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Invitro Evaluation of Antimicrobial Efficacy of Nisin and its Combination with Sodium Hypochlorite and Chlorhexidine On Enterococcus faecalis

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Abstract
Aim*: To evaluate the Antimicrobial Efficacy of Nisin and its combination with Sodium Hypochlorite and Chlorhexidine on Enterococcus faecalis.*
Materials and method*: A pure culture of Enterococcus faecalis (ATCC 29212) was inoculated on Mueller-Hinton agar plates incubated at 37°C overnight with sterile brain heart infusion broth. Forty single rooted human teeth were selected. Access cavity preparation was done.Working length was determined.Instrumentation was initiated with ISO hand files no. 15 k file followed by protaper rotary files upto size F_2 . 2ml of 5.25 % NaOCl was used as an irrigant after every instrumentation followed by a rinse with 3ml of distilled water .Samples were inoculated with bacterial strain and were divided into four groups Group I (20) 5 µL Normal saline Group II 5 µL Nisin (n = 10) Group III 5 µL Nisin + Na0Cl 1 % .1 min,Group IV (n = 10) 5 µL Nisin + Chlx 1%. On 8th day to evaluate the degree of infection dentin chips from root canals were extracted with sterile 6% Pro Taper rotary file.The dentin chips were transferred by placing files into 0.5 mL of Brain-heart infusion broth through sterile Eppendorf tubes and incubated at 37°C for 24 h. After 24 h, 5 µL of solution were inoculated on BHI agar plates from each tube and incubated at 37°C for 24 h to obtain bacterial colony forming unit (CFU) count. Statistical analysis was done using Kruskal– Wallis test with P value considered significant at ≤0.05 .Results :- Group 3 has minimum amount of bacterial count when compared with Group 4 , Group 2 and Group 1 Conclusion :- Nisin in combination with sodium hypochlorite has less amount of colony forming units .*

Key Words: *E. Faecalis, sodium hypochlorite, Nisin, Chlorhexidine*

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I. Introduction

For centuries mankind has searching for that magic drug that would cure all diseases and provide healing forever and searching for a drug to cure pulpal diseases is no different. Recent technical and scientific advances in Endodontics have improved the endodontic success rates. Despite the improvements in root canal instruments, instrumentation technique, irrigating solutions and medicaments, endodontic failures still persist.

Primary Endodontic infections are caused by necrotic pulp tissue colonized by microorganisms[1]. Success of Endodontic treatment depends on complete debridement and disinfection of root canal space. This is not always achieved completely because microorganisms may be found in root canals, dentinal tubules, apical ramifications, cementum or areas of root resorption thereby limiting the access of root canal systems by instruments and irrigants[1].

Irrigants are multifunctional in Endodontic treatment and are required to have antimicrobial effects, dissolve organic matter in the canal and flush out loose debris[3]. Enterococcus faecalis is one of such persistent organism of root canal system and frequently isolated in the root canals with pulpal infection[4]. It plays important role in the etiology of periradicular lesions after root canal treatment and seen in 22-77% root canal failure cases.

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses. E. faecalis is able to suppress the action of lymphocytes, potentially contributing to endodontic failure[5].

The term biofilm was introduced to designate the thin layered condensations of microbes that may occur on various surface structures in nature. Free-floating bacteria existing in an aqueous environment, so-called planktonic microorganisms are a prerequisite for biofilm formation. Such films may thus become established on any organic or inorganic surface substrate where planktonic microorganisms prevail in a water-

based solution[6]. Achieving predictable success of root canal treatment requires effective debridement and disinfection of root canal system and biofilm.

Sodium hypochlorite has been the gold standard for irrigation because of its ability to dissolve organic matter and high antimicrobial potential. However, there are certain major drawbacks associated with the use of sodium hypochlorite i.e irritant to periapical tissues, stains instruments, unpleasant taste, high toxicity, corrosion of instruments, inability to remove smear layer, burning of surrounding tissues and reduction in elastic modulus and flexural strength of dentin.

Chlorhexidine (CHX) is a broad spectrum antimicrobial agent that has substantive antimicrobial activity and relatively low toxic effects. However does not dissolve organic tissues (11). In vitro studies have shown CHX to exhibit sustained antimicrobial activity in the root canal for some time after being used as an endodontic irrigant. Therefore, CHX has been suggested as a root canal irrigant owing to its unique ability to bind to dentin, its effectiveness as an antimicrobial agent, and its substantivity in the root canal system.

