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Research article

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An Original Invitro Study for Evaluating Antimicrobial Efficacy of Ampicillin, Vancomycin, Ciprofloxacin, Gentamicin, Metronidazole and Calcium Hydroxide Against Enterococus Faecalis

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Abstract

Aim and objectives: *Aim of this study is to evaluate the antimicrobial effectiveness of Ampicillin, vancomycin, ciprofloxacin, Gentamicin, metronidazole and calcium hydroxide against enterococus faecalis.*

Materials and methods: *Ampicillin, vancomycin, ciprofloxacin, Gentamicin, metronidazole & calcium hydroxide were used to assess the antimicrobial efficiency against Enterococcus faecalis using the agar well diffusion test. Agar plates were incubated at 370 ˚C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm.*

Results: *Higher mean zone of inhibition was recorded in ciprofloxacin and calcium hydroxide followed by Ampicillin and vancomycin respectively. The lowest mean zone of inhibition was found in vancomycin.*

Conclusion: *From the present study, it can be concluded that ciprofloxacin shows comparable zones of inhibition with that of calcium hydroxide.*

Keywords: E. faecalis, Antibiotics, Calcium hydroxide.

Introduction

An important and fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent reinfection because micro organisms or their products are considered to be the primary etiologic factors of periapical lesions and root canal filling failure [1]. Root canal infections have a polymicrobial nature hence, anaerobic and facultative anaerobic bacteria's are usually found together in endodontic flare-ups and cases with post-treatment disease.

Elimination of microorganisms and complete removal of pulp tissue from the root canal system is of dominant magnitude during endodontic therapy. The root canal success mainly depends on mechanical preparation, irrigation, microbial control, and complete filling of the root canal system. Microorganisms, bacteria, and their products are considered as the etiological agents of pulp necrosis and periradicular lesions [2,3]. They may survive during endodontic procedures due to anatomical structural complexities and limitations of access by instrumentation and irrigants. To ensure complete elimination of root canal bacteria, effective antimicrobial agents are required for a predetermined time period for predictable eradication of remaining bacteria [4].

Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens, and intracanal medications [5]. since the chemomechanical preparation of the root canal reduces endodontic infection, but microorganisms are able to survive within

the complex anatomy of the root canal system. So, the antimicrobial intracanal medicaments are used to complement the disinfection of the root canal system.

In vitro studies have shown the ability of E. faecalis biofilm to calcify as it undergoes "maturation" within the root canal. So far, most studies on E. faecalis biofilm characteristics have been performed on monoculture biofilm. Although E. faecalis has sometimes been isolated as the sole infectious organism in root canals, the notion that endodontic infections are typically polymicrobial has become more and more favorable. E. faecalis was reported to coexist with several other taxa in root canal treated teeth. This study was thus conducted to evaluate and compare the antimicrobial effect of various medicaments against E. faecalis.

Material and methods

Agar-diffusion test: The bacterial stock culture Enterococcus faecalis was obtained and culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller - Hinton agar plates. Inoculation was performed by utilizing sterile swab brushed over the media. One round well, 4 mm deep and 8 mm diameter was punched in every agar plate utilizing sterile plug-borer and the prepared medicaments were added to the wells. Agar plates were incubated at 370 C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the closest size in mm.

The teeth were divided into six groups according to the solutions used for intracanal medicament. Ampicillin, vancomycin, ciprofloaxacin, Gentamicin, Metronidazole and calcium hydroxide.

Ampicillin- 500mg ampicillin sodium (Pamecil Medochemie Ltd, Limassol, Cyprus) pH = 8.5.

Vancomycin- 500mg vancomycin hydrochloride lyophilized powder (Vancomycin DBL, MaynePharma Pty, Ltd, Mulgrave VIC, Australia) pH = 5.5.

Ciprofloaxacin- 500mg ciprofloaxacin (Pamecil Medochemie Ltd, Limassol, Cyprus) pH = 12.5.

Gentamicin- 400mg gentamicin sulfate (Garasent, duopharma (M) sdn Bhd, Selangor Darul Ehsan, malaysia) pH = 5.5.

Metronidazole- 400mg Metronidazole (J.B Chemicals & Pharmaceuticals).

Calcium hydroxide - calcium hydroxide powder (calcium hydroxide p.a. merck, Darmstadt, germany) saturated solution, pH = 12.3.

Results

All medicaments showed well-defined zones of inhibition around their respective wells except the control well **(Figure 1 and 2)**.

Statistical analysis by one-way ANOVA showed significant difference between zone diameters of Ampicillin, vancomycin & ciprofloxacin against E. faecalis ($p < 0.05$). Maximum antimicrobial activity was shown by ciprofloxacin, followed by Metronidazole. Ciprofloxacin showed comparable zones of inhibition to Calcium hydroxide.

