Evaluation of the antimicrobial effect of endodontic sealers on microbiota associated with root canal infections

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الخلاصة

جاءت الدراسة الحالية لتقييم التأثير الضد مايكروبي لعدد من المواد المستخدمة في ختم قناة جذر السن ضد الاحياء المجهرية المعزولة من جذور الاسنان المخمجة. تم خلال هذه الدراسة جمع 120 مسحة مأخوذة من قنوات جذور الأسنان العائدة لمرضى يعانون من خمج قنوات جذور الأسنان ، من المراجعين لعيادة طب الأسنان في مستشفى الديوانية التعليمي.

تم بأستخدام نظام الـ VITEK 2- Compact تشخيص المحتوى المايكروبي المصاحب لأصابات قنوات جذور الأسنان، كانت أنواع المسبحيات *Streptococcus* هي الاكثر عزلا وبنسبة (105 عزلة)، حيث تضمنت *S.pyogenes* بنسبة (16.5%)، *S.mutans* (15.6%)، *S.sangius* (9.5%)، *S.angiosus* (6.8%)، *S. pneumonia* (6.3%)، *S.intermedius* (6.3%)، *S.mitis* (4%)، *S.salivarius* (3.4%)، وكذلك عزلت *Enterococcus faecalis* في 5 قنوات فقط (3.4%)،كما أظهرت النتائج أن العنقوديات *Staphylococcus* عزلت من 25 مسحةمتمثلة بأنواع *S.aureus* (11.5%)، والـ *S.epidermides* (5.4%)، وكذلك عزلت عصيات الحليب *Lactobacillus acidophilus* بنسبة ضئيلة (3.4%). اما المبيضات *Candida albicans* فقد عزلت في 10 من قنوات الأسنان (8.1%) فقط.

أستخدم اختبار الانتشار لتحديد الفعالية الضد مايكروبية لعدد من المواد التجارية المستخدمة في ختم قناة جذر السن المخمجة من خلال قياس قطر منطقة التثبيط ، أظهرت النتائج أن اقل معدل لقطر التثبيط كان يعود لهيدروكسيد الكالسيوم (1.2 ملم)، في حين اعلى قطر كان لاوكسيد الزنك الممزوج بزيت القرنفل (14.9 ملم)، منطقة التثبيط لاوكسيد الزنك الممزوج بزيت القرنفل (التأثير الضد مايكروبي) كانت اعلى معنويا وبمعدل 13.7 ملم مقارنة مع المادة المرجع (هيدروكسيد الكالسيوم). استخدام مادة الختم الصمغية قد رفع من قطر منطقة التثبيط بمقدار 2.2 ملم مقارنة بمادة الختم المرجع.التأثير الضد مايكروبي لكل من اوكسيد الزنك الممزوج بزيت القرنفل و مادة الختم الصمغية كان اعلى مقارنة مع مادة الختم المرجع وبفروقات معنوية عالية ($p< 0.001$).

Abstract

The present study aimed to evaluate the antimicrobial effect of the most commonly used sealers against the microorganisms that isolated from teeth with root canal infections. One hundred and twenty patients whom attended dental clinic of Al-Diwaniyah Teaching Hospital for root canal treatment on one tooth only were included.

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By using VITEK 2- Compact system to identify the microorganisms associated with endodontic infections, *Streptococcus* bacteria were more frequently isolated (105 isolates), and this number was including *S.pyogenes* 24(16.5%) , *S. mutans* 23(15.6%), *S.sangius* 14(9.5%), *S.angiosus* 10(6.8%), *S.pneumonia* 9(6.3%), *S.intermedius* 9(6.3%), *S.mitis* 6(4%), *S.salivarius* 5(3.4%), *Enterococcus faecalis* had been recovered from root canals of 5(3.4%)teeth,. Also this study revealed that *Staphylococcus* bacteria were isolated from 25 infected root canal, consisting of *S.aureus* 17(11.5%) and *S.epidermides* 8(5.4%), whereas *Lactobacillus acidophilus* isolated in small percentage (3.4%). *Candida albicans* represented 12(8.1%) of tested isolations,

