Determination of Minimum Inhibitory Concentration (MIC) of a PolyHexamethylene Biguanide (PHMB) Solution; a Potential Root Canal Irrigant.

ABSTRACT:

Background: A Polymeric Biguanide PHMB (PolyHexaMethylene Biguanide) was evaluated for its antimicrobial activity in terms of MIC (Minimal Inhibitory Concentration) against ATCC reference laboratory strain ~~29212~~ of *Enterococcus faecalis*.

Methods: The MIC was determined using criteria's laid down in DIN 58940-7 and 58940-8 and the corresponding supplementary sheets. Serial dilutions of PHMB were tested against Enterococcus faecalis in 96 well microtitre plates and MIC was read as the minimal concentration that allowed no visible growth.

Results: The MIC for PHMB against tested organism was found to be 2 mg/L.

Conclusion: Lower MIC values (2mg/L) against *Enterococcus faecalis* as compared to its contemporary bisguanide Chlorhexidine paves way to further research in its potential use as ~~Root~~ root canal ~~Irrigant~~irrigant.

Keywords: E. faecalis, Minimum Inhibitory Concentration (MIC), Polyhexamethylene Biguanide

(PHMB), Polyhexanide, Root canal Irrigant.

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INTRODUCTION:

Root canal disinfection is undoubtedly of paramount importance in Endodontics. Considering the enormous complexities in the root canal system [1] and having known that our instruments have only a very limited reach to the intricacies of root canals. [2, 3]~~,~~ Root canal

irrigants have come to the fore as ~~indispensible~~indispensable tools in cleaning of root canals. A vast majority of disinfectant solutions have been researched and numerous papers published in this domain. [4-7] However, owing to the resistant microflora residing in convolutions of root canals [8] and existence of bacteria in form of biofilms [9-10] that collectively shelter the microflora from disinfection procedures. We are yet to find a disinfectant which will achieve near ideal disinfection of root canals with minimal toxic effects on host cells.

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E. faecalis, a facultatively anaerobic, gram-positive cocci has omniviously been associated with root canal infections, more commonly with failed root canals than with primary root canal infections.[11] Its ability to form biofilms on root canal surface and survive in harsh environment renders it 1000 times more resistant to destruction as compared to their free floating counterparts.[12] Conventionally, 6% Sodium Hypochlorite (NaOCl) is the only agent capable of both physically eliminating the artificial biofilm and killing bacteria.[13] But NaOCl, particularly at high concentration is known to be cytotoxic.[14] Chlorhexidine, another commonly used root canal irrigant has been found to be inefficient in ~~eradiaction~~eradication of *E. faecalis* biofilms.[15] Moreover, It has been found to be more cytotoxic than NaOCl.[16]

MIC determination is the most commonly employed procedure to evaluate the physiological effects of an anti-microbial agent on microorganisms, and correlation of product concentration and effect. [17] National Committee on clinical laboratory standards [NCCLS] 1997 defined MIC as the lowest concentration that completely inhibits visible growth of the organism, as detected by the unaided eye after an 18-24 hour incubation period, with standard inoculums of approximately 105 colony forming units per milliliter (CFU/ ml).[17] MICs serve as an important research tool in determining the activity of novel antimicrobials under *in vitro* conditions and the data so obtained can be used to determine MIC breakpoints. [18]

Therefore, in an attempt to propose an alternative to such toxic concentrations of irrigants, a polymeric bisguanide PHMB was evaluated for its MIC against *E. faecalis* the most commonly isolated organism from failed root canals.