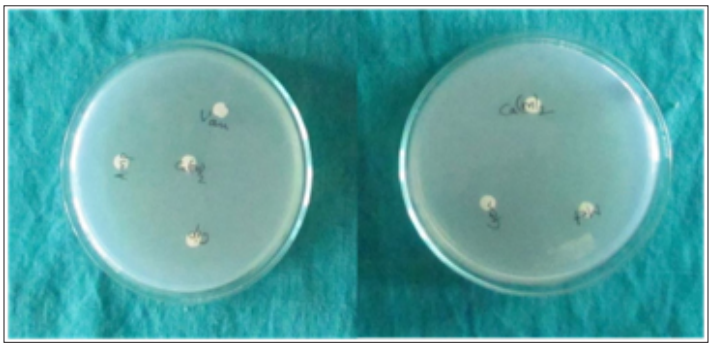

Figure 1: Ampicillin, vancomycin, ciprofloxacin, Gentamicin, Metronidazole and calcium hydroxide are added to the 4mm deep and 8mm diameter agar plate.

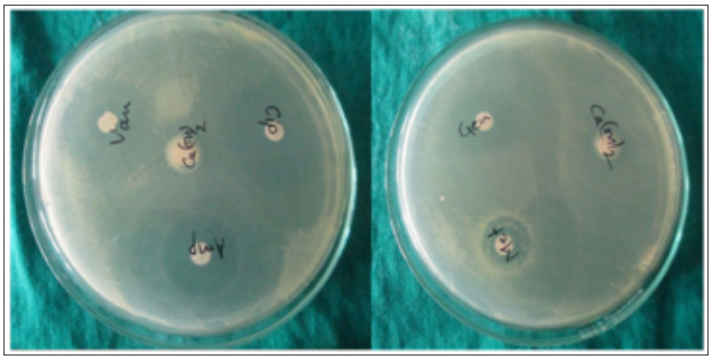

Figure 2: Zones of inhibition of Ampicillin, vancomycin, ciprofloxacin (highest), Gentamicin, Metronidazole (highest) and calcium hydroxide.

Discussion

The role of microorganisms in the development and maintenance of pulpal and periapical inflammation have been well documented. The success of root canal treatment largely depends on the elimination of microbial contamination from the root canal system. Although mechanical instrumentation of root canals can reduce bacterial population, effective elimination of bacteria cannot be achieved without the use of antimicrobial root canal irrigation and medication. This review will discuss the antimicrobial effects of the known root canal irrigants and medicaments and explore future developments in the field [6].

To increase the efficiency of instrumentation, root canal irrigating solutions and intracanal medicaments are used to eliminate the bacteria from the root canals. Antibiotics are used in dentistry both systemically and topically. During systemic administration of antibiotics, negligible concentrations reach the root canal, whereas during the local administration of antibiotics, greater concentrations can be used as intracanal medicaments, to decrease systemic consequences and complications. Because of the complexity of root canal infection, single irrigant or a medicament or an antibiotic could not result in effective sterilization of the root canal [7,8]. Combination of irrigants or medicaments decreases the development of resistant bacterial strains and produces synergistic effect, whose antimicrobial action lasts longer and also sustains release of medicaments occurs.

E. faecalis, facultative gram-positive anaerobic bacteria and most commonly isolated from retreatment cases. It is well recognized

as a pathogen associated with persistent apical periodontitis in endodontically treated teeth, easy to culture and has been used successfully in several studies on the efficacy of endodontic irrigants [9,10]. Various studies reported the use of paper points for collecting microbiological samples from the root canals. Paper point cultures of the root canal detected bacteria more frequently than dentin filling cultures on the reamers [11]. This study showed that both antibiotics were found to be more effective than the Calcium hydroxide in the infected tooth section models. This finding was in accordance with an in-vitro study which revealed that the amoxicillin, clavulanate, ciprofloxacin, clindamycin and doxycycline were significantly more effective than Calcium hydroxide in against the E. faecalis biofilm in root canal system.

It is known that the antibacterial activity of Calcium hydroxide depends on its high pH. The alkalinity of this agent destroys bacterial cell membranes and protein structures. However, the initial high pH of Calcium hydroxide at 12.3 would reduce to a pH of 10.3 when it was placed into the root canals. This pH reduction is due to the buffering effect of radicular dentine. It has been known that the E. faecalis can survive at a pH as high as 11.5; hence, with the lower pH value of Calcium hydrooxide, the E. faecalis in the dentinal tubules could not be removed effectively.

Conclusion

From the present study, it can be concluded that ciprofloxacin and Metronidazole has a significant antimicrobial effect against E. Faecalis. Preclinical and clinical trials are needed to evaluate biocompatibility before can conclusively be recommended as a medicament, but in vitro observation of ciprofloxacin & Metronidazole effectiveness appears promising.

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