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ORIGINAL ARTICLE

Antimicrobial Activities of Commercially Available Obturating Materials in Primary Teeth

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ABSTRACT

Background: *Preservation of primary dentition is a must for orofacial development as it helps to maintain the eruption of permanent teeth, aids in mastication, and phonation.* ***Objectives:*** *The present study compares the antimicrobial activities of commonly used obturating materials for filling the root canals of primary teeth against the microorganisms commonly infect the roots.* ***Methodology:*** *the antimicrobial activities of four commonly used obturating materials, ZOE; Iodoform; Vitapex; and Endoflas, were evaluated by three methods; the first method was electron microscope images which detected the presence of the bacteria in dentinal tubules. The second method was CFU count method in which thirty extracted deciduous mandibular molars were incubated in a mixed-species suspension, obturated, cultured and the numbers of the bacterial colonies were reported for each obturated group. The third method was the agar diffusion method in which the antimicrobial activities of the obturating materials were tested against four microbial isolates (E. faecalis; E. coli; S. aureus and Ps. aeruginosa).* ***Results:*** *The four obturating materials reported different antimicrobial effect in CFU test which was not statistically significant; however, Endoflas and ZOE were superior to Vitapex and Iodoform. Bacterial resistances were detected against Vitapex and Iodoform.* ***Conclusion:*** *Obturating materials containing eugenol were more effective than other materials without eugenol.*

INTRODUCTION

Various microorganisms were isolated from necrotic primary teeth as *Enterococcus faecalis, Streptococcus salivarius, Staphylococcus aureus, Neisseria catarrhalis, Lactobacillus casei, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and different anaerobic species [1,2].

In the past, extraction was the only solution to treat any pulpal infection for preventing complications. Nowadays pulp therapy solves those cases to maintain the integrity and health of the teeth and their supporting tissues [3]. Pulpectomy is removal of infected necrotic pulp of a tooth affected by caries, so the pain vanish and reserve the tooth function till it exfoliates normally [4].

Pulpectomy in primary teeth is challenging as the root canals have complex anatomy due to presence of numerous accessory and lateral canals which makes it difficult to remove infecting bacteria completely with instrumentation and irrigation. Failure to completely eradicate microorganisms can result in the failure of root canal therapy. Hence, the use of medicaments in the root therapy was introduced to reduce the intra-canal microbial growth [5].

Various materials have been tried in dentistry as intra-canal antimicrobials [6]. Zinc oxide eugenol is the most famous one used for filling the dentinal tubules of the primary teeth. Eugenol is an essential oil with germicide activity and was first used in 1876 by Chisholm who make Zinc oxide eugenol or ZOE [7]. Iodoform was also found to have an excellent healing properties and resorbtion of excess material and has been suggested as an alternate to ZOE [3,8].

Vitapex also reported a bactericidal effect and Endoflas disinfect the root canal, resorbable, and hydrophilic paste [9,10]. Dentists usually confused with different obturating materials and the common question which one shall we use? This study was done as a comparative assessment for the antimicrobial activities of these commonly used obturating materials.

METHODOLOGY

This in-vitro comparative study was carried out in the Department of Pedodontics and Preventive Dentistry in collaboration with Department of Medical Microbiology and Immunology, Mansoura University. According to the Clinical and Laboratory Standard Institute (CLSI) guidelines 2010 all tests were done [11]. The ethic committee of Faculty of Medicine, Mansoura University had approved the plan of our work.

Obturating Materials:

- Zinc Oxide Eugenol (ZOE), (Septodont, India).
- Iodoform and glycerine paste (Sigma-Aldrich, USA).
- Vitapex (Neo-Dent, Japan).
- Endoflas (Sanlor Lab, Colombia).

