



## Comparison of New and Conventional Root Canal Irrigants and their Efficacy on Treatment Outcomes in Endodontics

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### Introduction

Mechanical instrumentation is the core method for bacterial reduction in the infected root canal, but achieving bacteria-free root canals still proves to be difficult.<sup>2</sup> Various studies have demonstrated that mechanical preparation with hand instruments and irrigation with saline cannot predictably eliminate the bacteria from the infected root canals.<sup>3-5</sup> Therefore, the current focus of interest has been the use of irrigating solutions with strong antibacterial activity as the necessary supplement to mechanical preparation.

Even after meticulous mechanical procedures along with the use of irrigating solutions, some bacteria can still be recovered from the canals. The genera that most frequently persist include *Enterococci*, *Staphylococci* and Gram-negative enteric rods. *Enterococcus faecalis*, a Gram-positive facultative anaerobe, is commonly found in the root canals of failing endodontically treated cases.<sup>7</sup>

The persistence of these microorganisms in the root canals, even after the use of irrigating solutions suggests that the choice of the solution used is largely determined still on empirical data. There is a strong relationship between the long-term treatment success and root canal filled after

negative culture in teeth with apical periodontitis. Studies reveal complete periapical healing in 94% of teeth with apical periodontitis that yielded negative culture after root canal instrumentation. In contrast, the samples with positive culture before root canal obturation had only 68% of complete periapical healing<sup>(3)</sup>.

In infected and necrotic root canal systems, bacteria grow mostly in sessile biofilms, aggregates, and coaggregates in which they are embedded in an extracellular matrix material<sup>(4)</sup>. Factors that contribute to a persistent periradicular infection after root canal treatment include Most persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the aetiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of flora<sup>(8)</sup>. *Enterococcus faecalis* are gram positive cocci that occur singly, in pairs or in short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. *Enterococcus faecalis* is associated with different forms of pulpal and periradicular diseases including primary and

persistent endodontic infections. It possesses certain Virulence factors such as lytic enzymes, aggregation substance, cytolysin, and lipotechoic acid. It has also been shown to survive and persist as a pathogen in the root canals of teeth<sup>(9)</sup>.

Sundquist et al demonstrated the presence of *E faecalis* in the root canal system of 58% of teeth with endodontic treatment failure. Furthermore they showed that only 33% of the teeth which harboured *E faecalis* when the canals were being refilled demonstrated endodontic success to a greater extent<sup>(10)</sup>

It is unrealistic to assume that a linear streamlined instrument can reach out into the delicate intricacies of the pulp spaces to effectively debride its contents and the huge load of microorganisms along with their toxins. The webs, fins and anastomoses can be cleaned through the effective use of an irrigant. Hence, thorough flushing with intracanal irrigants goes hand in hand with mechanical cleaning of the root canal which is often referred jointly as “chemomechanical preparation”<sup>(11)</sup> of the root canal system.

The importance of root canal irrigation has widely been reported in endodontic literature. In a study conducted to evaluate the effect of mechanical shaping of root canals in endodontic treatment without the use of irrigants, it was concluded that, even after 5 visits, only 50% reduction in bacterial Count could be possible. This clearly explains the insufficiency of mechanical instrumentation alone in elimination of endodontic pathogens<sup>(12)</sup>

Various concentrations of sodium hypochlorite (NaOCl) have been used as root canal irrigant for many decades. The main advantage of NaOCl is its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms<sup>(13)</sup>. Combination of EDTA with NaOCl has also shown to be more effective at killing bacteria. Over the years, various root canal irrigants have been used like sodium hypochlorite (NaOCl), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), chlorhexidine. Out of these, sodium hypochlorite (NaOCl) is the most widely used irrigating solution.

Sodium hypochlorite has a disadvantage of reaction with periapical tissues if extruded beyond the apex, to overcome these problems it lead to development of chlorhexidine gluconate that has been recognized as an effective antimicrobial agent. It is a cationic chlorophenyl bisguanide with bacteriostatic and bactericidal action. Chlorhexidine possess many properties i.e. a broad spectrum antimicrobial agent, substantivity and relatively absence of toxicity, making it a potential endodontic irrigant<sup>(14)</sup>. The various concentrations of Chlorhexidine available are 0.12%, 0.2% and 2% of which 2% Chlorhexidine has shown to have maximum bactericidal effect, but couldn't eliminate the bacteria completely, which lead to development of new irrigants like MTAD, Nisin and Daptomycin.

