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Which treatment protocol, among classical methods and/or various laser applications is the most effective in root canal disinfection, *in vitro*? A systematic review

Joanna Theodosopoulou[1], Alexandra Tsigarida[2], Konstantinos Chochlidakis[3]

[1]Harvard School of Dental Medicine (HSDM), The Forsyth Institute, Private Practice, Athens, Greece;
[2]Department of Periodontology, The Ohio State University, Columbus, USA;
[3]Department of Prosthodontics, Eastman Institute for Oral Health, Rochester, USA.
Email: joanna_theodosopoulou@yahoo.gr

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ABSTRACT

Purpose: The aim of this systematic review was to answer the question "Which treatment protocol, among classical methods and/or various laser applications is the most effective in root canal disinfection, *in vitro*". Materials and Methods: A MEDLINE, a Cochrane and an Embase search (three specified searches) were conducted to identify randomized controlled trials (RCT) until June 2010, conducted on human teeth and published in English, German or French language, examining the root canal disinfection after the use of lasers with or without mechanical instrumentation. Additionally, hand search was conducted and contact with authors, when needed. Results: The MEDLINE, the Cochrane and the EMBASE search identified 240, 28, and 35 published articles, respectively. Ten articles from the MEDLINE and 5 articles from the Cochrane search (that were also identified in the MEDLINE search) met the inclusion and validity assessment criteria. In *E. faecalis* elimination, instrumentation of the root canal and diode laser/665 nanometer/1 Watt (diode laser/665 nm/1 W) irradiation with the combined effect of Methylene Blue (MB) as photosensitizing agent (logCFU/ml = 1.636) seemed to be the best method. In *P. aeruginosa* and in *A. naeslundii* elimination, instrumentation of the root canal followed by irrigation with 5.5% NaOCl (logCFU/ml = 0) seemed to be the best method. In general, instrumentation of the root canal followed by irrigation with 5.25% NaOCl (logCFU/ml = 0) and instrumentation of the root canal and Er: YAG laser/2940 nm/0.8 W irradiation (logCFU/ml = 1.924) seemed to be the best (polymicrobial studies). Conclusions: There are treatment protocols with the assistance or not of laser irradiation that can eliminate *E. faecalis*, *E. coli* and *S. aureus* inside the root canal. However, there is a serious number of *S. anginosus*, *F. nucleatum*, *A. naeslundii* and *P. aeruginosa* that remain inside the root canal even after laser irradiation. New research is needed in order to set a treatment protocol effective in the root canal disinfection from all bacteria that are related to endodontic origin pathology.

Keywords: Lasers; Classical Methods; Endodontic; Root Canal Therapy; Dentin; Bacteria; Disinfection

1. INTRODUCTION

One of the most crucial and fundamental stages of endodontic therapy is the root canal disinfection in its three-dimensional network of dentinal tubules. Nevertheless, persistence of infection in the root canal is responsible for long-term failures and need for endodontic therapy retreatments.

It is generally accepted that microorganisms tend to remain in the root canal even after proper preparation and are responsible for flare-ups, after the completion of the endodontic therapy. The most common of these microorganisms are: *Fusobacterium nucleatum*, *Enterococcus faecalis*, *Prevotella intermedia*, *Streptococcus anginosa*, *Treponima denticolla*, *Porphyromonas gingivalis* [1-4].

During the last years, laser irradiation has been additionally introduced in root canal preparation, trying to gain acceptance for its disinfection ability in comparison with the common mechanical instrumentation and irrigation procedures.

Many studies examine the effectiveness of Nd:YAG, diode, Er,Cr:YSGG and Er:YAG laser, when used in different wavelengths, solely, or in addition with various solutions in the bacterial elimination inside the root canals.

The purpose of this systematic review was to answer the question "Which treatment protocol, among classical methods and/or various laser applications is the most effective in root canal disinfection, *in vitro*".

2. MATERIALS AND METHODS

2.1. Literature Search

One electronic search of MEDLINE from 1966 to June 2010 (**Table 1**), one Cochrane (**Table 2**) and one Embase search from 1945 to June 2010 (**Table 3**) were conducted.

Table 1. Medline search strategy.