Purification of bacterial culture and preparation of inoculum:

Four human bacterial species, 10 isolates from each species, were isolated from oral, and throat samples as the following: *Escherichia coli, Pseudomons aeruginosa, Enterococcus fecalis* and *Staphylococcus aureus*. A single colony was picked with a sterile loop and transferred into fluid broth medium (Oxoid, UK), then aerobically incubated at 37°C. The density of the organism suspensions was adjusted by spectrophotometer to contain 10^8cfu/ml.

Preparation of the teeth samples:

Thirty primary mandibular molars were extracted from patients for unrelated study reasons. The inclusion criteria were extracted teeth with no external and internal root resorbtion and more than two-thirds of an intact root. The Collected teeth were placed in 5.25% NaOCl for one hour in order to disinfect the root surfaces after that the roots were stored in 0.9% sterile saline. The crown was cut perpendicular to the axis of the teeth from cementoenamel junction with a diamond disc mounted in micromotor into two roots (60 roots). Then root canal was manually adjusted to size 35, and then irrigation was performed with 5.25% sodium hypochlorite. EDTA (17%) was used to remove the smear layer followed by of 5.25 % NaOCL and roots were sterilized in the autoclave [12].

Roots contamination:

The roots were immersed in a mixture of the tested human bacterial species and were aerobically incubated at 37°C for 30 days with substitute 5 ml of the old broth by fresh one every 3 days [13]. After that the roots were obturated and according to the obturating materials, four groups (each group included 15 roots) were included. Group I: Roots were filled with ZOE paste; Group II: Roots were filled with Iodoform with glycerin paste; Group III: Roots were filled with Vitapex paste; and Group IV: Roots were filled with Endoflas paste.

Examination of the roots by electron microscope (EM):

The outer surfaces of all samples were disinfected with ethyl alcohol. Thin EM films were prepared after removing the obturating materials from the roots and they were examined by EM for the presence of microorganisms.

Counting the bacteria contaminating the obturated roots:

The outer surfaces of all samples were disinfected with ethyl alcohol, obturating materials were removed from roots with complete aseptic techniques and then each root was sectioned into three parts (cervical, middle and apical). Each part was vortex for 10 min in 1ml sterile nutrient broth (Oxoid, UK), diluted 1:2 by nutrient broth, and then the broth was plated onto blood agar plates, incubated for 24 hours, and the colony-forming units (CFU) per 1ml on the plates were counted and the numbers of the microorganisms in each root part were calculated and reported.

Antimicrobial susceptibility testing:

The freshly mixed obturating material was loaded in sterile syringe, and 0.5 ml material was injected on a sterile cellophane sheet placed on a marked glass slab. A standard weight was carefully placed on the obturating preparations in which the diameter of each disc was 6 mm [14]. According to Kirby-Bauer agar diffusion method broth cultures of *Escherichia coli, Pseudomons aeruginosa, Enterococcus fecalis* and *Staphylococcus aureus* (10^8 cfu/ml) were sub-cultured on Mueller-Hinton agar media (Oxoid, UK). Obturating materials disks were placed and the plates were incubated at 37°C. Inhibition zones diameters were measured and reported after 18 hours [15].

Statistical analysis:

Data were analyzed with SPSS version 21. The normality of data was first tested with Shapiro test. Continuous variables were presented as mean ± SD (standard deviation) for parametric data. ANOVA test was used to compare more than 2 means and in between groups comparisons were tested by post hoc LSD test while Kruskil Wallis test was used to compare more than 2 medians. The results were considered significant when the probability of error is less than 5% ($p \leq 0.05$). The smaller the p-value obtained, the more significant are the results (highly significant $p \leq 0.001$).

RESULTS

Examination of the roots by electron microscope (EM):

The films showed different microorganisms, inside the roots ducts which were numerous in group II (photo 1b) and group III (photo 1c), and were less in roots treated with ZOE (photo 1a) and Endoflas (photo 1d).