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a colourless liquid and has been used in dentistry in concentrations varying from 1% to 30%. H<sub>2</sub>O<sub>2</sub> degrades to form water and oxygen. It is active against viruses, bacteria, bacterial spores and yeasts<sup>(16)</sup> via the production of hydroxyl free radicals which attack proteins and DNA.<sup>(7)</sup> It has been shown that NaOCl, combined with H<sub>2</sub>O<sub>2</sub>, is no more effective against *E. faecalis* than NaOCl alone;<sup>(8)</sup> MTAD, a common intracanal irrigant, consists of 3% doxycycline, 4.5% citric acid, and 0.5% polysorbate 80 detergent and is used to remove pathogenic bacteria and smear layers during root canal procedures. MTAD has many advantages in root canal irrigation, but its bactericidal activity remains to be improved upon as its antibacterial effect has been largely attributed to doxycycline, a tetracycline that is bacteriostatic rather than bactericidal.<sup>(15)</sup>

Nisin which is a naturally occurring antimicrobial peptide (discovered in 1928) is found to be effective against the *E. faecalis*. Nisin is produced by *Lactococcus lactis* and which is a class I bacteriocin. It is used as food preservative and found to be safe in human beings<sup>(16)</sup>. Daptomycin is a novel lipopeptide antibiotic with bactericidal activity against a wide range of clinically

important Gram-positive bacteria, including vancomycin-resistant *enterococci* (VRE).

Thus, in the present study the relative antimicrobial efficacy of three different commonly used antimicrobial agents ie., hypo, hydrogen peroxide, chlorhexidine and three new antimicrobial irrigants ie., MTAD, MTAD in combination with nisin and MTAD in combination with Daptomycin were compared with regard to reduction in the number of *Enterococcus faecalis*.

### Aims and Objectives

The aim of this study is to evaluate the antimicrobial efficacy of Bio pure MTAD in combination with Nisin and Daptomycin against hypo, hydrogen peroxide, chlorhexidine which is calculated by Zone of inhibition, Time killing study and Optical density using spectrophotometer.

### Objective

- Irrigants that shows significant decrease in *Enterococcus faecalis* growth
- To check for the efficacy of MTAD in combination with Nisin and Daptomycin against hypo, hydrogen peroxide and chlorhexidine.
- To check which irrigant shows a reduction in bacterial count after the application to irrigants to inoculated solutions.

### Materials and Methodology

This study was conducted in the Department of Conservative dentistry and Endodontics, Panineeya Institute of Dental sciences and Hospital and in the Department of Microbiology, NRI Medical College, Vijayawada during 2012-2015.

### Methodology

#### Zone Inhibition Method

#### Preparation of blood agar medium plates

- The medium is prepared by adding sterile blood to sterile nutrient agar that has melted and cooled to 50°C.
- 10% concentration of sheep blood is used
- A thin layer of melted nutrient agar with blood about 14ml for a 9cm petri dish is poured and allowed to set. Sterility check is done by incubating 2 plates at 35°C overnight.
- Quality check is done by using ATCC 25923 *Staphylococcus aureus*.

#### Inoculum preparation of *Enterococcus faecalis*:

- Inoculum is prepared using ATCC 29212 *Enterococcus faecalis*
- Inoculum of Mc Farland 0.5 turbidity is prepared using nutrient broth

#### Preparation of discs containing chemical irrigants MTAD, 3% MTAD with 3% Nisin and 3% MTAD with 3% Daptomycin, sodium hypochlorite, hydrogen peroxide and chlorhexidine

- 3% of each irrigant solution w/ v were prepared by mixing 3.0 g of each material in 100ml of water and combination irrigants were prepared by mixing them in equal proportions.
- Whatman no 1 filter paper is used
- 6mm diameter is cut and dipped in working solution of chemical irrigants of MTAD, MTAD with Nisin and MTAD with Daptomycin using sterile forceps.

