Comparative Evaluation of Bactericidal Potential of Four Root Canal Filling Materials against Microflora of Infected Non -Vital Primary Teeth

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Background and objectives: *Since complete debridement of the root canals of the primary teeth is not practically possible due to the highly variable root canal anatomy, success of the endodontic therapy depends partly on the use of antibacterial irrigating agents and root canal filling materials. Recent literature indicates that anaerobes comprise a majority of the bacteria in necrotic root canals of primary teeth. The study determined the antibacterial effectiveness of four root canal filling materials namely Calcium hydroxide, Zinc oxide eugenol, Vitapex and Metapex against microbial specimens obtained directly from necrotic root canals of primary teeth.* ***Method:*** *Microbial specimens were collected using sterile paper points, from 15 primary maxillary and mandibular posterior teeth of randomly selected children in the age group of 4-10 years with infected non vital primary teeth, requiring pulpectomy procedure. The microbial specimens collected were subjected to microbiological analysis and the antimicrobial potential of root canal filling materials were tested using Agar diffusion technique.* ***Results:*** *were statistically analyzed using one-way ANOVA. Facultative/Aerobic organisms were isolated in all the cases, anaerobic organisms were isolated in 80% of the cases, and Candida albicans was isolated in 1 case. ZOE showed superior inhibitory activity against most of the organisms isolated followed by Vitapex, Calcium hydroxide and Metapex in descending order.* ***Conclusion:*** *Our data may be useful as a guide for relative antimicrobial effectiveness or non-effectiveness of the materials employed. In vivo studies are required to state the specific antimicrobial activity and merits and demerits of any of the test filling material.*

Keywords*: Zinc oxide eugenol, Vitapex, Calcium hydroxide, Metapex, micro-organisms, root canal, primary teeth.*

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INTRODUCTION

Success of endodontic therapy of primary teeth depends, in part, on the elimination or reduction of bacteria present in the root canal.[1]

This may be accomplished by mechanical debridement and use of antibacterial irrigating agents and root canal filling materials.[2] Since complete debridement of the root canals of the primary teeth is not practically possible, success of the endodontic therapy depends partly on the use of antibacterial irrigating agents and root canal filling materials.[2]

Thus, an endodontic filling material with considerable antibacterial property becomes a pre requisite particularly when treating infected non-vital primary teeth or those with necrotic pulp.

Antimicrobial activity of root canal filling materials has been extensively studied by agar diffusion techniques using pure cultures of oral bacteria.[2, 3] Most of these investigations focused on facultative *streptococci* and *staphylococci*, which may not have represented the predominant bacterial species found in infected root canals. A number of anaerobic species that are predominantly present in the infected root canals have not been included in agar diffusion testing of dental materials.[2]

Hence, our investigation is an evaluation of the bactericidal potential of four root canal filling materials against the microflora of infected non vital primary teeth.

The objectives of the present study were:

1. To identify the microflora of the root canals of infected non-vital primary teeth.

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2. To compare the bactericidal potential of root canal filling materials namely Calcium hydroxide, Zinc oxide eugenol, Vitapex and Metapex for primary teeth.

MATERIALS AND METHODS

The study group consisted of fifteen healthy randomly selected patients of both sexes, between the age group of 4-8 years, attending the out-patient block of the Department of Pedodontics and Preventive Dentistry, Yenepoya Dental College Hospital, Derelakatte, Mangalore. Fifteen primary and mandibular posterior teeth (one from each patient), which were infected and non-vital, requiring pulpectomy procedure and with the following inclusion criteria, were included in the study.

Inclusion criteria

1. Healthy children without any known systemic illness.
2. Antibiotics not received by the subject four weeks prior to the sampling.
3. Contained at least one necrotic root canal.
4. An abscess, sinus tract or obvious radiolucency must be present.
5. Did not have extensive root resorption or broken crowns.[4, 5]

Exclusion criteria for selection

1. Subjects known to have any systemic illness.
2. Subjects under antibiotic therapy.[4, 5]

A detailed medical, dental history, history of antibiotic use was recorded before the collection of the specimen. Informed oral and written consent was obtained from the parents of the participants before the commencement of the study.

CLINICAL PROCEDURE

The entire procedure was carried out under strict aseptic conditions. No endodontic procedure was performed before collection of the sample, so as to avoid disturbing the root canal flora.