#	Search history	Results
#13	Search **#12** Limits: **Humans, English, French, German, Greek, Modern**	240
#12	Search **#11 AND #1**	282
#12	Search **#11 AND #1**	13,136
#11	Search **#7 AND #10**	15,641
#10	Search **#8 OR #9**	14,900
#9	Search **(root canal therapy)**	1622
#8	Search **(endodontically treated teeth)**	4,830,685
#7	Search **#1 OR #2 OR #3 OR #4 OR #5 OR #6**	138,039
#6	Search **(tooth OR teeth)**	1,267,279
#5	Search **bacteria**	3,639,649
#4	Search **method**	19,546
#3	Search **dentin**	14,477
#2	Search **endodont***	137,179
#1	Search **laser**	240

Table 2. Cochrane search strategy.

#	Search history	Results
#1	Laser	6294
#2	Endodont*	1174
#3	Dentin	1427
#4	Method	241,235
#5	Bacteria	4978
#6	(Tooth OR teeth)	7062
#7	(#1 OR #2 OR #3 OR #4 OR #5 OR #6)	248,592
#8	(Endodontically treated teeth)	89
#9	(Root canal therapy)	401
#10	(#8 OR #9)	442
#11	(#7 AND #10)	422
#12	(#1 AND #11)	28

Table 3. Embase search strategy.

#	Search history	Results
1	Laser	106,390
2	Endodont*	1076
3	Dentin	2983
4	Method	785,208
5	Bacteria	121,213
6	(Tooth OR teeth)	40,114
7	(#1 OR #2 OR #3 OR #4 OR #5 OR #6)	5,138,833
8	(Endodontically treated teeth)	43
9	(Root canal therapy)	64
10	(#8 OR #9)	1,426,192
11	(#7 AND #10)	218,687
12	(#1 AND #11)	225,630
13	Limit 12 to humans, English or French or German or Greek from "1945-2007"	35

2.2. Inclusion Criteria-Validity

Three independent reviewers examined all the identified abstracts to determine whether they met the following criteria:

1) Study *in vitro*.
2) Conducted in human teeth.
3) Related to the question.
4) Experimental and control group.
5) Quantitative results provided.
6) English, German, French languages.

Whenever it was not possible to make this determination, the article was examined in full text. Subsequently, all relevant articles were obtained and a determination whether or not they met the inclusion criteria was made by three reviewers. It is important to state that only studies measuring the bactericidal effect of lasers and other procedures were included. The studies that examined the removal of smear layer or debris, the morphological or histological changes, the apical leakage after obturation and the dentin permeability were excluded, as irrelevant to our question (**Tables 8-12**).

All articles that met the inclusion criteria were assessed for validity. Validity was determined on a 7-point scale (**Table 4**) and studies not meeting 5 or more of the 7 validity criteria were excluded.

All articles were classified by evidence level (**Table 5**) (EBM://cebm.jr2.ox.uk/docs/levels.html) and then assessed for Validity (http://www.cebm.utoronto.ca/teach/materials/therapy.htm) (**Table 6**).

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Table 4. Validity assessment criteria[1].

1. Was the assignment of patients of treatment randomised?
2. Was the randomisation list concealed?
3. Was the follow-up of patients sufficiently long and complete?
4. Were all patients analysed in the groups to which they were randomised?
5. Were patients and clinicians blinded to the treatment being received?
6. Aside from the experimental treatment, were the groups treated equally?
7. Were the groups similar at the start of the trial?

Table 5. Clinical evidence levels[2].

Level of evidence	Study Type	#
1A	Randomized control trial (RCT) Systematic Review of RCTs	6
1B	Controlled Trial Systematic review of CCTs	2
2	Cohort Study(CCS) Systematic review of CSs	2
3	Case Control Study Systematic Review of CCSs	0
4	Case Series	0
5	Expert's Opinion Narrative Review	0
NA[3]	Cross Sectional Case Reports Animal Studies Laboratory Studies	0
Non validated	Because of language limitations	0

Table 6. Validity assessment criteria application.