In this study, the agar diffusion test had been used to evaluate the antimicrobial effect of commercially used endodontic sealers, the mean diameter of inhibition zone was the smallest with calcium hydroxide (1.2 mm), whereas the highest was with zinc oxide eugenol (14.9 mm). The inhibition zone of zinc oxide eugenol (antimicrobial effect) was significantly higher by a mean of 13.7 mm compared to the reference material (calcium hydroxide). The use of Resin based sealer would significantly increase the diameter of inhibition zone by 2.2 mm compared to reference sealer material. The antibacterial effect of zinc oxide eugenol and resin based were both very high compared to that of reference sealer material.

Introduction

Endodontic infection are polymicrobial, *Streptococcu, Staphylococcus, Eubacterium*, *Prevotella*, *Porphyromonas Fusobacterium*, *Spirochaetes* , *Candida albicans, Campylobacter* and *Actinomyces* are found in the oral cavity[(1)]. Theoretically, all microorganisms present in the oral cavity may invade root canal and participate in endodontic infection.

The selective process takes place over time that allows anaerobic bacteria to predominate, 98% of bacteria that cultured from the canals were strict anaerobes (such as *Prevotella, Porphyromona, Fusobacterium*, and *peptostreptococcus*)[(2)].

Root canal sealers are used in root canal with combination of core filling materials, such as gutta-percha or silver points. An ideal root

canal sealer in addition to its ideal "sealing" properties, should leave the tooth in the most biologically inert condition possible and must prevent reinfection and growth of any microorganisms remaining in the canal, thereby favoring periapical tissue repair[3].

Calcium hydroxide $Ca(OH)_2$ has been used extensively in dentistry since the 1920s. Today, it is still the most commonly used endodontic medicament throughout the world[4]. Byström *et al.* reported that calcium hydroxide was an effective intracanal medicament rendering 34 out of 35 canals bacteria free after 4 weeks[5]. The effectiveness of inter appointment calcium hydroxide was also reported by Sjögren *et al.*, who demonstrated that a 7 days dressing with calcium hydroxide eliminated all bacteria in the root canal[6].

Zinc oxide-eugenol sealer has shown good impermeability, volume stability, adherence and dissolution, although it is irritating to periapical tissues. It has also shown good antibacterial activity when compared with calcium hydroxide-based sealers[7]. It has been established by Leonardo *et al.* and Kont *et al.* that eugenol is a potent antibacterial agent and is conceivable that it plays a major role within the activity of zinc oxide-eugenol based sealers. Eugenol is a bactericidal at relatively high concentrations being able to induce cell death and inhibit cell growth and respiration, even in lower concentrations[8,9].

The resin sealer has good sealing properties, and is adhesive and antibacterial. It is based on an epoxy resin that sets slowly when mixed with an activator. The material can initially produce a severe inflammatory reaction if present in tissue, but this subsides over a few weeks, and it is then well tolerated. Extensive toxicological studies as well as endodontic studies in subhuman primates[10], in addition to subcutaneous and bone implantation experiments in rats, Zmener *et al.* have shown safety and efficacy of the sealer[11].

In conclusion, after 5 years, the sealer seems to be well tolerated by periapical tissues, whereas the patients reported being comfortable [11].

Chemo-mechanical preparation is undoubtedly of paramount importance in successful endodontic treatment. However, this does not negate the importance of the quality of the obturation, in which the sealer has a role to play, due to this fact, the present study aimed to

evaluate the antimicrobial effect of the most commonly used sealers against microorganisms' isolated from teeth with root canal infections.