Prior to the start of the procedure, the involved tooth and the surrounding area were wiped with 10% povidine iodine solution and the tooth was isolated with rubber dam.

Carious dentin was removed followed by access cavity was preparation using a sterile number 4 bur followed by pulp extirpation. Three sterile Nº20 paper points were introduced into the accessible root canals and left in place for one minute.[5, 6, 7] The three root canal samples so obtained were transferred immediately into:

A) Robertson Cooked Meat Media (R.C.M) for the cultivation of anaerobic organisms
B) Brain Heart Infusion (B.H.I) broth for cultivation of aerobic organisms
C) Sterile test tube for further smear preparation to observe the organisms by Gram stains respectively.

The antibacterial potential of the root canal filling materials namely: Calcium hydroxide (Deepti Dental Products of India, A.P, India),Zinc oxide eugenol (Vishal Dentocare Pvt Ltd., Gujarat, India), Vitapex (Neo Dental Chemical Products Co. Ltd, Tokyo, Japan), and Metapex (Meta Biomed Co. Ltd, Korea) towards the organisms isolated were assessed by Kirbey Baucer Agar plate diffusion technique.[8]

LABORATORY PROCEDURE

Smears were prepared on a sterile glass slide from each of the specimen collected in the sterile test tube and subjected to Gram staining and were observed under oil immersion lens for the presence of bacteria.

The specimens' inoculated into R.C.M and B.H.I were inoculated at 37^{O}C overnight. Subcultures were made from B.H.I broth into blood agar, heated blood agar and Mc Conkey's media and incubated at 37^{O}C overnight.

From R.C.M, subcultures were made on two blood agar plates. One blood agar plate was incubated in McIntosh field jar with Gas pack for anaerobic culture at 37^{O}C for 48 hours with metronidazole disk to screen out the growth of facultative anaerobic organisms. The second blood agar plate was incubated aerobically at 37^{O}C overnight.

The development of colonies on aerobic culture and anaerobic blood agar were studied for the colony morphology, Gram stain and biological characterization. The standard method for isolation and identification of microorganisms were followed as *per* Cowan and Steel's,[9] Vette and McCarter,[10] Topley and Wilson,[11] Mendel and Bennet.[12]

The powder liquid ratio of all the test root canal filling materials were standardized according to the formula given by Tchaou *et al.*[4] An electronic balance and micro pipette were used to measure the exact amount of powder and liquid to be dispensed.

AGAR DIFFUSION ASSAY

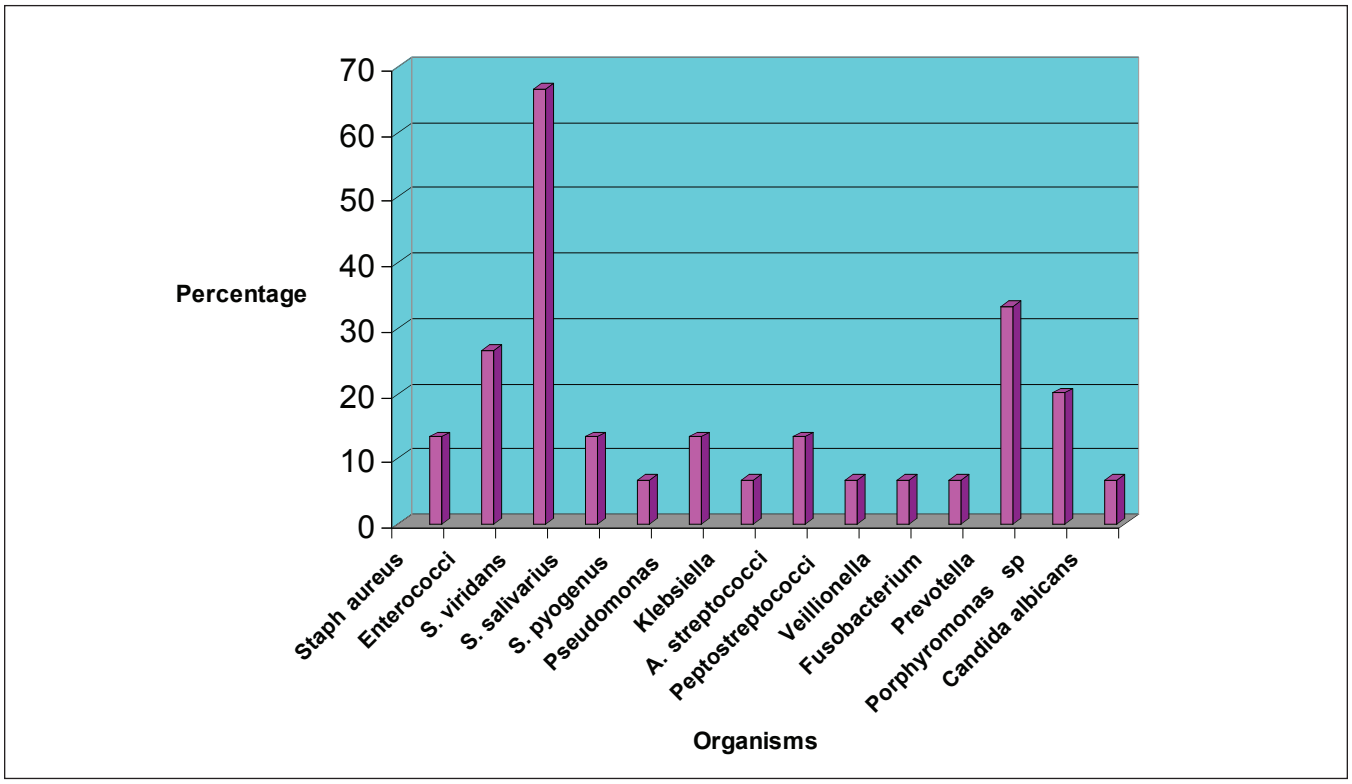
Sensitivity testing of the root canal filling materials against the isolates obtained was performed in Muller-Hilton agar by cup-plate method of Kirby-Bauer.[8]