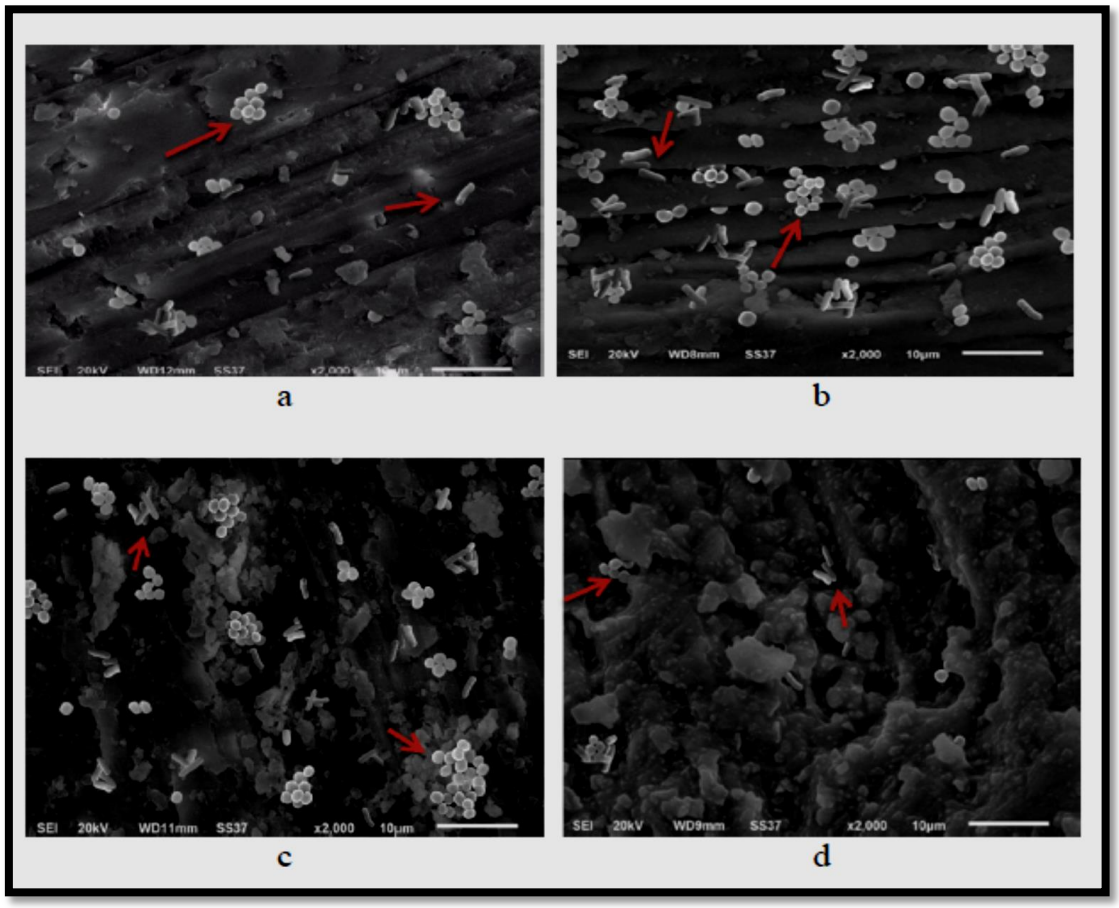


Photo 1: Electron microscope examination of the roots ducts X2000 showing the presence of microorganisms which were more obvious with group II and group III; a) ZOE group; b) Iodoform group; c) Vitapex group; d) Endoflas group.

Antimicrobial effects of the four obturating materials:

Group I (151.58±139) and group IV (123.22±208) were the best bactericidal materials and showed the lowest bacterial growth count; group II and group III reported 686.75±1327 and 339.72±996, respectively. However, no statistically significant difference was reported between the four obturating materials regarding their bactericidal power.

