



## STUDY OF ANTIMICROBIAL EFFICACY OF LINEZOLID, LANTIBIOTIC (NISIN), AND CALCIUM HYDROXIDE AGAINST ENTEROCOCCUS FAECALIS BIOFILM

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### ABSTRACT

**Background-** Persistent infection of the root canal due to the presence of resistance bacterial species, such as *Enterococcus faecalis*, has always been one of the most important reasons for endodontic treatment failure.

**Aim-** To evaluate the bactericidal effects of linezolid, lantibiotic (nisin), and calcium hydroxide against *Enterococcus faecalis* biofilm.

**Methods-** Single rooted human mandibular premolars were decoronated, biomechanically prepared, and vertically sectioned along the midsagittal plane to obtain a standardized tooth substrate. Standardized suspension of *E. faecalis* and tooth substrate was incubated for 3 weeks to allow growth of biofilm. At the end of 3 weeks, the grouping was done according to the medicament used, Group A-Linezolid, Group B-Lantibiotic (nisin), Group C-Calcium hydroxide, Group D-Negative treatment. The zones of inhibition were checked after 2 and 7 days.

**Results-** Group A gave the widest zone of inhibition ( $17\pm 1.14$  and  $17\pm 1.06$ ) followed by Group B ( $12\pm 1.68$  and  $12\pm 2.03$ ) and Group C ( $3.78\pm 0.64$  and  $2.56\pm 0.48$ ) both after 2 and 7 days. The antimicrobial efficacy against *E. faecalis* biofilm remain unchanged for Group A and B after 7 days whereas in Group C, the zone of inhibition decreased after 7 days.

**Conclusion-** Linezolid showed maximum antimicrobial potential against *E. faecalis* biofilm followed by lantibiotic (nisin) after 2 and 7 days. Antimicrobial efficacy against *E. faecalis* biofilm reduced with lapse of time for calcium hydroxide.

**KEYWORDS :** Biofilm, *Enterococcus faecalis*, linezolid, nisin

### INTRODUCTION

Persistent infection of the root canal due to the presence of resistance bacterial species, such as *Enterococcus faecalis*, has always been one of the most important reasons for endodontic treatment failure. One of the other characteristics of *E. faecalis* is its great ability to form a biofilm. Studies on the biofilm structure of *E. faecalis* have shown that after 6 weeks some signs of mineralization and complete maturation are observed in the structure of biofilm and a period of 6 weeks is considered a time interval for the maturation of biofilm.<sup>2,3</sup>

Calcium hydroxide has been extensively used intracanal medicament in dental practice. Certain reports have revealed that *E. faecalis* resists the highly alkaline environment produced by the calcium hydroxide dressing.<sup>4,5</sup> Hence, an alternative medicament for eradicating *E. faecalis* from complex root canals is required to achieve successful endodontic treatment. Lantibiotic (nisin) and Linezolid are the two new intracanal medicaments which have shown satisfactory results against *E. faecalis* biofilm.

To best of our knowledge and thorough literature search we did not find much reports on comparison of the antimicrobial efficacy of Linezolid, Lantibiotic (nisin) and calcium hydroxide against *E. faecalis* biofilm. Therefore, we planned this study to evaluate the bactericidal effects of linezolid, lantibiotic (nisin), and calcium hydroxide against *Enterococcus faecalis* biofilm.

### METHODS

This investigation was conducted at a dental college located in northern India. Convenient sampling technique was adopted. Forty five extracted single rooted human mandibular premolars free from cracks and caries were included. Teeth were scraped externally with sodium hypochlorite by using the sterile gauge to remove debris. The teeth were then washed with distilled water and stored in thymol until used. Samples were vertically sectioned along the midsagittal plane into two halves. The concave surface of the samples was minimally grounded to achieve a flat surface to enable *E. faecalis* to form a biofilm on the exposed root canal surfaces. The samples were then sterilized.

*E. faecalis* strain (RC115) was isolated from the root canal of a patient exhibiting repeated root canal treated failure. The isolate was identified and speciated based on conventional methods and polymerase chain reaction. *Enterococcus faecalis* suspension was prepared by adding pure culture of *E. faecalis* grown in Mueller-Hinton broth. The suspension was standardized. *E. faecalis* suspensions along with tooth samples were incubated at 37°C for a period of 3 weeks to allow the formation of biofilm. The culture medium was replaced every alternate day to avoid nutrient depletion and accumulation of toxic end products. The bacterial samples, incubated in Mueller Hinton agar, were taken every alternate day with a sterile paper point at 37°C for 24 h to check for cell viability and purity of the culture. At the end of 3 weeks, two samples were processed to confirm the presence of *E. faecalis* biofilm by scanning electron microscopy. At the end of 3 weeks, the biofilm was scraped from the tooth substrate. Bacterial emulsion was prepared by adding the scraped biofilm to 0.9% NaCl solution.

At the end of 3 weeks, the grouping was done according to the medicament used  
Group A -Linezolid  
Group B - Lantibiotic (nisin)  
Group C - Calcium hydroxide  
Group D - Negative treatment

Disk of the medicaments used were prepared. The disks of medicaments were placed upon Petri dishes with prepared bacterial emulsion and were incubated at 37°C on Mueller-Hinton agar. After a period of 2 days, Petri dishes of five samples of each group were checked for the zone of inhibition. The procedure was repeated after 7 days for remaining five samples of each group.