The agar plates were dried and 4 wells of 4mm diameter and 3 mm depth were made in the agar plates using sterile agar puncher. Using a sterile swab, the entire surface of the agar plate was swabbed 3 times to ensure even distribution of the inoculum and to obtain lawn culture.

The four root canal filling materials viz, Calcium hydroxide, Zinc oxide eugenol, Vitapex and Metapex were tested in each plate. Each of the four filling materials was filled in each well in the agar plate. These plates were incubated at 37^{O} degrees overnight. The diameter zones of inhibition in millimeter around the filling materials were measured after 24 hours for aerobic isolates and after 48 hours for anaerobic isolates.

RESULTS

In the present study, a total of 15 root canal samples were studied. Out of the total 15 samples, 12 samples (80%) exhibited polymicrobial infection, 3 samples (20%) exhibited the presence of facultative/ aerobic organisms. *Candida*



Graph 1. Microorganisms isolated

albicans was isolated from 1 specimen. The organisms isolated are given in Graph 1.

A total of 14 species so isolated were employed in the experimental procedure. The isolated organisms were divided into 5 groups based on bacteria or fungi, aerobic or anaerobic bacteria, gram positive or gram negative bacteria.

The mean zones of inhibition of the four root canal filling materials against the 14 organisms isolated are given in Table 1.

Table 1. Mean Zones Of Inhibition (Mm) Of 4 Filling Materials Against 14 Organisms

Organisms	Ca(OH)2	ZOE	Vitapex	Metapex
Staphylococcus aureus	12.5	16.5	14	12.5
Enterococci	12.75	16.75	14.75	13.5
Streptococcus viridans	13.2	18.1	13.3	11.9
Streptococcus salivarius	13.5	18	10.5	7.5
Streptococcus pyogenus	13	20	6	2
Pseudomonas	12	15	12	12
Klebsiella	14	20	18	13
Anaerobic streptococci	13.5	20	14.5	14
Peptostreptococci	13	18	13	13
Veillionella	14	20	17	15
Fusobacterium	12	20	14	12
Prevotella	11.6	18.8	12.6	11.2
Porphyromonas	13.33	18	13	12.66
Candida albicans	4	8	4	1

Measurements of the inhibitory zones are ranked arbitrarily into the following three categories according to the proportional distribution of the data set:[4,5]