Materials and Methods

Patient Selection:

One hundred and twenty patients whom attended participating dental clinic of Al-Diwaniyah Teaching Hospital for root canal treatment on one tooth only were involved in this study. These patients, ranging in age from 20 to 60 years, all patients were in good health, with teeth that had no previous pulpal treatment. Patients with a history of antibiotic treatment within the previous 3 months period were excluded.

Endodontic Samples Collection:

Samples from infected root canals were collected using a modification of published methods[12,13].

The tooth was isolated with a dental dam and the surrounding field was cleaned with 30 % hydrogen peroxide and decontaminated with 2.5 % sodium hypochlorite for 30 sec. After removal of caries, involved dentin and existing restorations, the area was swabbed with 2.5 % sodium hypochlorite. Access to the pulp chamber and root canal was made with a new, sterile, no. 4 round bur without water spray. After minimal canal enlargement with sterile saline irrigant to allow access to the working length, dry, sterilized paper points were placed within the canal space for 30 sec. The paper points containing the absorbed root-canal contents were placed in sterile vials containing transport media, samples were then taken directly to the laboratory.

Isolation and Identification:

Each transport media swab was inoculated into universal tube containing 5 ml of nutrient broth and incubated at 37 C° for 18- 24 hrs, a loop full of broth was streaked on surface of nutrient agar, blood agar, MacConkey agar, and Sabourauds dextrose agar, which then incubated at the same conditions. Colonies had been identified depending cultural characteristics including morphological colonies characteristics and microscopic properties *i.e.* Gram's stained slide was done for each different isolate of microorganism that recovered from the different media to study microscopic properties of genera.

Diagnosis Via VITEK 2 – Compact:

VITEK 2 Compact is the next generation of the gold standard in microbial identification and represent advanced colorimetric technology, this system is equipped with an extended identification database for all routine identification tests (over 330 claims for gram-negative, gram-positive and yeast microorganisms) that provide an improved efficiency in microbial diagnosis which reduce the need to perform any additional tests.

Procedure:

All the steps were done according to the manufacturer's instructions. (Biomerieux). Briefly, 3 ml of normal saline was placed in plane test tube and inoculated with a lope full of isolated colony, the test tube was inserted into a DensCheck machine for standardization of colony to McFarland standard solution ($1.5*10^8$ cell / ml), the standardized inoculums was placed into the cassette and a sample identification number entered into the computer software via barcode, the VITEK 2 card type was then readied from barcode placed on the card during manufacture and the card was thus connected to the sample ID, the cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader/ incubator module. All subsequent steps were handled by the instrument. When the test cycle is completed, the system automatically ejected the cards into a waste container.

Effect of Endodontic Sealers on Root Canal Microbiota:

Three types of commercial sealers had been used in this study including zinc oxide eugenol, calcium hydroxide, and resin based sealer; each sealer was prepared according to manufacturer's instructions (Meta Biomed -Korea). Microbial inoculation was done using sterile cotton-tipped application, and three wells of 4mm depth and 6mm diameter wide were punched in each Muller Hinton agar plate and filled with the freshly prepared sealers.

Plates were incubated at 37°C for 18-24hrs. Afterward, the diameters of the zones of microbial inhibition were measured and recorded for each sealer tested.

Statistical Analysis:

Statistical analysis were computer assisted using SPSS version 13. P value less than 0.05 level of significance was considered statistically significant.

