–49 years, visited Osaka University Dental Hospital from July 2012 to December 2013. Only those who were systemically healthy and had not taken antibiotics for at least 3 months were included. Following mouth washing with water, non-stimulated expectorated whole saliva was collected from each participant in a sterile plastic tube and immediately placed on ice. The saliva specimens were then immediately transported to our laboratory, where the following procedures were performed. Saliva specimens from participants found to harbor AMPC-resistant strains were obtained more than twice at an approximately 3-month interval.

2.2. *Isolation of AMPC-resistant oral streptococcal strains*

Isolation of AMPC-resistant strains was performed as previously described [8,9,17]. Briefly, each saliva specimen was serially diluted in sterile saline, streaked onto *mitis salivarius* (MS) agar (Difco Laboratories) plates with or without AMPC (32 μg/mL), and incubated anaerobically at 37 °C for 48 h. The total number of streptococci was estimated based on the number of colonies on the plate, then four representative colonies, or all colonies if fewer than four on plates with MS agar containing AMPC were isolated and grown anaerobically in brain–heart infusion broth (Difco Laboratories) at 37 °C for 18 h.

2.3. *Identification of same and different clones using molecular biological approach*

To determine whether the strains isolated from each participant were the same or different clones, randomly amplified polymorphic DNA (RAPD) analysis was performed using Ready-To-Go RAPD analysis beads and primers (Amersham Biosciences), as previously described [18]. Briefly, PCR was performed using six primers (P1, 5′-GGT GCG GGA A-3′; P2, 5′-GTT TCG CTC C-3′; P3, 5′-GTA GAC CCG T-3′; P4, 5′-AAG AGC CCG T-3′; P5, 5′-AAC GCG CAA C-3′; and P6, 5′-CCC GTC AGC A-3′) and comprised 45 cycles of denaturation at 94 °C for 30 s, annealing at 36 °C for 30 s and extension at 72 °C for 1 min. Amplicons were separated by electrophoresis on 1.5% agarose gels.

2.4. *Determination of minimum inhibitory concentrations for AMPC and other antibiotics*

The minimum inhibitory concentrations (MICs) of AMPC were determined using a macrodilution broth method [19]. Briefly, samples consisting of 950 μL of Mueller–Hinton broth (Difco Laboratories) supplemented with 5% defibrinated sheep blood (Nippon Biotest Laboratories) and containing a two-fold serial dilution of AMPC were placed in sterile 13 × 100 mm test tubes. Test strains were cultured in brain-heart infusion broth at 37 °C for 18 h, then washed and adjusted to 10^7 colony forming units (CFU)/mL. Thereafter, 50 μL samples of the diluted test strains (5×10^5 CFU) were added to tubes containing the antimicrobial agents and incubated anaerobically at 37 °C for 18 h. The breakpoints of AMPC concentrations were set based on the Clinical and Laboratory Standards Institute recommendations [19], as follows: susceptible, ≤ 0.25 μg/mL; intermediate, 0.5–4 μg/mL; and resistant, ≥ 8 μg/mL. The MICs of other antibiotics, including ampicillin (ABPC), penicillin G (PCG), erythromycin (EM), and levofloxacin (LVFX), were also analyzed using the same method. In addition, the MICs for AMPC and the other four antibiotics were also evaluated using representative strains from three different oral streptococcal species (*Streptococcus oralis* ATCC 10557, *Streptococcus mitis* ATCC 49456, and *Streptococcus salivarius* HHT). The breakpoint of each antibiotic was set based on the Clinical and Laboratory Standards Institute recommendations [19].

2.5. *Identification of bacterial species*

Identification of bacterial species was performed based on 16S rRNA alignments as previously described [8,9,17]. Briefly, genomic DNA was extracted from each strain and the 16S rRNA gene sequence of ~1500 bp was amplified by PCR using AmpliTaq Gold polymerase (Applied Biosystems) with primers 8UA (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1540R (5′-AAG GAG GTG ATC CAG CC-3′), as described previously [20]. The sequences obtained were compared with those available in GenBank using the gapped BLASTN version 2.0.5 program obtained from the National Center for Biotechnology Information server (http://blast.ddbj.nig.ac.jp/blastn?lang=en).

2.6. *Statistical analysis*

Intergroup differences for various factors were determined by statistical analysis of variance for factorial models. Fisher's protected least-significant difference test was used to compare individual groups. Student *t* test was performed to compare the total bacterial numbers of streptococci in saliva