1. No/Weak inhibition (NW)
2. Medium inhibition (M)
3. Strong inhibition(S).

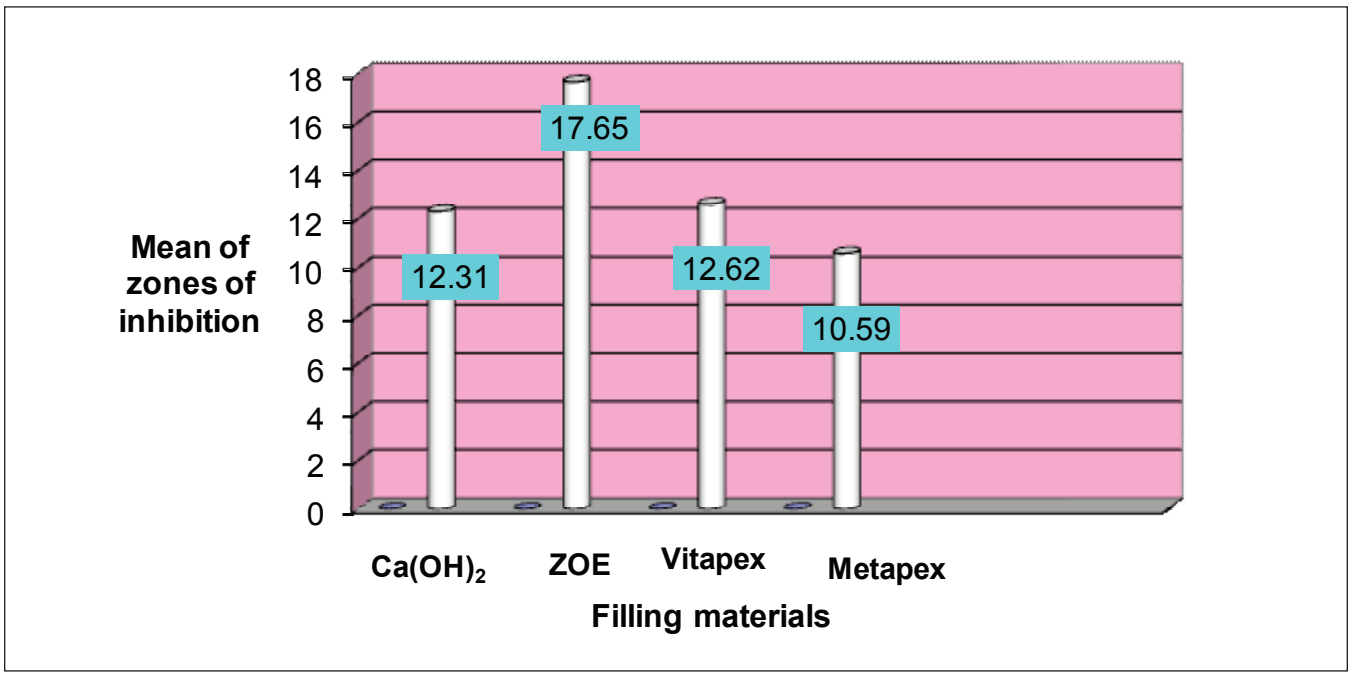
Zone size categories and proportions of data represented in each category are represented in Table 2.

Table 2. Ranking Scheme For Microbial Inhibition Rank

RANK	RANGE OF ZONE DIAMETERS (mm)	% OF DATA SET REPRESENTED	FREQUENCY (N= 56)
NO/WEAK	1-8	12.5	7
MEDIUM	8.1-13.5	46.42	26
STRONG	>13.5	41.07	23

Statistical Analysis

Statistical analysis was carried out by one-way ANOVA using SPSS version 11.5 with post-hoc tests to compare the statistical difference of antimicrobial effects between the materials tested with each of the four bacterial groups (Aerobic Gram positive, Aerobic Gram-negative, Anaerobic Gram-positive and Anaerobic Gram negative).



Graph 2. Mean of zones of inhibition of Filling materials

Statistical difference of the antimicrobial effects between the materials against the fifth group (fungi) could not be done as Candida albicans was obtained in only one case.

ZOE exhibited the maximum inhibitory effect followed by Vitapex, Calcium hydroxide and Metapex. The mean zones of inhibition of the filling materials are given in Graph 2.

Statstically there was very high significant difference in the inhibitory activity between the materials. (p=0.001)

The statistical difference in the inhibitory activities between ZOE and Vitapex were highly significant (p=0.008).

The differences in the inhibitory activities between ZOE and Metapex were statistically very highly significant (p=0.001).

The statistical difference in the inhibitory activities between ZOE and Ca (OH) 2 were highly significant (p=0.004).