Results and Discussion

Table (1) shows the different microorganisms isolated from root canal samples .The *Streptococcus* species were more frequently isolated (105 isolates), and this number including *S.pyogenes* 24(16.5%) , *S.mutans* 23(15.6%), *S.sangius* 14(9.5%), *S.angiosus* 10(6.8%), *S. pneumonia* 9(6.3%), *S.intermedius* 9(6.3%), *S.mitis* 6(4%), *S.salivarius* 5(3.4%)(Figure 1). These results consistent with study done by Lamont and Jenkinson (2010) [14] whom showed that most prevalent genus associated with root canal infections was *Streptococcus* . *E. faecalis* had been recovered from root canals of 5(3.4%) teeth, and such result agree with that of Ercan *et al.* [15], who showed that among 100 root canal samples, *E. faecalis* presented in 3(1.5%)teeth only. Also the recent study revealed that *Staphylococcus* bacteria isolated from 25 infected root canal, consisting of *S.aureus* 17(11.5%), *S. epidermides* 8(5.4%), and such outcome was consistent with Gomes *et al.* [16] who reported that Streptococci spp. were the most frequently isolated bacteria followed by Staphylococci (13.3%), Lactobacilli spp. (8.3%). The results showed that *Lactobacillus acidophilus* also isolated in small percentage (3.4%), and this findings were similar to that of Moüller [17] and Sundqvist *et al*. [18] whom both recorded that samples from root canal had frequently shown Gram-positive facultative including Streptococci, Lactobacilli and Enterococci.

The present study showed that *Candida albicans* represented 12(8.1%) of tested isolations (Figure 3.1), and such finding was consent with Ercan *et al.* [15] who showed that among 100 root canal samples, *C. albicans* had been recorded in only 8 (4.1%) samples.

These differences in results of the present study may due to great inter-individual variability in endodontic communities associated with the same clinical disease *i.e.* each individual harbors a unique endodontic microbiota in terms of species richness and abundance; and this inter-individual variability is still more pronounced when individuals from different geographical locations are analyzed [19,20,21].The type and combination of microbial microbiota are developed in response to the surrounding environment. Factors that influence whether species shall die or survive include the particular ecological niche, nutrition, an aerobiosis, PH, and competition with other microorganisms[3].

Table (1): Relative frequency of isolated microbiota.

Positive Microorganism (N[1]=120)	Microbial species	No.[2]	% of patients	95% Confidence interval
Staphylococcus	*S. aureus* *S. epidermides*	25	20.8	(14.2% - 29.4%)
Streptococcus	*Strept. pyogenes* *Strept. mutans* *Strept. sangius* *Strept. angiosus* *Strept. pneumonia* *Strept. intermedius* *Strept.mitis* *Strept. salivarius* *E. faecalis*	105	87.5	(79.9% – 92.6%)
Candida	*C. albicans*	12	10	(5.5% – 17.2%)
Lactobaciillus	*L. acidophilus*	5	4.2	(1.5% - 9.9%)
1:Number of patients, 2 :Number of isolates (mixed infections).				

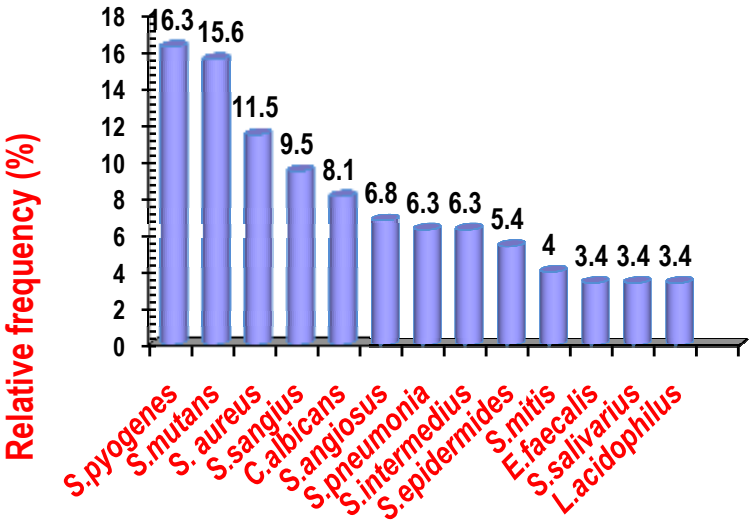


Figure (1): Bar chart showing the relative frequency of isolated microbiota.