METHODOLOGY:

The MIC was determined using criteria's laid down in DIN 58940-7 and 58940-8 [19, 20] and the corresponding supplementary sheets. To summarize, the test organism ATCC laboratory strain (29212) of *Enterococcus faecalis* was cultivated on Blood agar at 36˚ C for 18 hours. Following this, one colony of cultivated *E. faecalis* was transferred into 1 mL of Mueller–Hinton bouillon (Figure 1) and then diluted to reach 10^5cfu/mL. 96-well microtitre plates (Figure 2) were used to perform the test procedure. PHMB in white crystalline form was obtained from Sinobio Chemistry Co., Ltd, China. Hundred mL of defined antiseptic dilution was placed in each well ~~alongwith~~along with 100 mL of test organism suspension. PHMB solution was subjected to serial dilution and a variety of concentrations ranging from 5000 to 1mg/ L dilutions were made. After a period ~~of 24~~of 24 h, the turbidity was evaluated. Presence of turbidity was defined as indicator for bacterial growth or viability. After 24 h, plating was done for solutions to confirm their efficacy. (Figure 3)

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Figure 1: Mueller-Hinton broth for culture of *E. faecalis*

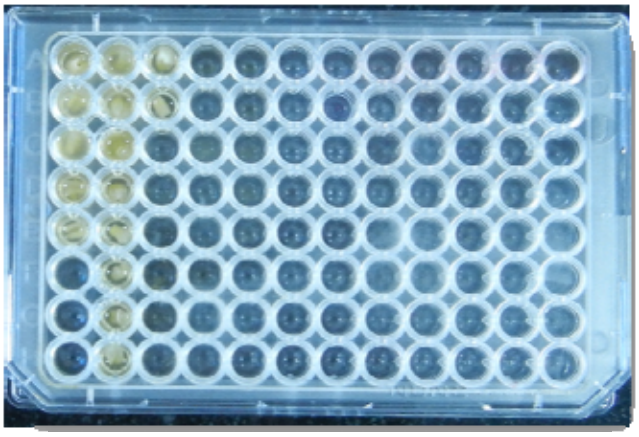

Figure 2: Serial dilution of 96 well plates

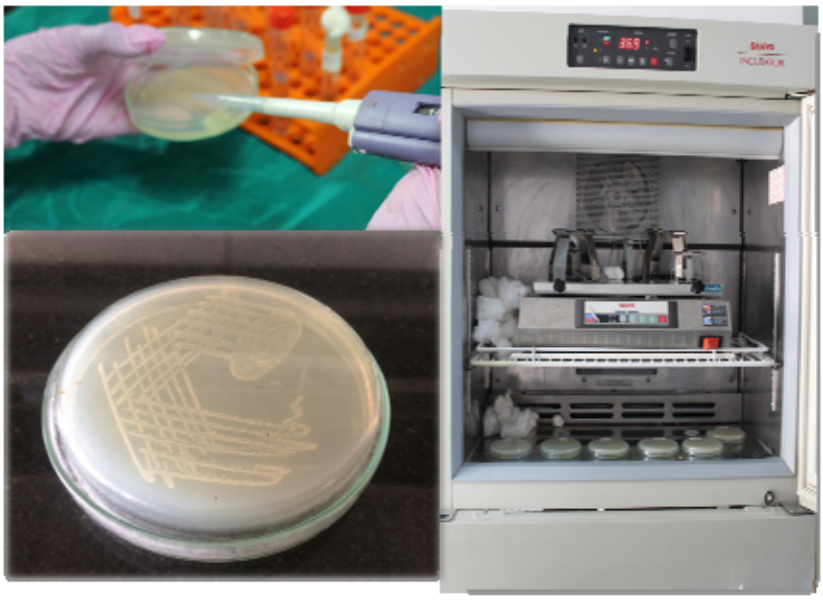

Figure 3: Plating, incubation and culture of bacteria to confirm antibacterial efficacy

RESULTS AND DISCUSSION:

A Value of 2mg/ L was obtained as MIC after 24 hours for PHMB solution against E. ~~Faecalis~~faecalis.

Antimicrobial activity is a central role in any root canal irrigant. This study was carried out to determine antimicrobial activity of a broad spectrum antimicrobial PHMB against *E. faecalis*, the most commonly associated microorganisms with failed root canals.

