

Advantages of new generation sequencing by synthesis for the study of bacterial communities in root canal therapy.

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Today new generation sequencing (NGS) technologies allow a more accurate analysis of the bacterial composition of a microbiota. Additionally, they have contributed to the understanding of the role that prokaryotes play in endodontic pathogenesis. The sequencing of the hypervariable regions V3 and V4, a segment of the 16S rRNA gene, to evaluate diversity together with sequencing by synthesis (*e.g.* Illumina MiSeq) has made it possible to elucidate how clinical patterns influence the structure of the bacterial community. The 16S rRNA gene has nine hypervariable regions, and Illumina sequencing allows for the generation of a slightly larger amplicon than when only a single hypervariable region is sequenced, as in some cases of pyrosequencing, another type of NGS. As regions V3 and V4 are very close to each other, they are analyzed in a single sequence, improving the efficiency of taxa assignment. In the same context, many previous studies have been carried out by sequencing using 454 pyrosequencing.^{1,2} However, although 454 pyrosequencing is a better tool for evaluating bacterial composition compared with other molecular methods of DNA-based techniques such as restriction fragment length polymorphism (RFLP), microarrays or denaturing gradient gel electrophoresis (DGGE), it is limited by a relatively high reading error rate. In contrast, sequencing by synthesis technology such as that of Illumina has a substantially lower error rate than the 454 pyrosequencing platform, and the read length is deterministic rather than random. Therefore, the Illumina platform is not capable of misinterpreting the number of nucleotides in homopolymer regions. In NGS technologies, bases are deduced from signals of light intensity, a process commonly known as "base-calling", and errors in this process are more common in pyrosequencing.³ Although there are many studies reporting the composition of the microbiota associated with persistent chronic apical periodontitis, there are only six reports to date performed with new generation sequencing, and of these only one, carried out in a Brazilian population, included the Illumina MiSeq system.⁴ All these results corroborate the idea that new generation sequencing by synthesis is a much more sensitive and accurate technique for describing microbial communities. Consequently, its use is suggested when conducting comparative studies between different world populations.³

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