#### Testing of chemical irrigant

- On a blood agar medium plates, ATCC 29212 *Enterococcus faecalis* is streaked using inoculating wire loops in a Biosafety cabinet level 2.
- The inoculum is streaked in four quadrants of 2 plates
- The discs containing chemical irrigants of MTAD, MTAD with Nisin and MTAD with Daptomycin, sodium hypochlorite, hydrogen peroxide and chlorhexidine to be

tested are placed on the streak line using sterile forceps

- One quadrant of inoculum is used for quality check
- The plates are incubated at 35<sup>0</sup>c, overnight in an incubator
- Inhibition of growth is checked after incubation period

### Optical Density and Time Kill Study

Optical density, measured in a spectrophotometer, can be used as a measure of the concentration of bacteria in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacterial or other material is present.

Time kill curves are procedure to check the amount of bacteria killed at different intervals of time.

Eight 10ml test tubes are taken and one each labelled as

- Saline
- *Enterococcus faecalis*
- Saline
- MTAD
- 3% MTAD with 3% Nisin
- 3% MTAD with 3% Daptomycin
- Sodium Hypochlorite
- Hydrogen peroxide
- Chlorhexidine
- The first test tube contains saline
- Second test tube contains only saline inoculated with *Enterococcus faecalis*
- Third test tube contains 3% MTAD irrigant solution which is inoculated with *Enterococcus faecalis*
- Fourth test tube contains 3% MTADN solution which Is inoculated with *Enterococcus faecalis*
- Fifth test tube contains 3% MTADd which is inoculated with *Enterococcus faecalis*
- Sixth test tube contains hypo solution which is inoculated with *Enterococcus faecalis*

- Seventh test tube contains hydrogen peroxide which is inoculated with *Enterococcus faecalis*
- Eighth test tube contains chlorhexidine which is inoculated with *Enterococcus faecalis*

All the test tubes inoculated with *enterococcus faecalis* were checked for reduction in the bacterial count at 30mins, 60mins,90mins intervals under spectrophotometer at 540nm

The results were charted according to the percentage of reduction in bacteria

### Results

**Table (No 1)** – Zones of inhibition by MTAD, MTAD+ Nisin , MTAD + Daptomycin and Control

CHEMICAL IRRIGANT	ZONE OF INHIBITION
Control	6mm
MTAD	10mm
MTAD + Nisin	20mm
MTAD + Daptomycin	12mm
Hypo solution	10mm
Hydrogen Peroxide	10mm
Chlorhexidine	11mm

The results of this in vitro study showed that the combination of MTAD with Nisin showed a relative larger zone of inhibition for the growth of *Enterococcus Faecalis* ,when compared to the zones by MTAD alone MTAD in combination with Daptomycin and other irrigants.

The Zones of inhibition produced by MTAD alone and MTAD in combination with Daptomycin were not significantly different from each other.

The control did not show any inhibition of *E.faecalis* growth.

**Table (No 2):** Optical Density And Time Kill Study

GROUPS	0 HRS	30 MINS	60 MINS	90 MINS
SALINE	100%	100%	100%	100%
SALINE+INOCULUM	76%	76%	76%	76%
MTAD	81.2%	87%	89.3%	90.5%
MTAD+ NISIN	88.6%	98%	98.30%	98.90%
MTAD+ DAP	84.2%	88.20%	91%	91.20%
HYPO	81.2%	87%	89.3%	90.5%
HYDROGEN PEROXIDE	80%	84%	86%	90%
CHLORHEXIDINE	85%	88%	91%	95%

## Discussion

It is well established that pulpal and periapical diseases as well as failure of endodontic therapy are due to the presence of microbes in the root canal system. Eliminating microbes from the infected root canals and prevention of re-infection is one of the fundamental aims of endodontic therapy<sup>(48)</sup>. Although the root canal flora is dominated by obligate anaerobic bacteria, some facultative strains like *Enterococci*, *Staphylococci* and fungi such as *candida* species etc. have been involved in persistent infections, influencing the prognosis of the root canal therapy<sup>(49)</sup>.