DISCUSSION

There are very few studies concerning root canal microflora of the primary teeth. Marsh and Largent[13] reported alpha hemolytic streptococci as the predominant microorganism whereas other studies[14, 15] reported that the most predominant microorganism in root canals of primary teeth with necrotic pulp and periapical lesions were *Streptococcus salivarius*. Anaerobic organisms represented over 70% of the microflora of the primary molars that had been treated unsuccessfully and were also the most prevalent bacteria in teeth indicated for extraction.

In the present study, aerobic and anaerobic bacteria, black-pigmented *bacilli, streptococci* and Gram-negative aerobic rods were found. This is in agreement with Toyoshima *et al*[16] who reported that in root canals of primary teeth with necrotic pulp and periapical lesions there is a polymicrobial infection, similar to microbiota of permanent teeth.

The present study also reported the presence of *Candida* in 1 case; there are considerably few reports in the literature regarding the presence of *Candida* in the root canal.[6]

Facultative / aerobic organisms were present in all 15 cases (100%). However, this finding differs from that of Silva *et al* [17] who reported aerobic organisms in 60% of necrotic root canals of primary teeth. Streptococci were present in 86.65% of the cases, which is consistent with the findings of Silva *et al* [17] who reported 85% prevalence and also comparable with the findings of Marsh and Largent[13] who reported 82% prevalence.

In our study, *Streptococcus viridans* was the organism was present in majority of the cases (66.66%), which is comparable to that of the findings of Marsh and Largent[13] who reported *Streptococcus viridans* (alpha hemolytic *streptococci*) as the predominant organism. *Streptococcus salivarius* were present in 13.33% and Streptococcus pyogenus in 6.66% of the cases which differs from that of Cohen *et al* [14] who isolated *Streptococcus salivarius* in 70% of the cases. However, in the study by Cohen *et al* [14] the organisms were isolated from open, infected primary teeth whereas in our study only those teeth which did not have broken crowns were taken to avoid the possible contamination of the root canal microflora with that of the salivary organisms.

Enterococci were found in 26.66% of the cases which is consistent with the findings of Rocas *et al* [18] who reported 33%.

Staphylococcus aureus were isolated in 5% of the cases by Cohen *et al* [14] while 13% in our study.

In the present study, aerobic Gram-negative organisms were found in 20% of the cases [*Pseudomonas* (13.33%) and

Klebsiella (6.66%)], which is comparable with the findings of Silva *et al* who isolated Gram-negative aerobic rods in 15% of necrotic primary molars.

Anaerobic bacteria were present in 12 canals (80%) in the present study.

Veillonella was found in 6.66% which is comparable with the findings of Peters *et al*[19] who isolated *Veillonella* species in 4% of infected root canals.

Among the anaerobic organisms, black-pigmented bacilli have frequently been isolated from root canals of permanent teeth with necrotic pulp.[17] Sundqvist *et al* [20] reported their presence in 30% of the cases, Toyoshima e*t al*[16] isolated black pigmented bacilli in 44.4% of retreatment cases. While another study,reported their presence in 30% of the cases.[17] In the present study, black-pigmented bacilli (*Prevotella* and *Porphyromonas*) were present in 53.33% of the cases which may be comparable with the findings of a previous investigation that found 49%. *Peptostreptococcus* were isolated in 6.66% of the cases, which is consistent with the findings of Chu *et al* [21] [10%] and Adib *et al* [22] [6.7%] of the cases and differs from that of Gomes *et al* [23] who isolated their presence in 35% of infected dental root canals.

Anaerobic streptococci were found in 13.33% in the present study, which is comparable with the findings of Adib *et al* [22] who reported 17.5%. Fusobacterium were isolated in 6% of the cases in our study.

Reported rates of yeast incidence in root canals vary widely (3 to 55%), depending upon the population sampled and the sensitivity of the sampling technique.[6] *Candida albicans* were isolated in 6.66% of the cases in our study, which may be comparable with a previous study by Adib *et al* [22] who isolated 2.4% of *Candida* species.

Most of the previous studies that tested antibacterial activity of filling materials against pure cultures used Gram-positive cocci, Gram-negative rods, or other bacteria that may not have represented the predominant bacterial species found in infected root canals, i.e. *Peptostreptococci*, *Prevotella* species and obligate and facultative *streptococci*.[2]

While our objective was to test bacteria that were representative of endodontic microbiota, comparing our data with previous studies is difficult because of the different test strains, media and culture conditions involved.