PHMB in its Polymeric form, has been found to be an effective broad spectrum antimicrobial with efficacy against some fungi and protozoa in addition to gram positive and gram negative bacteria.[21]

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Mechanism of action of PHMB has been described by Ikeda *et al.* [22] PHMB acts on negatively charged species in the bilayer composed of neutral and acidic phospholipids. After its adsorption into Phosphatidyl glycerol bilayer, its biguanide groups interact with the polar headgroups of the lipids and hexamethylene groups with the hydrophobic interior. The Phosphatidyl glycerol bilayer hence becomes disorganized with resulting greater fluidity, lateral expansion and raised permeability of the bilayer.

No evidence of microbial resistance against PHMB can be found in literature. This in part can be attributed to its superficial interaction with the membrane in polymeric form which merely involves physical bridging of the molecule in the phospholipids. Lack of any chemical interaction avoids any possibility of generation of any mechanism for reduced susceptibility. [23]

Traditionally, a plethora of methods have been employed for determining susceptibility to antimicrobials; Broth microdilution test [24, 25], Disk diffusion test [26, 27] and automated

generated systems [28] to name a few. Amongst these, Agar dilution and Broth microdilution are the popular methods for quantitative determination of Antimicrobial activity in terms of MIC.

Broth Microdiluton test was chosen for determination of MIC in this study because it is reproducible, easy to perform as channels are ~~pre~~prepared, ~~costeffective~~cost-effective and saves reagents and space. [29]

Polyhexanide showed an MIC of 2 mg/L in this study. Our results were in agreement with the results of Koburger and Colleagues[30] who employed a microdilution test and a quantative suspension test to determine MIC values of a variety of antiseptic solutions at 24 h and 48 h and Minimum bactericidal concentration (MBC) at 24 h. They found octenidine and polyhexanide to be most efficacious with ~~with~~ equally low MIC and MBC values at all times. However, CHX after a period of 24 h was found to eliminate *E.faecalis* at 16 mg/ L. [30] Moreover it has a high toxicity and low tissue tolerability. [6] On the contrary, Polyhexanide has been shown to have better safety profile. [31, 32]

Muller and Kramer in 2008 introduced a Biocompatibiity Index (BI) as a measure of microbicidal activity of Antimicrobials against cytotoxicity. A BI greater than 1 indicates good antiseptic efficacy with a relatively low cytotoxicity, whereas antiseptics with BI less than 1 present a relatively high cytotoxicity. Polyhexanide was found to have BI greater than 1. [33]

Hirsch and colleagues tested a variety of antiseptics for antimicrobial activity and ~~cytotoxicity against~~cytotoxicity against primary human keratinocytes, primary human fibroblasts and human keratinocyte cell line. Their results were in favour of Lavasept and Prontosan which showed best antimicrobial efficacy with low or no cytotoxicity on different cell lines. [34]

CONCLUSION:

We believe that this *in vitro* study will be a valuable guide for determining the optimal first-line drug at higher concentrations but at lower toxicity levels for endodontic irrigation, particularly retreatment cases. Therefore, based on this study results confirming antimicrobial activity of PHMB against *E. Faecalis*, we suggest that PHMB may be useful as an effective endodontic irrigant. However, the limitation of this study was that MIC was determined against pure cultures without load of debris/ proteins which might be an interfering factor. Further research should be carried out to determine its antimicrobial activity against various other microorganisms found in infected root canals and its effect on removal of root canal biofilm.

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COMPETING INTEREST: None

AUTHORS' CONTRIBUTIONS:

AUTHOR 1 designed the study, wrote the protocol and wrote the first draft of the manuscript.

AUTHOR 2 guided the study design and protocol, reviewed the manuscript and made modifications.

AUTHOR 3 managed the literature searches and execution of the study.

All authors read and approved the final manuscript.

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