Of the *Enterococcus* species, *E. faecalis* the most frequently isolated or detected species from oral infections, including marginal periodontitis, infected root canals, and periradicular abscesses<sup>(9)</sup>. *Enterococcus faecalis* is an unrelenting organism that, despite making up a small fraction of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after orthograde endodontic therapy. It has been hailed as the “star survivor” and is commonly found in a lofty percentage of root canal failures<sup>(50)</sup>. It thrives in treated root canals as a single organism or as a major portion of flora<sup>(51)</sup>.

*E. faecalis* is a Gram-positive, Catalase negative, fermentative, non-sporing facultative, coccus<sup>(50)</sup>. Its cells are ovoid, ranging from 0.5 to 1u m in diameter. These organisms occur singly, in pairs, or in short chains. Most strains are non hemolytic and non-motile. Surface colonies on blood agar are creamy white, circular, smooth, and entire<sup>(52)</sup>. It has the ability to survive in various adverse circumstances. These comprise the capability to survive in hyper-osmotic conditions, at

temperatures ranging from 10 c to60 c and at a pH of over 9.6. Growth on bile-esculin is a useful trait to identify Enterococci.

Studies have shown that these bacteria attain an increased resistance or phenotypic tolerance to numerous disinfectants or physical agents. *Enterococci* possess a number of virulence factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immunomodulation and cause toxin-mediated damage. These factors include: 1.aggregation 2.enterococcal surface proteins 3. Gelatinase 4.Cytolysintoxin 5.Extracellular superoxide production, 6.capsular polysaccharides and 7.antibiotic resistance determinant<sup>(48)</sup>.

The survival of *E. faecalis* within infected root canals is aided by dentinal fluid. Dentinal fluid also aids in their invasion into dentinal tubules and helps these microbes to endure long-term starvation. Therefore, even in a well-debrided and obturated root canal, *E. faecalis* may grow by utilizing local sources of energy and nutrients.

An assortment of antimicrobial solutions have been tested for their ability to eradicate *E. faecalis* from the intricacies of the root canal system. These encompass both inter appointment dressings, such as calcium hydroxide, camphorated paramonochlorophenol etc, as well as irrigants such as NaOCl, Chlorhexidine gluconate, chlorhexidine acetate and iodine compounds and newer irrigants like MTAD, MTAD with Nisin and MTAD with Daptomycin. Among these *E. faecalis* appears to be extremely resistant to the medicaments used during treatment and is known to resist the antibacterial effects of calcium hydroxide which is generally a

highly effective antimicrobial agent. This is because *E. faecalis* can endure a high alkalinity up to around pH 11.5. Certain mechanisms have been postulated which enable *E. faecalis* to survive the high pH of calcium hydroxide. These include a stress induced protein synthesis and the presence of a potassium/proton antiport system in maintaining cytoplasmic pH in an alkaline environment. The natural buffering effect of dentine, affords further protection since pH levels in dentine do not reach higher than 9.7-10.8. Thus, the highly complex nature of this organism poses a great challenge to endodontists.

Whilst the ability of *E. faecalis* to resist the antimicrobial effect of calcium hydroxide remains a significant clinical challenge in endodontic retreatment, other treatment regimens aimed at eradicating *E. faecalis* during each of the phases of endodontic therapy are being continuously evaluated.

Among various treatment steps involved with infection control, the chemo mechanical preparation assumes a pivotal role in root canal disinfection. In addition to the mechanical effects exerted by instruments and the flow and backflow of the irrigant solution during preparation, the use of an antimicrobial substance for irrigation has been shown to be necessary to enhance bacterial elimination.

A large number of substances have been used as root canal irrigants, including acids (citric and phosphoric), chelating agents (EDTA), proteolytic enzymes, detergents (cetrimide), alkaline solutions (sodium hypochlorite, urea), oxidative agents (hydrogen peroxide and Gly-oxide), MTAD, peptides like Nisin and Daptomycin

An ideal root canal irrigant should a) Have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms b) Dissolve necrotic pulpal tissue remnants c) Inactivate endotoxins d) Prevent the formation of a smear layer during instrumentation or dissolve the latter once it has formed. Furthermore, as endodontic irrigants come in contact with vital tissues, they should be

systemically nontoxic, noncaustic to periodontal tissues and have a little potential to cause an anaphylactic reaction