The bacterial specimens in this study were collected from the root canals of infected non-vital primary teeth which did not have broken crown to prevent contamination of the root canal microflora by the organisms from saliva and carious lesion of the teeth.

An *in vitro* study cannot stimulate perfectly an *in vivo* study but it can control factors that an *in vivo* study cannot, such as quantitative evaluation of antibacterial activity by a variety of filling materials. As the *in vitro* method also required the filling materials to diffuse into the agar, the net inhibitory effect was a combination of diffusion potential and anti-bacterial activity. The ability to diffuse into dentinal tubules is a desired characteristic of an antibacterial agent.[4]

Hobson found that microorganisms penetrated into the tubules of dentinal walls in root canals in 70% of extracted teeth with necrotic tissue.[24]

Even if several filling materials have been used through the years, the most common ones are Zinc oxide eugenol, Iodoform and Calcium hydroxide. Generally after a good filling and irrigation, the final outcome of the pulpectomy procedure depends on the quality of the root canal filling materials, which can neutralize any remaining pulp tissue and microorganisms.

In this study, ZOE exhibited strong inhibitory action against all the four groups of bacteria except against *Candida albicans*. Broisman *et al,*[25], Cox *et al,*[3] Grossman,[26] Pupo *et al*,[27] Rahmat,[28] Canalda and Pumarola,[29] and Pumarola *et al*[30] agree that the sealers with ZOE base are those that have greater inhibitory effect against the microorganisms found in root canals. The antimicrobial activity of ZOE is attributed to the eugenol content of the material. Cox *et al* [3] demonstrated that zinc oxide had no inhibitory effect and the addition of eugenol to zinc oxide retarded the growth of only the Gram-positive organisms. The inclusion of zinc acetate as a setting accelerator inhibited both Gram-positive and Gram-positive bacteria.

Calcium hydroxide showed medium or moderate inhibition against all the four bacterial groups and showed least inhibition against *Candida albicans* in our study.

This is in agreement with the results of Tchaou *et al* [4] who found that calcium hydroxide produced medium or medium-strong inhibition against *P.intermedia* strains. The result of our study contradicts with that of a previous study [5] in which calcium hydroxide exhibited weak antimicrobial activity against facultative/aerobic Gram-positive, facultative/aerobic Gram-negative organisms but failed to inhibit anaerobic bacteria. The result of our study also differs from other studies by Difore *et al,*[31] Abdulkader *et al,*[32] Siqueira and Gonclaves.[33] They demonstrated that calcium hydroxide associated with an inert substance (distilled water, saline or glycerine) was ineffective against several obligatory and facultative anaerobic bacteria. The weak inhibitory effect of calcium hydroxide in agar diffusion assay can be explained by the fact that blood or buffer present in the agar media might have neutralized calcium hydroxide, a phenomenon that may also occur in vivo where blood and buffering systems are present.

Vitapex showed strong inhibitory action against facultative/aerobic Gram-negative bacteria and anaerobic Gram-negative organisms, moderate inhibition against facultative/aerobic Gram-positive and anaerobic Gram-positive organisms but showed weak inhibitory action against *Candida albicans*. This finding differs from the results of Pabla *et al* [34] and Tchaou *et al* [4] according to whose studies, Vitapex showed the least or no antibacterial activity.

However, Nurko and Garcia-Godoy[35] studied the effectiveness of Vitapex in the root canal treatment of primary teeth. The treatment was deemed successful if, clinically, the tooth was painless, without pathological mobility, and the gingiva was healthy without a sinus or fistula. The authors

recommended the use of Vitapex as a root canal filling material as they observed various advantages of this material.

On the other hand, Estrela *et al* [36] verified the influence of iodoform on the antimicrobial potential of calcium hydroxide on *Staphylococcus aureus*, *Enterococci faecalis*, *P. aeruginosa, B. subtilis* and *C.albicans* by direct exposure test and agar diffusion test. The results showed significant antimicrobial effectiveness for calcium hydroxide paste or iodoform plus saline.

Metapex showed the lowest antimicrobial activity when compared to the other three root canal filling materials tested in this study. However, it showed moderate inhibitory activity against facultative/aerobic Gram-negative bacteria, anaerobic Gram-positive bacteria and anaerobic Gram-negative bacteria but showed weak inhibitory activity against facultative/aerobic Gram-positive organisms and failed to inhibit *Candida albicans*.