By comparing the bacterial growth count in the three parts of the tooth, Group I showed highly statistically significant differences followed by group IV (P=0.001, and 0.009, respectively) and Group II & III showed statistically significant differences (P=0.015, and 0.046, respectively). The apical part was the one which was reported with the maximum inhibition of the bacterial growth, followed by the middle and finally the cervical as in table 1, figure 1, and photo 2.

Table 1: Comparison between the obturated roots parts culture results

	Cervical (CFU/ml)	**Middle** (CFU/ml)	**Apical** (CFU/ml)	**P**
Zinc oxide	335±296	113.92±186**a**	5.83±14.4**ab**	0.001**
Iodoform with glycerin	1071.2±1886	903.92±1917**a**	85.16±193**ab**	0.015*
Vitapex	487.50±1423	473.08±1428**a**	58.58±140**a**	0.046*
Endoflas	334.41±595	26.58±44.3 **a**	8.67±19.3**a**	0.009*

*significant p ≤0.05; ** highly significant p ≤0.001; a: significant with cervical, b: significant with middle

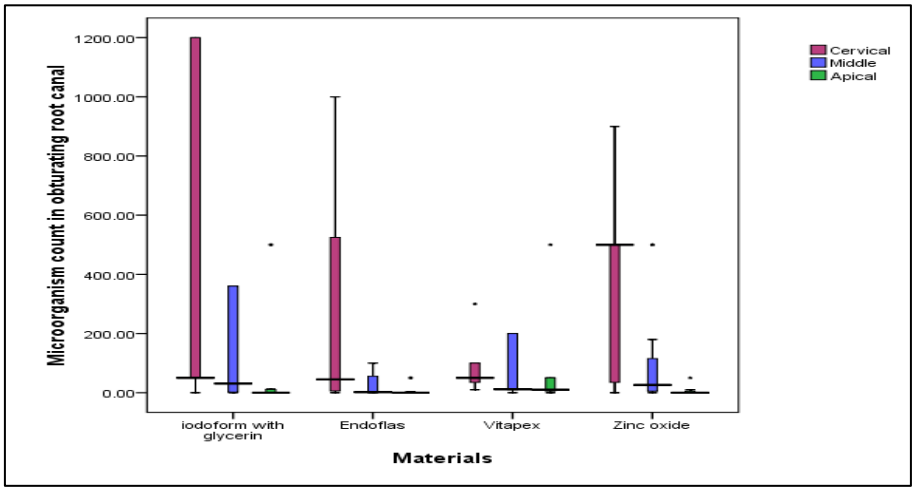


Fig. 1: Box plot for comparison between the obturated roots parts culture results