Nisin (Mol. Wt 3500) is a naturally occurring antimicrobial peptide and was discovered in 1928 (14, 15). It is a cationic peptide antibiotic produced by streptococcus lactis .Nisin is safe to humans and is used extensively as a food preservative in over 40 countries , mainly in preservation of meat and daily products. To date there appears to be few reports investigating the effects of Nisin with regard to Endodontic infections. This antibiotic peptide is a Class I bacteriocin. It is effective against Gram- positive bacteria and spores,including strains of E. faecalis.

As there are inherent drawbacks of using conventional irrigants, therefore the purpose of this study was to evaluate other alternatives for root canal irrigation with high antimicrobial activity or similar to that of conventional irrigants with low toxicity. Therefore, the present study was aimed to explore newer irrigants probably be as more effective and at the same time would be less irritating to the tissues than NaOCl.

II. Material and Methods

E. faecalis culture preparation:-

A pure culture of Enterococcus faecalis (ATCC 29212) was inoculated on Mueller-Hinton agar plates incubated at 37°C overnight with sterile brain heart infusion broth.

Tooth Samples Preparation

Eighty single rooted type I Vertucci's classification human mandibular premolar teeth with fully formed apices were taken for the study. The specimens were cleaned of superficial debris, calculus, tissue tags and stored in normal saline.The specimens were sectioned below the cementoenamel junction with a diamond disk to obtain a standardized tooth length of 18mm. The root canals were then instrumented using the crown down technique and rotary instruments to an apical size of ProTaper F3.A total volume of 2 ml of 5% sodium hypochlorite was used between each instrument during the cleaning and shaping procedure. The specimens were then cleaned in distilled water for 30 min . After rinsing, teeth were stored in sterile water. Then the roots were dried, coated externally with clear nail varnish, and sterilized in an autoclave for 30 min at a temperature of 121°C and at a pressure of 15 p. Each root canal was inoculated with 24 h cultured broths of E . Faecalis OG-1 and ATCC 29212 bacterial solution up till the canal orifice using a sterile endodontic needle in a microbiological safety cabinet. After inoculation, the samples were kept in cotton plugged test tube and incubated at 37°C for 21 days. Every 3rd day, the canals were reinoculated with fresh bacterial samples.

Root canal Medication

The Specimens randomly divided into four treatment groups:

- **Group I, (20)5 µL Normal saline;**
- **Group II,(20) 5 µL Nisin**
- **Group III, (20) 5 µL Nisin + Na0Cl 1 % .**
- **Group IV,(20) 5 µL Nisin + Chlx 1%**

In all the samples, the prepared medicaments of 5 µL were injected in the root canals and completely filled. On 8th day, to evaluate the degree of infection, dentin chips from root canal of specimens were extracted with a sterile 6% Pro Taper Rotary file. The dentin chips were transferred by placing files into 0.5 mL of Brain-heart infusion broth through sterile Eppendorf tubes and incubated at 37°C for 24 h. After 24 h, 5 µL of solution were inoculated on BHI agar plates from each tube and incubated at 37°C for 24 h to obtain bacterial colony forming unit (CFU) count.

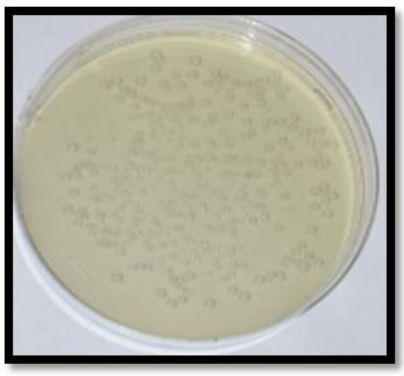
Statistical analysis

Statistical analysis was done using Kruskal– Wallis test with P value considered significant at ≤ 0.05.

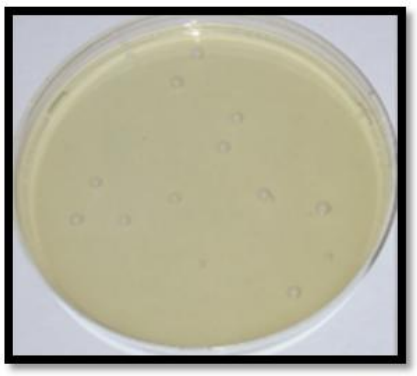
III. Results

The means CFUs count of the present study were showed in Table 1 .