One of the most routinely used root canal irrigant is sodium hypochlorite, because of its potent advantages. However the use of sodium hypochlorite has various inherent disadvantages, principally due to its toxicity- it injures all living tissues except keratinized epithelia. At very low concentrations, contact with vital tissues induce an inflammatory reaction. Accidental extrusion beyond the apex causes excruciating pain, immediate swelling, and profuse bleeding and also pharyngeal edema and oesophageal burns when swallowed unintentionally. Due to these biocompatibility issues other irrigants have come into the endodontic scenario such as MTAD, Nisin and Daptomycin

Bio Pure (DENTSPLY, Tulsa Dental, Tulsa, Ok, USA) otherwise known as MTAD was introduced by Torabinejad and Johnson in 2003. This solution contains Doxycycline hyclate (at a concentration of 3%), citric acid (concentration 4.25%) and a detergent, Polysorbate 80 (concentration 0.5%, also known as tween 80). Several studies have evaluated the effectiveness of MTAD for disinfection of root canals. Torabinejad et al have shown MTAD is able to remove the smear layer and is effective against *E. faecalis*.

Doxycycline, an important component of MTAD belongs to the tetracycline family of antibiotics. Tetracyclines have been used to remove the smear layer from instrumented root canal walls, for irrigation of retrograde cavities during periapical surgical procedures, and as intracanal medicaments. Tetracyclines readily attach to the dentine and are subsequently released without losing their antibacterial activity. This property creates a reservoir of active antibacterial agent, which is then released from the dentine surface in a slow and sustained manner. Thus, the presence of doxycycline may be accountable for substantivity of MTAD. Doxycycline has also been shown to possess anticollagenase activity.

Root canal instrumentation produces a layer of organic and inorganic material called the smear layer that may also contain bacteria and their by-products. Removal of the smear layer remains controversial, however it can prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling material to canal walls. Thus demineralizing agents such as ethylenedimine tetraacetic acid (EDTA) and citric acid have been recommended as adjuvants in root canal therapy. Loel 1975 and Tidmarsh 1978 demonstrated the effectiveness of citric acid as a root canal irrigant. It has been shown to be more effective than NaOCl Layer (Baumgartner et al 1984). Although citric acid appears to be slightly more potent at similar concentration than EDTA, both agents show high efficiency in removing the smear layer. In addition to their cleaning ability, chelators such as EDTA and citric acid may detach biofilms adhering to root canal walls. Thus, the presence of citric acid in MTAD accounts for removal of the smear layer.

Nisin, an antimicrobial peptide produced by *Lactococcus lactis*, has been extensively used as a preservative in dairy products. The peptide is composed of 34 amino acid residues. Nisin inhibits the proliferation of most gram-positive bacteria and is heat-stable, odourless, colourless, tasteless and active at low pH. Nisin also has a strong bactericidal effect and microorganisms generate little resistance to Nisin. Furthermore Nisin has low toxicity. These advantages of Nisin suggest it has potential as an intracanal irrigant. Nisin attaches to the plasma membrane through a specific docking molecule, creating a preforming unit comprised of several Nisin molecules. These interactions lead to the formation of transient pores in the cell membrane, which dissipate the cell's ion gradients and cause the leakage of cellular components, ultimately leading to cell death.

Daptomycin is a novel lipopeptide antibiotic with bactericidal activity against a wide range of clinically important Gram-positive bacteria,

including vancomycin-resistant enterococci (VRE). Daptomycin is believed to act by  $\text{Ca}^{2+}$ -dependent insertion of its acyl tail into the Gram-positive cell membrane, which is followed by the development of potassium efflux channels, depolarization of the membrane and cell death. For synergy screening, Daptomycin was tested with the following antibiotics by Etest: ampicillin, oxacillin, piperacillin, ceftriaxone, cefepime, imipenem, tetracycline, chloramphenicol, clindamycin, linezolid, synercid, and rifampicin.

This study was undertaken to evaluate the antimicrobial activity of MTAD and MTAD with Nisin and Daptomycin, hypo, hydrogen peroxide and chlorhexidine.