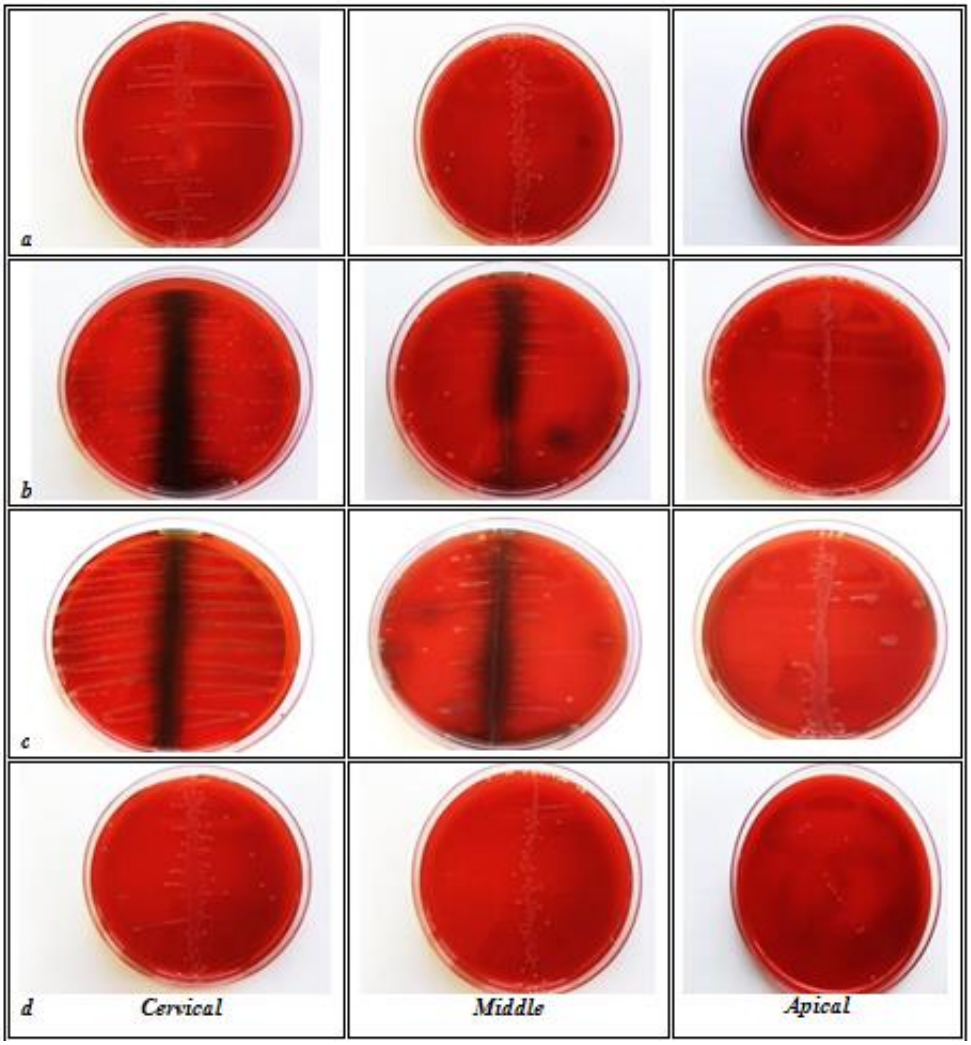


Photo 2: Comparison between the CFU numbers after cultures of the obturated roots parts by different materials a) ZOE; b) Vitapex; c) Iodoform; d) Endoflas.

There were no statistically significant differences between numbers of CFU/ml of the four obturating materials as regard the inhibition of bacterial growth in the cervical and middle parts but a significant statistical difference (P< 0.022) was reported in the apical part in which ZOE was the best followed by Endoflas, Vitapex and finally the Iodoform as in table (2).

Table 2: Comparison between the obturated roots apical parts culture results

Apical	Mean ± SD (CFU/ml)	P – value
Zinc oxide	5.83±14.4	0.022*
Iodoform with glycerin	85.16±193	
Vitapex	58.58±140	
Endoflas	8.67±19.3	

*significant p ≤0.05

Antimicrobial susceptibility testing:

There was high statistically significant difference (P< 0.001) between the four groups as regard the sensitivity for *Enterococcus faecalis* in which Endoflas was the best and followed by ZOE; the reported inhibition zone diameters were 24.80±5.06, (mean ± SD), and 22.00±5.09, respectively. *Enterococcus faecalis* isolates were highly resistance to Vitapex and the Iodoform with glycerin.

There was a high statistically significant difference (P< 0.001) between the four groups as regard the sensitivity for *S. aureus* in which Endoflas was the best and reported an inhibition zone diameter by 18.60±3.51, followed by ZOE (14.20±2.77). *S. aureus* isolates were highly resistance to Vitapex and the Iodoform with glycerin.

There was high statistically significant difference (P< 0.001) between the four groups as regard the sensitivity for *E. coli* in which Endoflas was the best and reported 21.40±4.7, followed by ZOE (18.40±4.09). *Escherichia coli* isolates were highly resistance to Vitapex and the Iodoform with glycerin.

