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16S rDNA PCR detection of Actinobacillus actinomycetemcomitans from periodontitis-free plaque

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Objective: 16S rDNA PCR assisted detection and isolation of Actinobacillus actinomycetemcomitans was studied. Methods: Prevalence of A. actinomycetemcomitans in subgingival plaque samples (2/person) of 47 20-24 year-old periodontitis-free dental students was independently assessed by 16S rDNA PCR (Ashimoto et al 1996) or TSBV culture. Presumptive cultural identification of A. actinomycetemcomitans was based on colony morphology and positive catalase test. For samples 16S rDNA PCR positive but culture negative, representative colonies were selected and PCR screened. Identity of presumptive A. actinomycetemcomitans isolates was confirmed by partial 16S rDNA gene sequencing. Results: 11 (11.7%) sites or 8 (17.0%) subjects were 16S rDNA positive, while 4 (4.3%) sites or 3 (6.4%) subjects were culture positive for A. actinomycetemcomitans. From the 7 PCR positive but culture negative plaque samples, gram-negative coccobacilli strains weakly reactive to A. actinomycetemcomitans 16S rRNA PCR primers were identified: 3 Neisseria subflava, 2 Haemophilus segnis, 1 each of Campylobacter showae, and Haemophilus paraphrophilus. Conclusions: Prevalence of A. actinomycetemcomitans was low in subgingival plaque of periodontitis-free adults. The Ashimoto et al. 16S rDNA PCR protocol is specific but did not assist A. actinomycetemcomitans isolation. Rather, false positive PCR signal from non-A. actinomycetemcomitans isolates was observed.

Microbiology / Immunology and Infection Control

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