Different approaches have been used to test the effectiveness of antimicrobial agents in the laboratory. These include: incubation of broth cultures of selected bacteria with the antimicrobial agent (O'Hara et al 1993, D'Arcangelo et al. 1999), growth of selected bacteria as 'lawns' on agar surfaces and use of the disc diffusion method (Siqueira et al. 1998). Use of calorimeter or spectrophotometer for checking of optical density seen with reduction of bacterial count.

In the present study the antimicrobial efficacy of different irrigants was evaluated by incubation of broth cultures of selected bacteria with the antimicrobial agent using zone inhibition method and optical density seen with reduction of bacterial count. According to present study results MTAD with combination of Nisin has shown greater zone of inhibition of 12mm.

(*Zhongchun tong*, Lin zou et al 2011) reported there was no significant difference between the viable bacterial counts in root canals with and without MTAD applied as a final rinse after root canal preparation, indicating that *E. faecalis* was strongly resistant to MTAD.

It can be explained by saying; It is difficult to completely eradicate *E. faecalis* from root canals with MTAD, in part because doxycycline, the primary antibacterial agent in MTAD, is bacteriostatic rather than bactericidal. For endodontic treatments, a bactericidal agent is

needed to achieve a greater killing effect. Nisin, an antibacterial peptide, has strong bactericidal activity and is associated with little drug resistance, although there are no reports on its ability to eradicate the smear layer. In contrast, MTAD can effectively remove the smear layer. A combination of Nisin and MTAD could compensate for the weaknesses of each drug and kill and eradicate *E. faecalis* from the root canal. Furthermore, MTAD provides an acid environment that is favourable for Nisin because Nisin has higher antibacterial activity and stability at low pH.

In MTAD, polysorbate 80 is a detergent rather than an antibacterial agent. Citric acid has weak antibacterial activity at a concentration of 4.25%, and only when the concentration is greater than 10% can citric acid exert effects on *E. faecalis*. Therefore, the greater antibacterial activity of MTADN can be attributed mainly to the synergistic action of Doxycycline and Nisin. Doxycycline is a semisynthetic tetracycline antibiotic that exerts its bacteriostatic action by penetrating the bacterial cell. The antibiotic can bind to the 30S ribosomal mRNA complex at the donor site to block aminoacyl-tRNA in microorganisms and inhibit protein synthesis at the ribosomal level. Nisin exerts its antibacterial activity through pore formation on the cell membrane and disruption of cell wall synthesis, resulting in the rapid efflux of small required cytoplasmic compounds.

Nisin, a cationic peptide, can stably bind to cell membranes by adhering to anionic lipid II, which is a principal component of the membranes of gram-positive bacteria. By using lipid II as a “docking molecule” to form pores on the surface of cell membranes in a targeted manner, nanomolar concentrations of Nisin are able to effectively kill bacteria. When doxycycline is applied in combination with Nisin, the pores formed by Nisin on the surface of the cell membrane can facilitate the penetration of doxycycline molecules into the microorganism, and thus more intracellular protein synthesis can

be inhibited by the doxycycline molecules. SEM revealed that MTADN caused the most serious damage to *E. faecalis*, indicating that it is difficult for *E. faecalis* to resist this double attack.

(Zhongchun Tong, Rong et al. 2012) conducted a similar study to check for synergistic effects of MTAD with Nisin. He concluded as MTAD in combination with Nisin improved antibacterial efficacy against pathogens, especially for some gram-positive bacteria associated with persistent intracanal infection. Therefore, the combination had the potential to be used as an effective intracanal irrigation.

(Zhongchun Tong, Junqi Ling et al 2014) reported a study where MTAD was used in combination with Nisin at sub-inhibitory concentration against *E. faecalis* growth and expression of pathogenic genes. The study reported similar results where MTAD with Nisin combination showed complete elimination of *E. faecalis*. *E. faecalis* showed the least resistance to alkaline environments after treatment with MTADN. This result indicated that Nisin, in combination with doxycycline, could help calcium hydroxide intracanal dressing to better inhibit the pathogenic bacteria *E. faecalis*. Calcium hydroxide dissociates into calcium and hydroxyl ions on contact with aqueous fluids, and hydroxyl ions cause the lethal effect on bacterial cell by damage to the bacterial cytoplasmic membrane, denaturing of proteins, and damage to DNA. In MTADN and MTAN, the antibacterial peptide Nisin exerts its antibacterial activity by forming pores in cell membranes, thus disrupting cell wall synthesis and causing a rapid efflux of essential cytoplasmic small molecules. The pores made by Nisin facilitate the penetration of hydroxyl ions into bacteria, resulting in rapid death.