In this study, the agar diffusion test had been used, which is the most widely used *in vitro* method for the evaluation of antimicrobial activity. This method allows direct comparisons between materials and also indicates which sealers are more likely to have antimicrobial activity within the root canal system. Besides that, its results are highly influenced by the diffusibility of the material across the medium[(22)].

As shown in Table 2, three different sealers were tested for their antimicrobial effect through diameter of inhibition zone. The mean diameter of inhibition zone was the smallest with calcium hydroxide (1.2 mm), therefore it was used as a reference material to compare the effect of the other two materials. On the other hand the mean inhibition zone diameter was the highest with Zinc oxide eugenol (14.9 mm). The difference in mean inhibition zone diameter was significantly different between the three types of sealers. The inhibition zone of Zinc oxide eugenol (antimicrobial effect) was significantly higher by a mean of 13.7 mm compared to the reference material, *i.e.* its effect is stronger than that of reference sealer by 11.4 times. The use of Resin based sealer would significantly increase the diameter of inhibition zone by 2.2 mm (1.8 times greater) compared to reference sealer material. The antibacterial effect of Zinc oxide eugenol and resin based were both very high compared to that of reference sealer material (Calcium hydroxide)(Figure 2,3). It was higher for Zinc oxide eugenol (Cohen's d=13.2) than for resin based (Cohen's d=3.8).

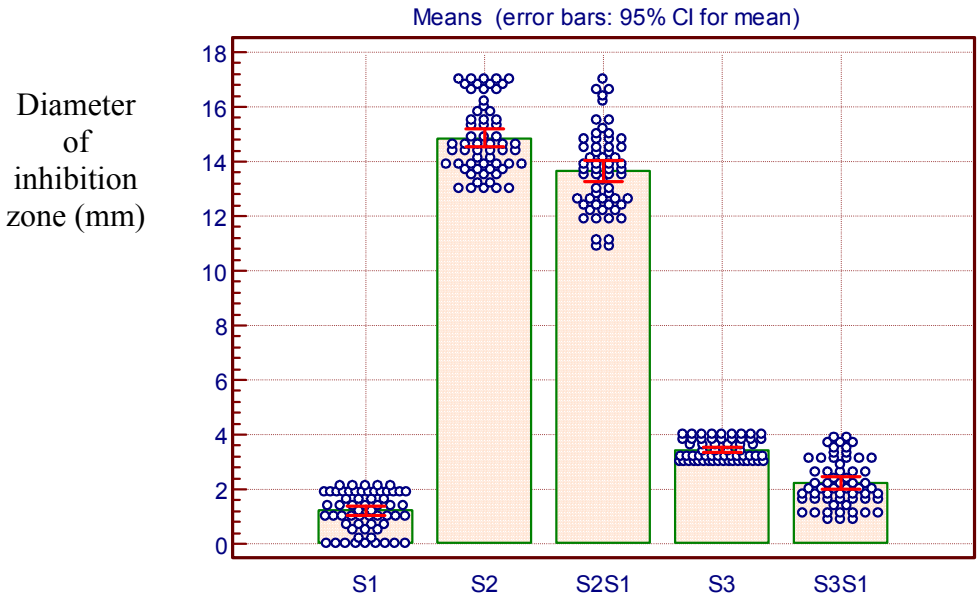
Table (2) : The difference in mean inhibition zone diameter (mm) of three different sealer materials.

	Calcium hydroxide	**Zinc oxide eugenol**	**Resin based sealer**	**P value**
Range	(0 - 2.2)	(13 - 17)	(3 - 4)	<0.001
Mean	1.2	14.9	3.4	
SD	0.72	1.28	0.37	
SE	0.093	0.165	0.048	
Mean difference compared to Calcium hydroxide (sealer)	**Reference***	13.7	2.2	
SE of mean difference		0.189	0.11	
Change ratio		11.4	1.8	
Cohen's d (effect size)		13.2	3.8	
Bonferonni adjusted paired T-test		<0.001	<0.001	

* :calcium hydroxide as a reference material to compare the effect of the other two materials.

