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Best Practice of in vitro Methods on Measuring Anti Microbial of Chemical Substance on Root Canal Treatment: Literature Review

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ABSTRACT

Root canal or known as endodontic therapy use chemo-mechanical treatment of the root canal to eliminate the microbial infection. Chemical substance that contains drugs and mechanical has its own role on eliminating microbes, but in result those two were combined as a combination causes. This review reveal best practice on laboratory methods on measuring of anti microbial activity using the required data of chemical substance that were used as irrigant or sealer, and also reviewing some of the theoritical pharmacodynamic mechanism to explain how chemical substance role. In Conclusion, some of the methods reveal will be useful for those need to analyze or compare various chemical substance to be used as an irrigant or sealer in endodontic treatment, whether for standardize branded substance or even new substance from natural resources.

Keyword: Chemical substance, drugs, root canal, laboratory methods

INTRODUCTION

Antimicrobial susceptibility testing is essential for guiding the treatment of many types of bacterial infections, especially in the current context of rising rates of antibiotic resistance. Bacteria have long been recognized as the primary etiological factor in the development of pulp and periapical lesions. Successful root canal therapy depends on thorough debridement of pulpal tissue, dentin debris, and infective microorganisms. Currently, it is impossible to eradicate intraradicular infection with mechanical instrumentation alone.[1]

Clinical studies have demonstrated that chemo-mechanical preparation and use of antimicrobial medicaments are effective in reducing the bacterial load in root canal systems.[2] *Enterococcus faecalis* is often isolated from previously treated teeth presenting with persistent disease.[3] In terms of chemical substance that being used on endodontic treatment, is the substance that has antimicrobial activity. The substance is used as irrigant and sealers as medicaments.

Consequently, recent laboratory studies have focused on evaluating the effectiveness of root canal irrigants and medicaments against *Enterococcus faecalis*. The challenges on this research area was about the method on creating the closest environment and relevant to day to day of endodontic treatment implementation using irrigants and medicaments.

In order to have clinical relevance, researchers should develop standardized methods to test antimicrobial activity and to evaluate the antimicrobial effectiveness of root canal chemical substance against multiple endodontic pathogen species associated with persistent endodontic disease.[4]

Main Bacteria

Enterococcus faecalis is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24% to 77%. This finding can be explained by various survival and virulence factors possessed by *Enterococcus faecalis*, including its ability to compete with other microorganisms, invade dentinal tubules, and resist nutritional deprivation.[5]

Chemical Substance in Endodontic

Irrigation is defined as “to wash out a body cavity or wound with water or medicated fluid” and aspiration as the process of removing fluids or gases from the body with a suction device. Disinfectant, meanwhile, is defined as “an agent that destroys or inhibits the activity of microorganism that cause disease.[6] The purpose of irrigation in endodontics are mechanical, chemical and biologic. The mechanical and chemical objectives are as follows; (1) flush out debris, (2) lubricate the canal, (3) dissolve organic and inorganic tissue, and (4) prevent the formation of a smear layer during instrumentation or dissolve it once it has formed.[1]

Antimicrobial Test

Agar Diffusion Test

The principle of disk diffusion testing has been used in microbiology laboratories for over 70 years. Alexander Fleming used a variant of this technique when working with penicillin in the 1950s. At that time, there were as many different procedures in use as there were microbiologists. Drs. Bauer, Kirby, Sherris, and Turck painstakingly tested all of the variables used in the procedure, such as the media, temperature, and depth of agar. In 1966, they published their landmark paper describing the test that is used today. NCCLS adopted the basic procedural steps in the Bauer paper as the disk diffusion reference method. These steps must be followed precisely to obtain accurate results.[7]

Once isolated colonies are available from an organism that has been identified as a potential pathogen, it is necessary to proceed as follows to perform the susceptibility test in steps as followed : 1) Select colonies; 2) Prepare inoculum suspension; 3) Standardize inoculum suspension; 4) Inoculate plate; 5) Add antimicrobial disks; 6) Incubate plate; 7) Measure inhibition zones; and 8) Interpret results.[7]

Microdilution MIC Test

The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest (i.e. minimal) concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system. We determine the concentration in the laboratory by incubating a known quantity of bacteria with specified dilutions of the antimicrobial agent. Using NCCLS interpretive criteria the results are interpreted as susceptible, intermediate, or resistant. MIC tests can be performed using broth or agar media, but broth microdilution is the most widely used method in clinical laboratories. Several companies manufacture MIC panels that contain dilutions of one or multiple antimicrobial agents in a broth microdilution format.[7]

Broth microdilution MIC testing is performed in a polystyrene panel containing approximately 96 wells. A panel may contain 7–8 dilutions of 12 different antimicrobial agents. One well serves as a positive growth control (broth plus inoculum), and one serves as a negative control (broth only). Most systems have a volume of 0.1 mL in each well.