In the present study optical density of the various irrigants were compared with Saline and Saline inoculated with *E. faecalis*, which was recorded using spectrophotometer. Saline and Saline inoculated with *E. faecalis* were used as control groups whose optical density values remained constant. Saline OD value was 100% due to

complete absence of bacteria and Saline inoculated with *E. faecalis* was recorded to be 76% due to the presence of bacteria, which was taken as a comparative reading after being added by three different irrigants used in the present study.

According to the results of the present study the optical density value of MTAD with Nisin was greater when compared to MTAD with Daptomycin and MTAD alone, which is better explained by (Zhongchun Tong, Junqi Ling et al 2014) the synergistic action of doxycycline and Nisin. In the present study the OD value of MTAD with Daptomycin was superior than MTAD alone. No study used MTAD in combination with Daptomycin for killing *E. faecalis*. The zone of inhibition was recorded at 12mm for MTADd and 10mm for MTAD. A plausible explanation for the synergy between Daptomycin and MTAD would be that, Daptomycin binds and opens channels that alone are insufficient to produce killing but can allow specific entry of Doxycycline to carry out the further lysis of the cell.

(Kenneth H, Herbert Houck et al 2004) conducted a study where synergy between Daptomycin and 18 other antibiotics against 19 strains of high-level vancomycin-resistant enterococci was tested, which included tetracycline groups. He concluded there was no significant synergy between Daptomycin and any other antibiotic by this screening method. If confirmed by further studies, Daptomycin with either rifampicin or ampicillin may be useful in the management of infections caused by VRE.

Time-killing curves were used to evaluate the antibacterial activities of MTAD, MTAD with Nisin and Daptomycin, where the solutions were tested of OD at different time intervals to check for the reduction of bacterial count, by using saline and saline with inoculum as control group.

According to the results in the present study Doxycycline and Nisin have showed the superior antimicrobial activity at 30 mins of 98%, followed by MTADd of 88.2% and least being MTAD of

87%. Which showed a significant difference between the three groups. The OD value of MTAD with Nisin was greater with increase of time where it could eliminate complete bacteria of 98.9% at 90mins, when compared to MTADd and MTAD which was 91.2% and 90.5. MTAD alone showed a least killing of *E. faecalis* at 30mins and 90mins interval.

(Bradley M. Newberry, Shahrokh Shabahang. et al 2007) conducted a study to determine the Antimicrobial effect of MTAD as a final irrigant on eight strains of *Enterococcus faecalis* (*E. faecalis*) and to measure the minimum inhibitory concentration (MIC) of MTAD. He concluded that this treatment regimen was effective in completely eliminating growth in seven of eight strains of *E. faecalis*. Which can be explained by that the superior bactericidal effect of MTAD caused by a carryover effect of the doxycycline in the MTAD preparation

(Trisha A. Krause . et al 2007) carried out a study to check for the Antimicrobial effect of MTAD, Sodium hypochlorite, Doxycycline and citric acid on *E. faecalis* using zone inhibition method. Zone inhibition of doxycycline and MTAD was superior than NaOCl.

(Joshua M. Davis, James Maki .et al 2007) conducted a study using Zone inhibition method to check for Antimicrobial Effects of Various Endodontic Medicaments on *Enterococcus faecalis*. The

Largest diameter of the zones of microbial inhibition was measured in millimetres and recorded. Where Bio Pure MTAD showed significantly more zones of microbial inhibition than 5.25% NaOCl, 2% CHX, and Dermacyn.

(Shahrokh Shabahang, Manouchehr Poursmail. et al 2003) Antimicrobial efficacy of MTAD and Sodium hypochlorite. The efficacy of MTAD in disinfecting the internal and external surface of roots is a result of the presence of the antibacterial effect of doxycycline – its ability to remove organic and inorganic substances from the surface of roots, which is facilitated by the presence of citric acid and presence of detergent that aids its

propensity to diffuse into the root canal and the dentinal tubes.