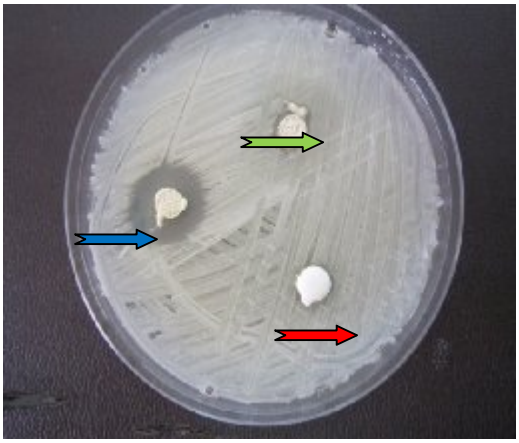
The antimicrobial effect of root canal sealers containing zinc oxide-eugenol cement was at attributable to free eugenol liberated from the set material, a phenolic compound, which is effective against mycotic cells and vegetative form, a previous study had shown that ZnOE sealer can release formaldehyde after setting and the combined effects of eugenol and formaldehyde might be the reason why ZnOE is highly

antimicrobial. In the same way, the antimicrobial effect of resin-based sealers may be related to release of formaldehyde in the polymerization process[8], whereas calcium hydroxide sealer shown to be appropriate for elimination of bacteria depends on ionization that releases OH–ions, causing an increase in PH. A PH >9 may reversibly or irreversibly inactivate cellular membrane enzymes of the microorganism, resulting in a loss of biological activity[23].



Figure(2): Dot diagram with error bars showing the mean (with its 95% confidence interval) of inhibition zone diameter (mm) between three different sealer materials.

S1	Calcium hydroxide
S2	Zinc oxide eugenol
S3	Resin based
S2S1	Difference between Zinc oxide eugenol andCalcium hydroxide
S3S1	Difference between Resin based and Calcium hydroxide



Figure(3): Agar diffusion test showing the antimicrobial effect of three types of endodontic sealers on root canal microbiota.

Calcium hydroxide
Zinc oxide eugenol
Resin based sealer

Results of this study agree with studies of Leonardo *et al.*[8], Siqueira *et al.*[22]. Also agree with Chang *et al.*[24],who studied the antimicrobial properties of four commonly used endodontic sealers: two epoxy-resin-based sealers one zinc-oxide eugenol-based sealer and one calcium hydroxide-based sealer, the testing microbes were four facultative anaerobic species and four obligate anaerobic species; their study showed that the ZnOE sealer most effective against the microorganisms followed by resin base sealer then calcium hydroxide sealer.

As a conclusion, our findings indicated a potential complex interactions of species resulting in characteristic clinical pictures which cannot be achieved by individual species alone. The sealers evaluated in this study showed different inhibitory effects in which root canal sealers containing eugenol proved to be the most effective against the microorganisms.

References

1. Aas, A. J. ; B. J. Paster; L. N. Stokes; I. Olsen; & F. E. Dewhirst (2005) " Defining the Normal Bacterial Flora of the Oral Cavity Journal of Clinical Microbiology. 43(11): 5721- 5732.
2. Ingle, J. (2008)" Ingle's Endodontics". 6th edition. Peoples Medical Publishing House USA. DDS, Leif Bakland