To facilitate testing appropriate antimicrobial agents against specific isolates, a laboratory usually has one type of panel for gram-positive bacteria and another for gram-negative bacteria. For testing urine isolates some laboratories may have a different type of panel that contains drugs appropriate for treating lower urinary tract infections. Panels containing special media are required for testing fastidious bacteria.

Mueller-Hinton broth is recommended as the medium of choice for susceptibility testing of commonly isolated, rapidly growing aerobic, or facultative organisms. The broth must have the appropriate divalent cation content provided by the manufacturer (Ca++ and Mg++). Each batch must be tested with a pH meter after the medium is prepared. The pH should be between 7.2 and 7.4 at room temperature (25 ℃). For fastidious organisms Mueller-Hinton broth may be supplemented with 2–5% lysed horse blood.

The performance of each batch of broth is evaluated by using a standard set of quality control organisms. If a new lot of broth does not yield the expected results, the cation content of the broth as well as each step of the test should be investigated. Meanwhile a different lot should be evaluated.

PCR Test

PCR-based techniques (both conventional and real time) rely on the sequence-specific amplification of nucleic acids. For this reason, PCR was initially used in microbiological testing for the rapid identification and quantification of causative agents of infections through the amplification of sequences specific to a particular pathogen. With increased knowledge of the genetic basis of antibiotic resistance, PCR-based approaches have been developed for detecting the presence of genetic determinants of resistance to a variety of antibiotics for a number of different bacterial species.[8]

Among the most popular molecular techniques to detect bacteria are those based on PCR amplification of the 16S or other ribosomal DNA sequences. In contrast to endpoint PCR methods that essentially provide qualitative data, quantitative real-time PCR (qPCR)

detects both the specific gene targets in bacteria and allows quantification of bacteria in samples.

In addition to using PCR for detecting the presence of genetic determinants of resistance, the ability of real-time PCR to accurately quantify the number of copies of a specific nucleic acid in a sample has led to the development of approaches that employ this method for measuring bacterial growth. This approach monitors the number of bacterial genome copies present during growth of the isolated bacteria in the presence of the antibiotic being tested. Since quantitative real-time PCR can provide precise information regarding genome copy numbers, very short incubation times can be used for differentiating susceptible from resistant strains.[8]

Recent investigations have employed molecular methods, based in particular on 16S rRNA-based endpoint polymerase chain reaction (PCR). Using PCR-based methods, *Enterococcus faecalis* was detected in 77% of samples from 22 failed endodontically treated teeth undergoing retreatment, and in 67% of 30 cases of persistent endodontic infections associated with root-filled teeth in Brazil and in 22% of samples from 37 retreatment cases with nonhealing periradicular lesions in the United States.[9]

The PCR reaction was performed in a final volume of 20 µl and loaded in an optical 96-well plate, which was then covered with an optical adhesive sheet. The primers used amplified enterococcal DNA sequences in the tuf gene. The PCR conditions were as follows: The initial denaturation was at 94°C for 15 seconds, annealing at a temperature of 55°C and extension at 72°C for 45 seconds. The final extension was at 72 for 5 minutes and then cooled to 4°C until removed. All PCR experiments had positive and negative controls. The qPCR assay was carried out in a thermal cycler (7900 HT Real-time PCR system). The reaction mix contained 16Sr DNA primers, sterile water, template and SYBR Green master mix.[10]

The major advantage of these PCR-based approaches is that they can be carried out in a relatively short period of time, in some cases using clinical samples without the need for purity culture. PCR thus clearly has the potential to significantly reduce turnaround times and rapidly provide information on antibiotic resistance.[8] However the major limitation of this approach, is that the presence of resistance genes may not always correlate with phenotypic resistance.

CONCLUSION

There are options available for antimicrobial susceptibility test on chemical substance to be used in root canal treatment. Classic method such as agar diffusion and MIC microdilution still useful for screening any substance resource. One of the important thing need to be addressed on advance scale on using that classic method is how to create similar environment just like in vivo had. On the other hand, real time PCR have a promising methods not only for in vitro, but also for in vivo on clinical situation, the presence of persistent root canal infection detected by culture test before obturation is one of the most significant biological factors influencing the outcome of root canal treatment.[11] Advances in

DNA sequencing technology have made it possible to sequence entire bacterial genomes extremely rapidly. These methods, coupled with bioinformatic tools that can quickly assemble and analyse the massive amount of data obtained from these sequencing runs, open the possibility of using these techniques for detecting antibiotic resistance as a standardized in vitro tests for antimicrobial activity of endodontic.

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