Bleach products contain many compounds other than sodium hypochlorite itself. NaOCl solutions require careful handling to maximize their advantages as endodontic irrigants and their costs vary widely. Sodium hypochlorite solutions are effective endodontic irrigants over a wide range of concentrations, but stale solutions are an expensive combination of salt and sodium chlorate. As with other therapeutic items, timely purchase of the solution in the strength required, storage in the original light-proof container which is kept sealed at all times, and discarding remnants well within the recommended time span will ensure that the product is always effective.

Most of the research on the use of CHX in endodontics is carried out using in vitro and ex vivo models and gram-positive test organisms, mostly *E faecalis*. It is therefore possible that the studies have given an over positive picture of the usefulness of CHX as an antimicrobial agent in endodontics. More research is needed to identify the optimal irrigation regimen for various types of endodontic treatments. CHX is marketed as a water-based solution and as a gel (with Natrosol). Some studies have indicated that the CHX gel has a slightly better performance than the CHX liquid but the reasons for possible differences are not known. Iodine compounds have antibacterial activity when used alone, and do not dissolve tissue either. Water and saline solutions bear the risk of contamination if used from containers that have been opened more than once. Iodine potassium iodide (eg, 2% and 4%, respectively) has considerable antimicrobial activity but no tissue dissolving capability and it could be used at the end of the chemo mechanical preparation like CHX. However, some patients are allergic to iodine, which must be taken into consideration.<sup>64</sup>

A potential problem with the prescription tray delivery system for hydrogen peroxide is that it requires daily use to be effective. Patient compliance is always a concern with any oral hygiene instruction. However in the Putt et al.

study, subjects were receptive to using properly fitted trays, especially after observing rapid improvements in their oral condition<sup>65</sup>.

### Conclusion

Within the limits of the present study, it can be concluded that –

- The antimicrobial efficacy of MTAD with Nisin (MTADN) is significantly greater when compared to MTADd and MTAD alone.
- The inhibition zone size was seen greater with MTADN followed by MTADd and MTAD.
- The optical density value of MTAD with Nisin was significantly greater when compared to MTADd and MTAD, which can be concluded that MTAD and Nisin have a greater synergistic effect.

### Summary

Successful endodontic therapy largely depends on the adequate cleaning and shaping of the root canal and elimination of the main etiological factors i.e. the microorganisms. For this purpose various irrigants and intracanal medicaments have been introduced. Among which to of the most common irrigants are sodium hypochlorite and chlorhexidine, but due few potent disadvantages and its inability to completely eliminate the bacteria lead to the development of few new irrigants like MTAD, Nisin and Daptomycin. This study was therefore undertaken to evaluate the antimicrobial efficacy of MTAD, MTADN AND MTADd. The present study was undertaken in Panineeya Institute of Dental Sciences, Hyderabad and NRI medical college Vijayawada.

Total four protocols were taken to calculate the antimicrobial efficacy of three different irrigants, which include Zone inhibition method, where blood agar medium was taken and divided in four different compartments and labelled as *E. faecalis*, *MTAD*, *MTADN* and *MTADd*. All the four compartments were streaked with *E. faecalis* and incubated. 6mm disc dipped in each irrigant to be

tested is placed in their in their respective compartments and check for the amount of zone of inhibition.

Second and third method was check for reduction in bacterial count by checking the optical density at different time intervals under spectrophotometer. Five 10ml test tubes were taken each filled with Saline, Saline inoculated, MTAD to inoculation, MTADN to inoculation and MTADd to inoculation and checked of OD at 0hr, 30mins, 60mins and 90mins interval. Morphological changes were observed under SEM.

Results of the present study reveal significant zone of inhibition and decrease in optical density for all the irrigants. Among the three irrigants used, MTADN showed a large zone of inhibition of 20mm and reduction in OD of 98.9% at 90mins interval, which is significantly higher than MTADd and MTAD.

The present study concluded that MTAD with NIsin showed a superior antimicrobial efficacy and have a greater synergistic effect than MTADd, MTAD, hypo, hydrogen peroxide and chlorhexidine alone.

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