The weak inhibitory activity may be explained by the fact that calcium hydroxide an ingredient of Metapex has been demonstrated to interfere with the antiseptic capacity of dyadic combinations of endodontic medicaments.

Most of the studies related to antimicrobial activity of root canal filling materials have been done using standardized bacterial strains (ATCC- American Type Culture Collection). Very few studies were reported in the dental literature exclusively on bacterial strains isolated from infected primary teeth.[4, 5, 34]

The mean zone of inhibition of the materials in this study cannot be compared with previous studies because of the variability's bacterial strains, culture media, culture conditions and powder and liquid ratio of the test materials.

Based on the results of this study, ZOE showed superior antimicrobial activity against most of the organisms isolated, followed by Vitapex, Calcium hydroxide and Metapex in descending order.

CONCLUSION

The following conclusions were drawn from the present study:

1. The root canals of infected primary teeth are polymicrobial in nature, with anaerobic and facultative/ aerobic organisms predominately present.
2. All the test filling materials showed varied antimicrobial activity against the microorganisms tested.
3. ZOE showed superior inhibitory activity against most of the organisms isolated followed by Vitapex, Calcium hydroxide and Metapex in descending order.

It is difficult to draw conclusions based on in vitro evaluation of antimicrobial activity with isolated bacteria. It is well known that endodontic infections are mixed with complex floral interactions. The effect of the test filling materials against a single strain may not be effective against a mixed variety of infection. The use of artificial media also plays an important role in determining the experimental results. It is possible that different results might have been obtained if other methods of testing antimicrobial activity i.e. Agar dilution method, Direct contact test etc. were employed.

Microbial interactions in the oral cavity should be considered before concluding the ideal test results. In vivo studies are required to state the specific antimicrobial activity and merits and demerits of any of the test filling material.

The clinical relevance of the findings from this study, however, can only be determined in clinical trials. Our data may be useful as a guide for relative antimicrobial effectiveness or non-effectiveness of the materials employed.

REFERENCES

1. Tronstad L. Recent development in endodontic research. Scand J Dent Res, 100: 52–9. 1992.
2. Tchaou WS, Turng BF, Minah GE, Coll JA. Inhibition of pure cultures of oral bacteria by root canal filling materials. Pediatr Dent, 18(7): 444–49, 1996.
3. Cox ST, Hembree JH, Mcknight JP et al. The bactericidal potential of various endodontic materials for primary teeth. Oral Surg, 45(6): 947–54, 1978.
4. Tchaou WS, Turng BF, Minah GE, Coll JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials. Pediatr Dent, 17(5): 351–55, 1995.
5. Reddy S, Ramakrishna Y. Evaluation of antimicrobial efficacy of various root canal filling materials used in primary teeth: A microbiological study. J Clin Pediatr Dent, 31(3): 195–99, 2007.
6. Akdeniz BG, Koparal E, Sen BH, Ates M, Denizci AA. Prevalence of candida albicans in oral cavities and root canals of children. J Dent Child, 289–92, 2002.
7. Pazelli LC, Freitas AC, Ito IY, Souza-Gugelmin MC, Medeiros AS, Nelson- Filho P. Prevalence of microorganisms in root canals of human deciduous teeth with necrotic pulp and chronic periapical lesions. Pesqui Odontol Bras, 17(4): 367–71, 2003.
8. Bauer A, W, Kirby W, Sherris JC and Turch M. Antibiotic susceptibility testing by standardized single disc method. Amer J Clin Pathol, 45: 493, 1966.
9. Cowan and Steel. Manual for identification of Medical bacteria. 3rd edition. Cambridge university press. 112–14.
10. Y.Vette S, McCater. Oral and Maxillofacial infection. Topazian, 4th edition; chapter 3: 43–61.
11. Topley and Wilson. Microbiology and microbial infections. 9th edition: vol 3, 225–27.
12. Mendel and Bennet. Principle and practice of infectious disease. 5th edition, 286–89.
13. Marsh SJ, Largent MD. A bacteriological study of the pulp canals of infected primary molars. J Dent Child, 34: 460–470, 1967.
14. Cohen MM, Joress SM, Calisti LP. Bacteriologic study of infected deciduous molars. Oral Surg Oral Med Oral Pathol, 13(11): 1382–86, 1960.
15. Tomic-Karovic K, Jelinek E. Comparative study of the bacteriological flora in the surroundings, the root canals and sockets of deciduous molars. Int Dent J, 21: 375–88, 1971.
16. Toyoshimo Y, Fukusima H, Inoue JI, Sasaki Y, Yamamoto. Bacteriological study of the periapical pathosis on deciduous teeth. JPN Dent J, 26: 449–58, 1988.
17. Silva LAB, Nelson-Filho P, Faria G et al. Bacterial profile in primary teeth with necrotic pulp and periapical lesions. Braz Dent J, 17(2): 144–48, 2006.
18. Rocas IN, Siqueira JF, Santos KRN.Association of Enterococcus faecalis with different forms of periradicular diseases. J Endodon, 30(5): 315–20, 2004.
19. Peters LB, Wesselink PR, van Winkelhoff AJ. Combinations of bacterial species in endodontic infections. Int Endod J, 35(8): 698–702, 2002.