Reference	1	2	3	4	5	6	7
De Souza, E. B. *et al.*, 2008	Y	Y	Y	Y	N	Y	Y
Fimple, J. L. *et al.*, 2008	Y	Y	Y	Y	N	Y	Y
Fonseca, M. B. *et al.*, 2008	N	N	Y	Y	N	Y	Y
Bergmans, L. *et al.*, 2008[4]	Y	Y	Y	Y	N	Y	Y
Foschi, F. *et al.*, 2007	Y	Y	Y	Y	N	Y	Y
Wang, Q. Q. *et al.*, 2007[4]	Y	Y	Y	Y	N	Y	Y
Gordon, W. *et al.*, 2007	N	N	Y	Y	N	Y	Y
Schoop, U. *et al.*, 2007	N	N	Y	Y	N	Y	Y
Soukos, N. S. *et al.*, 2006	N	N	Y	Y	N	Y	Y
Vezzani, M. S. *et al.*, 2006	N	N	Y	Y	N	Y	Y
Bergmans, L. *et al.*, 2006[4]	Y	Y	Y	Y	N	Y	Y
Perin, F. M. *et al.*, 2004	N	N	Y	Y	N	Y	Y
Kreisler, M. *et al.*, 2003	N	N	Y	Y	N	Y	Y
Dostαlovα, T. *et al.*, 2002	N	N	Y	Y	N	Y	Y
Schoop, U. *et al.*, 2002	N	N	Y	Y	N	Y	Y
Piccolomini, R. *et al.*, 2002[4]	Y	Y	Y	Y	N	Y	Y
Folwaczny, M. *et al.*, 2002[4]	Y	Y	Y	Y	N	Y	Y
Moritz, A. *et al.*, 1999	Y	Y	Y	Y	N	Y	Y
Mehl, A. *et al.*, 1999	Y	Y	Y	Y	N	Y	Y
Moritz, A. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Ramskold, L. O. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Gutknecht, N. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Moshonov, J. *et al.*, 1995	N	N	Y	Y	N	Y	Y
Fegan, S. E. *et al.*, 1995	N	N	Y	Y	N	Y	Y
Hardee, M. W. *et al.*, 1994	N	N	Y	Y	N	Y	Y

[1]Evidence-Based Medicine. How to Practise and Teach EBM, 2nd edition, Sackett, D. *et al.*, Churchill Livingstone, Edinburgh, UK.
[2]http://www.cebm.net/index.aspx?o=1025
[3]Not applicable.
[4]Also identified in Cochrane search.

2.3. Data Analysis

All results were converted and finally expressed in log-CFU/ml (logarithm of the Colony Forming Units per milliliter) of the bacteria that were found after root canal preparation and laser or not laser irradiation. This was done for all specimens of all the studies.

Data from the studies that met validity criteria were extracted and classified by the method used for each bacterial group (**Tables 15-21**), for the polymicrobial studies (**Table 22**) and for all of the studies together (**Table 23**).

3. RESULTS

3.1. Medline Search

The MEDLINE search from 1966 to June 2010 identified 240 articles (**Table 7**). From 240 articles identified by the search, the hand examination of titles, abstracts and articles in full text revealed that 120 were irrelevant and 120 appeared to be relevant. Of the 120 relevant articles, 6 were *in vivo* studies, 5 expert's opinion, 6 narrative reviews, 3 case reports, 1 was an animal study and the remaining 99 were relevant *in vitro* studies.

Of the 99 relevant *in vitro* studies:

- Seventy were excluded because they were not related to the question.
  Analytically:
  Seventeen examined the removal of smear layer and debris (**Table 8**).
  Twenty four examined morphological changes of the root canal (**Table 9**).
  Twelve examined obturation and apical leakage after obturation (**Table 10**). Eleven examined dentin permeability (**Table 11**).
  Five examined the thermal effects on the dentin (**Table 12**).
  One examined adhesion of root canal sealers [5].
- One was excluded because it was not conducted on human teeth but on human teeth slices [6].
- Three were excluded because they did not provide quantitative results [7-9].

From the remaining 25 articles, 15 [10-24] were excluded because they met less than 5 of 7 validity criteria (**Table 13**). Ten articles [25-34] were finally included. Details of the included 10 studies are presented in **Table 14**.

3.2. Cochrane Search

The Cochrane search identified 28 articles. From these 28 articles the hand examination of titles, abstracts and articles in full text, revealed that 16 were irrelevant to the question and 12 appeared to be relevant. Of the 12 articles, 7 did not meet the inclusion criteria and the remaining 5 were relevant *in vitro* studies. All of the 5 relevant *in vitro* studies were also identified by the Medline

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Table 7. Search results.