3. Cohen, S. ; & R. Burn (2002) "Path way of pulp".6th edi. 13: 363-376
4. Tang, G. ; L. P. Samaranayake; & H-K Yip (2004) "Molecular evaluation of residual endodontic micro-organisms after instrumentation, irrigation and medication with either calcium hydroxide or Septomixine". Oral Dis.10:389-397.
5. Byström, A. ; R. Claesson; & G. Sundqvist (1985) "The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals". Endod. Dent. Traumatol.1:170-175.
6. Sjo¨gren, U. ; D. Figdor; L. Spa°ngberg; & G. Sundqvist (1991) "The antimicrobial effect of calcium hydroxide as ashort term intracanal dressing". Int. Endod. J.24:119-125.
7. Kaplan, A. E. ; M. Picca; M. I. Gonzalez; R. L. Macchi;& S. L. Molgatini (1999) " Antimicrobial effect of six endodontic sealers: an in vitro evaluation". Endod. Dent. Traumatol.15(1): 42-5.
8. Leonardo, M. R. ; L. A. da Silva; M. Tanomaru Filho; K. C. Bonifacio; & I.Y. Ito (2000)"In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics". J. Endod.26(7): 391-4.
9. Kont, F.; H. Cenk; & O. Erganis (2004) "In vitro antibacterial activities of root canal sealers by using two different methods". J. Endod.30 (1): 57 – 60
10. Louw, N. P. ; C. H. Pameijer; & G. Norval (2001) "Histopathological evaluation of a root canal sealer in subhuman primates". J. Dent. Res. 79:654. (abstr).
11. Zmener, O. ; G. Banegas; & C. H. Pameijer (2005) "Bone tissue response to a methacrylate-based endodontic sealer: a histological and histometric study". J. Endod.31:457-9.
12. Fouad, A. F. ; J. Barry; M. Caimano; M. Clawson; Q. Zhu; R. Carver; K. Hazlett; & J. D. Radolf (2002) "PCR-based identification of bacteria associated with endodontic infections". J. Clin. Microbiol. 40: 3223-3231
13. Hoshino, E. (1990) "Sterilization of carious lesions by drugs". Journal of the Japanese Association for Dental Science .9:32-7.

14. Lamont, R. J. ; H. F. Jenkinson (2010) "Oral Microbiology at a Glance "Wiley-Blackwell. Singapore, Hong Kong.
15. Ercan, E. ; M. Dalli; İ. Yavuz; T. Özekinci (2006)"Investigation of microorganisms in infected dental root canals". Biotechnol. & Biotechnol. Eq. 2 (166).
16. Gomes, B. P. F. ; R. C. Jacinto; E. T. Pinheiro *et al*.(2004) *Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia* and *Prevotella nigrescens* in en- dodontic lesions detected by culture and by PCR. Endodontic Topics. 20(4):1–8.
17. Möller, A. J. R. (1966) "Microbial examination of root canals and periapical tissues of human teeth". Odontol. Tidskr.74:1-380.
18. Sundqvist, G. ; D. Fidgor ; S. Persson ; U. Sjogren (1998) " Microbiologic Analysis Of Teeth With Failed Endodontic Treatment and The Out Come Of conservative re-treatment" .Oral. Surg. Oral Med. Oral Patho. Oral Radiol. Endod. 85; 86-93.
19. Narayanan, L. ; & C. Vaishnavi (2010) "Endodontic microbiologyp" J. of conservative dentistry 13(4):233-239.
20. Peters, L. B. ; A. J. van Winkelhoff; J. F. Buijs; & P. R. Wesselink (2002) "Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions"Journal of Endodontics Research.
21. Siqueira, J. F. ; I. N. Rocas (2009) "Distinctive feature of the microbiota associated with different forms of apical periodontitis". J. O. M.1.
22. Siqueira, J. F. ; A. Favieri; S. M. Gahyva; S. R. Moraes; K. C. Lima; & H. P. Lopes (2000) "Antimicrobial activity and flow rate of newer and established root canal sealers". J. Endod.26(5): 274-7.
23. Estrela, C. ; G.B. Sydney; L. L. Bammann; & O. Jr. Felippe (1995) "Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria". Bras. Dent. J. 6:85-90.
24. Chang, Y. C. ; F. M. Huang;K. W. Tai; & M. Y. Chou (2001) 'The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells". Oral Surg. Oral Med.Oral Pathol. Oral Radiol. Endod. 92: 446-450.