20. Sundqvist G, Johansson E, Sjogren U. Prevalence of Black-pigmented Bacteroides species in root canal infections. J Endodon, 15(1): 13–19, 1989.
21. Chu FCS, Tsang CSP, Chow TW, Samaranayake LP. Identification of cultivable microorganisms from primary endodontic infections with exposed and unexposed pulp space. J Endodon, 31(6): 424–29, 2005.
22. Adib V, Spratt D, Ng YL, Gulabivala K. Cultivable microbial flora associated with persistent periapical disease and coronal leakage after root canal treatment: a preliminary study. Int Endod J, 37(8): 542–51, 2004.
23. Gomes BP, Pinheiro ET, Gade-Neto CR et al. Microbiological examination of infected dental root canals. Oral Microbiol Immunol, 19(2): 71–6, 2004.
24. Hobson P. Pulp treatment of deciduous teeth. Br Dent J, 128: 232–8, 275–82, 1970.
25. Broisman H, Van Houte J, Gron P and Krakow A.A. Antimicrobial effects of N2 in vitro. Oral Surg, Oral Med, Oral Pathol, 45: 116–122, 1978.
26. Grossman L.I. Antimicrobial effect of root canal cements. J of Endod, 6: 594–507, 1980.
27. Pupo J, Biral R.R, Benatti O, Abe A and Valrighi L. Antimicrobial effects of endodontic filling materials on microorganisms found in root canal. Oral Surg, 74: 216–220, 1992.
28. Rahmat AB. Evaluation of antimicrobial activity of 10 root canal filling materials on Streptococcus sanguis and Streptococcus mutans. Oral Surg, Oral Med, Oral Pathol, 68: 99–102, 1989.
29. Canalda C, Pumarola J. Bacterial growth inhibition produced by root canal sealer cements with calcium hydroxide base. Oral Surg, Oral Med, Oral Pathol, 68: 99–102, 1989.
30. Pumarola J, Esteban B and Canalda C. Antimicrobial activity of 7 root canal sealers. Oral Surg, Oral Med, Oral Pathol, 74: 216–220, 1992.
31. DiFiore PM, Peters DD, Setterstrom JA, Lortan L. The antibacterial effects of calcium hydroxide apexification pastes on Streptococcus sanguis. Oral Sur,g 55(1): 91–94, 1983.
32. Abdulkader A, Duguid R and Saunders E.M. The antimicrobial activity of endodontic sealers to anaerobic bacteria. Int Endod J, 29: 280–283, 1996.
33. Siqueria J.F and Gonclaves R.B. Antibacterial activities of root canal against selected anaerobic bacteria. J Endod, 22: 890, 1996.
34. Pabla T, Gulati MS, Mohan U. Evaluation of antimicrobial efficacy of various root canal filling materials for primary teeth. J Ind Soc Pedod Prev Dent, 15(4): 134–40, 1997.
35. Nurko C, Garcia-Godoy F. Evaluation of a calcium hydroxide / iodoform paste (vitapex) in root canal therapy for primary teeth. J Clin Ped Dent, 23: 289–94, 1999.
36. Estrela C, Estrela CRA, Hollanda ACB, Decurcio DA, Pecora JD. Influence iodoform on antimicrobial potential of calcium hydroxide. J Appl Oral Sci, 14: 33–37, 2006.