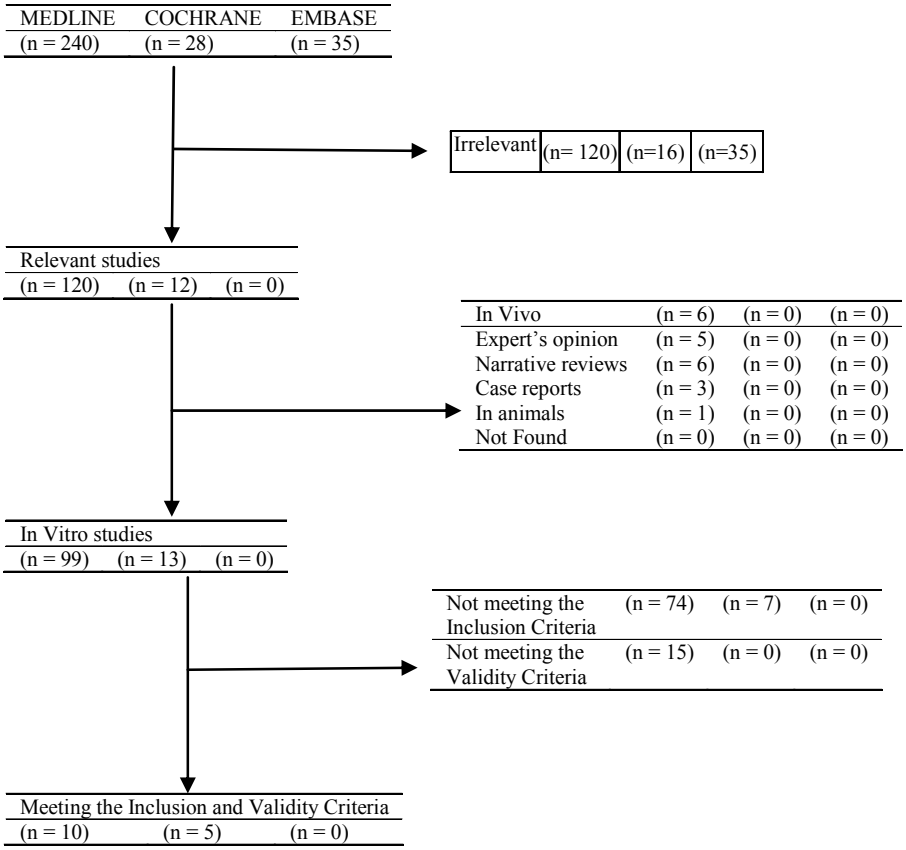

Table 8. Excluded studies: remove smear layer/debris.

Reference	Detail
Soares F. *et al.*, 2008	Remove smear layer
Moshonov J. *et al.*, 2004	Remove smear layer
Moshonov J. *et al.*, 2003	Remove smear layer
Matsuoka E. *et al.*, 1998	Remove smear layer
Takeda F. H. *et al.*, 1998	Remove smear layer
Arrastia-Jitosho A. M. *et al.*, 1998	Remove smear layer
Takeda F. H. *et al.*, 1998	Remove smear layer
Radatti D. A. *et al.*, 2006	Remove debris
Blum J. Y. *et al.*, 1997	Remove debris
Harashima T. *et al.*, 1997	Remove debris
Saunders W. P. *et al.*, 1995	Remove debris
Machida T. *et al.*, 1995	Remove debris, Thermal effects
Moshonov J. *et al.*, 1995	Remove debris
Bahcall J. K. *et al.*, 1993	Remove debris, Morphological
Frentzen M. *et al.*, 1991	Remove debris
Liesenhoff T. *et al.*, 1989	Remove debris, Morphological
Pini R. *et al.*, 1989	Remove debris

search, met all the inclusion criteria and 5 or more of the validity assessment criteria. So, they were finally included (**Table 6**).

3.3. Embase Search

The Embase search from 1945 to June 2010 identified 35 articles. From these 35 articles identified by the search, the hand examination of titles, abstracts and articles in full text, revealed that all of them were irrelevant.

In order to be able to compare the bactericidal ability of the various treatment protocols that were applied on the human teeth *in vitro*, by each different group of researchers, the results were expressed in logCFU/ml. The CFU/ml of the bacteria that were found in the root canals after bacterial contamination and consecutive standard root canal preparation and/or laser irradiation was calculated and converted in logCFU/ml. The closer the value of logCFU/ml is to 0, the greater the positive effect the treatment protocol has to the root canal disinfecttion.

LogCFU/ml of all bacteria is plotted in **Tables 15-23** (Clustered bars and 3-D clustered bars). On the X-axis lies the logCFU/ml and on the Y-axis the various treatment protocols.