Written and informed consent was obtained from study subjects. Permission of ethical committee was obtained from the Institutional Ethics Committee. All the questionnaires were manually checked and edited for completeness and consistency and were then coded for computer entry. After compilation of collected data, analysis was done using Statistical Package for Social Sciences (SPSS), version 21 (IBM, Chicago, USA). The results were expressed using appropriate statistical variables.

## RESULTS

Group A gave the widest zone of inhibition ( $17\pm 1.14$  and  $17\pm 1.06$ ) followed by Group B ( $12\pm 1.68$  and  $12\pm 2.03$ ) and Group C ( $3.78\pm 0.64$  and  $2.56\pm 0.48$ ) both after 2 and 7 days. The antimicrobial efficacy against *E. faecalis* biofilm remain unchanged for Group A and B after 7 days whereas in Group C, the zone of inhibition decreased after 7 days. (Table 1)

**Table 1: Comparison of mean values of zones of inhibition of different groups after 2 and 7 days**

Variable	Zones of inhibition		P value
	2 Days (Mean $\pm$ SD)	7 Days (Mean $\pm$ SD)	
Group A	$17\pm 1.14$	$17\pm 1.06$	>0.05 (Non-Significant)
Group B	$12\pm 1.68$	$12\pm 2.03$	>0.05 (Non-Significant)
Group C	$3.78\pm 0.64$	$2.56\pm 0.48$	<0.05 (Significant)

## DISCUSSION

Linezolid has gained popularity on the basis of its wide spectrum of activity against Gram positive organisms, including vancomycin resistant *E. faecalis*. Linezolid is a synthetic antibiotic belonging to a new class of antimicrobials called the oxazolidinones. Linezolid disrupts bacterial growth by inhibiting the initiation process in protein synthesis.<sup>6</sup>

Nisin, a naturally occurring antimicrobial peptide, is produced by *Streptococcus lactis subspecies lactis*. It has antimicrobial activity against a wide range of Gram positive bacteria and their spores,<sup>7</sup> even against drug resistant *E. faecalis* isolates.<sup>8</sup> Its use in dentistry has so far been limited.

A meta-analysis<sup>9</sup> on the antibacterial effect of  $\text{Ca}(\text{OH})_2$  as an intracanal medicament showed limited disinfection activity within the root canal system. In a recent systematic review by Saatchi M et al.<sup>10</sup> authors reported no improvement in antibacterial activity by mixing chlorhexidine and  $\text{Ca}(\text{OH})_2$ .

Another study<sup>11</sup> assessed the mature biofilm of *E. faecalis* against various antimicrobial agents such as calcium hydroxide, amoxicillin, and clavulanate potassium, ciprofloxacin, clindamycin, and doxycycline. Authors concluded that these agents were significantly better than calcium hydroxide, whereas in the present study, there is a reduction in biofilm forming ability of *E. faecalis* in the presence of liquorice at a concentration of 4 and 3 g and 24 and 48-h-old biofilm than calcium hydroxide, thus supporting the observations of the previous study.

In this study we observed that Group A gave the widest zone of inhibition ( $17\pm 1.14$  and  $17\pm 1.06$ ) followed by Group B ( $12\pm 1.68$  and  $12\pm 2.03$ ) and Group C ( $3.78\pm 0.64$  and  $2.56\pm 0.48$ ) both after 2 and 7 days. Similar results were obtained by Taneja S.<sup>12</sup> in that study, the negative treatment group showed no zone of inhibition. In calcium hydroxide group zone of inhibition formed had significantly lower values in comparison to nisin and LZ group, both after 2 and 7 days.

The antibacterial properties of calcium hydroxide are attributed to its high alkalinity and its ability to destroy the cytoplasmic membrane, denature bacterial proteins, and damage bacterial DNA.<sup>13</sup> The proton pump of *E. faecalis* carries protons to the interior of the cell, acidifying its cytoplasm, in situations of increased alkalinity in its environment when treated with calcium hydroxide.<sup>14</sup> We observed that antimicrobial efficacy against *E. faecalis* biofilm remain unchanged for Group A and B after 7 days whereas in Group C, the zone of inhibition decreased after 7 days. It could be due to the fact that with time calcium hydroxide diffuses into agar media resulting in dissolution of calcium and hydroxyl ions which in turn decreases the pH of the media.<sup>14</sup> Another study by Pavaskar R et al.<sup>15</sup> observed that the antimicrobial efficacy of linezolid against *E. faecalis* planktonic suspension lasted for 14 days although that of calcium hydroxide declined after 72 h.

## CONCLUSION

This study observed that Group A gave the widest zone of inhibition.

Means linezolid showed maximum antimicrobial potential against *E. faecalis* biofilm followed by lantibiotic (nisin) after 2 and 7 days. Antimicrobial efficacy against *E. faecalis* biofilm remain unchanged for linezolid and nisin after 7 days but that of calcium hydroxide reduced with lapse of time.

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