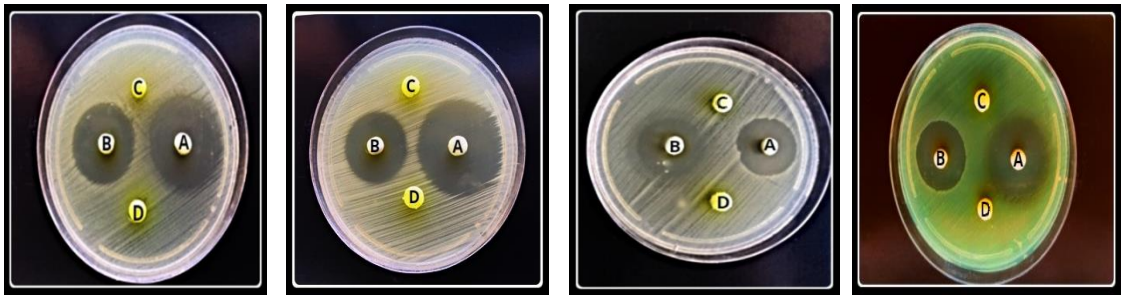
There was high statistically significant difference (P< 0.001) between the four groups as regard the sensitivity for *Ps. aeruginosa* in which Endoflas was the best and reported 17.0±4.6, followed by ZOE (14.20±5.8). *Ps. aeruginosa* isolates were highly resistance to Vitapex and the Iodoform with glycerin.

Endoflas reported a significant difference (P< 0. 046) in the inhibition of the four bacterial species; in which *E. fecalis* isolates were the most sensitive to it followed by *E. coli*, *S. aureus* and finally *Ps. aeruginosa*. ZOE showed a statistically significant difference (P< 0. 047) in inhibiting the growth of the four bacterial species; in which *E. fecalis* isolates were the most sensitive to it followed by *E. coli*, and then both *S. aureus* and *Ps. aeruginosa* reported similar sensitivity to it. Both Vitapex and Iodoform with glycerin were non-significant in affecting the growth of the four bacterial species which were resistant for them as shown in table 3 and photo 3.

Table 3: Comparison between the inhibition zones diameters of the obturating materials

Inhibition zones of	*E. faecalis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*ANOVA test*	*P*
Zinc oxide	22.00±5.09	14.20±2.77 a	18.40±4.09	14.20±5.8 a	3.34	0.046*
Iodoform with glycerin	0.0±0.0	0.0±0.0	1.60±3.5	0.0±0.0	1.00	0.418
Vitapex	2.00±4.47	0.0±0.0	0.0±0.0	0.0±0.0	1.00	0.418
Endoflas	24.80±5.06	18.60±3.51 a	21.40±4.7	17.0±4.6 a	2.84	0.047*

a: significant with Enterococcus faecalis by post hoc LSD test; *significant p ≤0.05



E. coli *S. aureus* *E. faecalis* *P. aeruginosa*

Photo 3: Antimicrobial susceptibility testing for the four obturating materials by the disk diffusion method according to Kirby-Bauer method. Obturating materials labeling were; (A) Endoflas; (B) ZOE; (C) Vitapex, and (D) Iodoform with glycerin.

DISCUSSION

All bacteria that inhabit the oral cavity can invade the pulp space during and after pulp necrosis in primary teeth. Failure of pulp therapy in primary teeth usually occurs in spite of mechanical preparation and irrigation due to the entrapped microorganisms in the tortuous and complex canal space [4]. Thus, for optimal success of endodontic treatment, substances with antimicrobial properties are used as obturating materials in deciduous teeth [16,17]. In 2008, ZOE has been the recommended material for treatment of the deciduous teeth root canals [18]. In 2009, iodoform paste was reported as alternative to ZOE [4].

