TITLE: COMPARISON BETWEEN BIOCHEMICAL AND MOLECULAR IDENTIFICATION OF BACTERIA ISOLATED BY CULTURE IN ENDODONTIC INFECTIONS

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ABSTRACT

Culture and biochemical methods for microbial identification, frequently used in clinical laboratory, require the recognition of differences in morphology, growth requirements, enzymatic activity, and metabolism to define genera and species. Different from the phenotypic methods, the 16S rRNA sequencing allows a better identification of microorganisms and overcome some limitations of conventional biochemical bacterial identification. The aim of this present study was to compare the identification of cultivable bacteria by biochemical tests (phenotypic method) and by 16S rRNA gene sequencing (genotypic method). Samples were collected from the root canals of 20 patients who attended the Piracicaba Dental School, State University of Campinas - UNICAMP, for endodontic treatment. Two hundred twenty strains isolated by microbiological culture were initially characterized according to their gaseous requirements, Gram-stain characteristic, and ability to produce catalysis. They were then biochemically identified using specific kits (Rapid ID 32 A , API Strep, API Staph, Rapid NH, BioMérieux, Marcy-l'Etoile, France). The same isolate was submitted to DNA extraction and amplification of the 16S rRNA gene (PCR), followed by sequencing by BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The nucleotides sequences obtained were compared to those from gene bank of the National Center of Biotechnology Information through the BLAST. Sequencing allowed the identification of 97% of isolates (215/220) against 70,5% (155/220) identified biochemically. Thirty-four different species were identified by biochemical methods and 57 by sequencing. Strains not identified biochemically were characterized in 97% (63/65) by sequencing. There was an agreement of 49% between the biochemical and 16S rRNA gene sequencing identification, 27.7% at genus level and 21.3% at species level. There was a statistically significant agreement between the methods in the identification of *Prevotella buccae, Parvimonas micra, Fusobacterium nucleatum* and *Propionibacterium acnes*. It was concluded that 16S rRNA sequencing can offer a more consistently reliable and accurate method for bacterial identification, allowing the characterization of biochemically unidentified strains. [Supported by FAPESP (2011/09047-4 & 2015/23479-5); CNPq: (308162/2014 -5); CAPES; FAEPEX & EDUCORP].

Keywords: Bacterial identification, Biochemical methods, 16S rRNA Sequencing, Endodontic Microbiota, Acute Apical Abscess

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