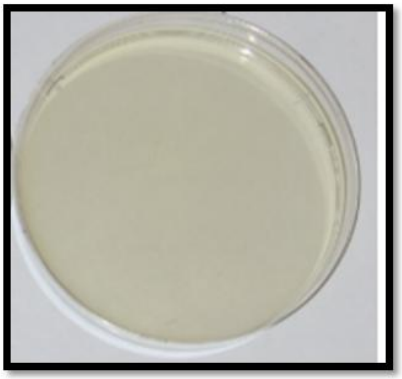
CFU of Different Groups



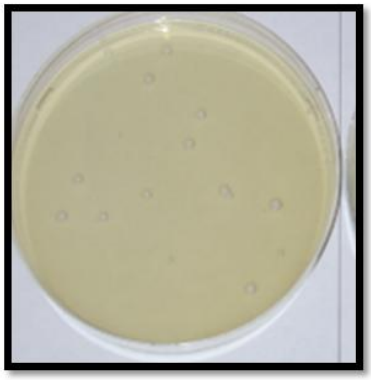
(Group 1) 5 µL Normal saline



(Group 2) 5 µL Nisin



(Group 3) 5 µL Nisin + Na0Cl 1 %



(Group 4) 5 µL Nisin + Chlx 1%

Treatment Groups	Enterococcus faecalis CFUs Mean±SD Min - Max	Enterococcus faecalis CFU Mean - Maximum
1.5 µL Normal saline;	93.00±2.45 90–97	90–97
2. 5 µL Nisin	84.00±3.09	80–88
3.5 µL Nisin + Na0Cl 1 %	5.00±1.33	03–07
4. 5 µL Nisin + Chlx 1%	30 .00 ± 1.33	10 - 20

IV. Discussion

The most common reason for the failure of Endodontic treatments and retreatments is the persistence of pathogenic bacteria within the root canal systems. Neither irrigation nor current intracanal medicaments can render the root canals completely free of bacteria. Thus, the discovery of new root canal irrigating agents with improved antimicrobial properties could benefit both the patient.

Recently, there has been a growing interest in using nisin for biomedical applications and for targeting oral biofilms and resistant microorganism. The 2 most well-known natural variants of nisin are nisin A and Z, which differ by a single amino acid substitution at position 27.

Previously, Tong et al reported that the MIC and MBC against E. faecalis was 1 and 2 mg/mL using nisin A (2.5%, Sigma-Aldrich), which is at least 10-fold higher than that re- ported for nisin ZP in this study .In addition, Shin et al showed that low concentrations of nisin ZP exhibited strong antibiofilm properties against saliva-derived multispecies biofilms.

In this study four different irrigating agents has used , comparing with saline and Nisin in combination with sodium hypochlorite and chlorhexidine .Based on the results in Group 1 saline has high amount of bacterial count as it does not has antimicrobial property. In Group 2 Nisin has bacterial count less than saline as it inhibits the bactericidal activity by targeting through specific lipid molecule . Nisin uses Lipid II as a ‘‘docking

molecule'' to form pores on the cell membrane surface in a targeted manner; at a nanomolar level, then, nisin is able to effectively kill bacteria. Sodium hypochlorite is an effective root canal irrigant which causes irreversible oxidation of hydro sulfuric groups of essential enzymes, disturbing metabolic functions in the microorganism[18].

CHX, a cationic bisbiguanide, crosses the cell wall, presumably by passive diffusion, and subsequently attacks the cytoplasmic membrane. According to reports, the antimicrobial effect lasts for up to 12 weeks, which directly corresponds to the CHX concentration. Hence, the concentration of CHX retained in the root canal gradually decreased which could be the reason, the mean CFU values increased gradually at 72 h.

Nisin with low doses of NaOCl enhanced the antibiofilm properties of these antimicrobial agents. As an irrigating agent, NaOCl is stable at a pH of 11, whereas nisin A is only soluble at an acidic pH, thereby limiting the compatibility of these agents at their optimal pH. However, because nisin ZP is stable at a neutral and basic pH, this allows preparations of nisin-NaOCl combinations for potential use as irrigating solutions.

As a unique bacteriocin, nisin has minimal cytotoxicity on mammalian cells and an absence of stable and transmissible resistance propert. Here, we showed that nisin ZP exerts antimicrobial and antibiofilm properties against E. faecalis. In addition, when combined with low concentrations of NaOCl, nisin's antibiofilm effects were enhanced in removing E. faecalis biofilms from their substratum. In conclusion, because novel bacteriocins, such as nisin ZP, can potentially improve the prognosis of endodontic therapy, their exploration is important for future clinical advancement[19].

V. Conclusion

Novel bacteriocins, such as Nisin, can potentially improve the prognosis of Endodontic therapy, when used with an adjunct their exploration is important for future clinical advancement .

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