In the present study, deciduous molars were the target for two reasons; the first was the limited researches applied on them and the second was because they were more available than the anterior teeth. The roots were incubated in a broth culture that contains a mix of four bacterial isolates, *E. coli*, *Ps. aeruginosa*, *E. fecalis* and *S. aureus*, as a trial to simulate the real situation in which poly-microbial infections are the usually faced challenge in primary teeth root canals [19,20]. The time for incubation was similar to the one used in Haapasalo and Orstavik study to confirm the bacterial penetration into dentinal tubules [13].

In the instant study although there was no statistically significant result between the antibacterial effects of the four tested obturating materials, Endoflas showed better antibacterial activity when compared to ZOE. Endoflas also reported a statistically significant difference ($P<0.046$) in inhibiting the growth of the four bacterial species when compared with other materials; in which *E. fecalis* were the most sensitive to it followed by *E. coli*, *S. aureus* and finally *Pseudomonas aeruginosa*. Parallel results were reported by many studies [21,22,23,24,25]. However, Hegde et al found that the Endoflas was not the best bactericidal material when compared with ZOE paste, zinc oxide-calcium hydroxide mixture, calcium hydroxide paste and Metapex [26]. The antimicrobial effect of Endoflas is probably due to the two main components in it; Eugenol acts by protein denaturation while iodoform acts by the liberation of iodine which oxidizes and inactivates proteins, nucleotides and fatty acid resulting in cell death [27].

In the current study, ZOE followed the Endoflas ($P< 0. 047$) in inhibiting the growth of the four bacterial species; in which *E. fecalis* was the most sensitive to it followed by *E. coli*, and then both *S. aureus* and *Ps. aeruginosa* reported similar sensitivity to it. These results are proven with Hegde et al. [26]. On the other hand, this wasn't true with Reddy research who reported that ZOE was with a limited antimicrobial activity [28].

In the existing study the bacterial resistance was reported against Vitapex and Iodoform and these results were parallel to those reported by other studies [29,30]. Also, Aydos and Milano concluded that Iodoform lack the antimicrobial activity in vitro but it in vivo results were divergent, suggesting that its function may be helped by stimulating the biological body reaction [31]. The weak activity of vitapex was explained by that one of the ingredient of vitapex (calcium hydroxide), had been detected to interfere with its antiseptic capacity [32], and this explanation had been supported by other studies [33,34]. On the other hand, Katerine et al disagreed with this and reported that the pure Iodoform paste and Vitapex were the most effective obturating materials [35].

Several factors could be responsible for this dissimilarity among studies antibacterial activity results of the obturating materials. The most cleared one might be heterogeneity of the tested species or the concentration of the chemical substances in the obturating materials like the concentration of eugenol. Also different methods with different sensitivities for detection of the obturating materials bactericidal effect may lead to different and incomparable results [36].

In the current study, obturating materials that contain eugenol (Endoflas and ZOE) showed better antimicrobial activity against tested microorganisms when compared to the non-eugenol containing materials (Iodoform and Vitapex). Eugenol is a phenolic substrate that can affect microorganisms in vegetative form by making protein denaturation and convert functioning proteins to non-functional one which kill the microorganisms [23,37].

In the ongoing study there were statistically significant differences as regard the inhibition of bacterial growth in the apical part of the teeth root when compared to middle and the cervical in which the final one reported the heaviest bacterial growth and a significant statistical difference ($P< 0.022$) was reported also between the obturating materials in the antibacterial effect on the apical part, and ZOE was the best one which reported the lowest CFU numbers followed by Endoflas, Vitapex and finally the Iodoform. Presumably, the study of the antibacterial effect on these three parts of the teeth did not go too far as a research point, however, two studies supported these results and found that bacterial count in the dentinal tubules was more pronounced cervically than apically [38,39]. These results need more studying as the low reported number of bacteria in the apical part may not be totally due to the better antimicrobial action of the obturating materials in this part but it may be related to that reported by Mjor et al[40] who detected that the apical third of the root contains fewer dentinal tubules.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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