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Table 9. Excluded articles: morphological changes.

Reference	Details
Gurbuz, T. *et al.*, 2008	Morphological study
Da Costa Ribeiro, A. *et al.*, 2007	Morphological, Thermal effects
Jahan, K. M. *et al.*, 2006	Morphological
Altundasar, E. *et al.*, 2006	Morphological and Histochemical Changes
Matsuoka, E. *et al.*, 2005	Morphological study
Biedma, B. M. *et al.*, 2005	Morphological study
Camargo, S. E. *et al.*, 2005	Morphological study
Niccoli-Filho, W. *et al.*, 2005	Morphological study
Ali, M. N. *et al.*, 2005	Morphological study, Smear layer removal
Ishizaki, N. T. *et al.*, 2004	Morphological, Thermal effects
Khabbaz, M. G. *et al.*, 2004	Morphological study
Kesler, G. *et al.*, 2002	Morphological study, Remove smear layer
Kaitsas, V. *et al.*, 2001	Morphological and Histological changes
Matsuoka, E. *et al.*, 2000	Morphological study
Yamazaki, R. *et al.*, 2001	Morphological, Thermal effects
Barbakow, F. *et al.*, 1999	Morphological study, Remove smear layer
Takeda, F. H. *et al.*, 1999	Morphological study, Remove smear layer
Eto, J. N. *et al.*, 1999	Morphological study, Remove smear layer
Takeda, F. H. *et al.*, 1998	Morphological study, Remove smear layer
Harashima, T. *et al.*, 1998	Morphological study, Remove smear layer
Khan, M. A. *et al.*, 1997	Morphological, Thermal effects
Komori, T. *et al.*, 1997	Morphological, Thermal effects
Goodis, H. E. *et al.*, 1993	Morphological study, Remove smear layer
Gutknecht, N., Behrens, V. G. 1991	Morphological study

Table 10. Excluded articles: obturation/apical leakage.

Reference	Details
Ebihara A. *et al.*, 2002	Apical leakage after obturation
Kimura Y. *et al.*, 1999	Apical leakage after obturation
Kimura Y. *et al.*, 2001	Apical leakage after obturation
Park D. S. *et al.*, 2001	Apical leakage after obturation
Goya C. *et al.*, 2000	Apical leakage after obturation, Removal of smear layer
Yamazaki R. *et al.*, 1999	Apical leakage after obturation
De Moura-Netto C. *et al.*, 2007	Apical sealing
Gekelman D. *et al.*, 2002	Apical sealing of root canal fillings
Carvalho C. A. *et al.*, 2002	Apical sealing of root canal fillings.
Varella C. H., Pillegi R., 2007	Obturation
Gharib S. R. *et al.*, 2007	Obturation
Wang X. *et al.*, 2005	Obturation, Morphological, Thermal effects, Apical leakage

Table 11. Excluded articles: dentin permeability.

Reference	Details
Winik, R. *et al.*, 2006	Marginal permeability, Tubular penetration,
Al-Azzawi, L. M. *et al.*, 2006	Dentin permeability
Aranha, A. C. *et al.*, 2005	Dentin permeability
Oliveira, R. G. *et al.*, 2004	Dentin permeability after apicoectomy
Gouw-Soares, S. *et al.*, 2004	Dentin permeability
Arisu, H. D. *et al.*, 2004	Dentin permeability, Morphological
Brugnera, A. Jr. *et al.*, 2003	Dentin permeability
Lee, B. S. *et al.*, 2002	Dentin permeability
Pecora, J. D. *et al.*, 2000	Dentin permeability
Stabholz, A. *et al.*, 1992	Dentin permeability
De Souza, F. D. *et al.*, 2005	Coronal microleakage

Table 12. Excluded articles: thermal effects/adhesion of root canal sealers

Reference	Details
Nammour S. *et al.*, 2004	Thermal effects
Deutsch A. S. *et al.*, 2004	Thermal effects
Amyra T. *et al.*, 2000	Thermal effects, Morphological
Cohen B. I. *et al.*, 1996	Thermal effects
Neev J. *et al.*, 1993	Thermal effects

Table 13. Excluded studies: not meeting validity criteria.

Reference	1	2	3	4	5	6	7
Fonseca, M. B. *et al.*, 2008	N	N	Y	Y	N	Y	Y
Gordon, W. *et al.*, 2007	N	N	Y	Y	N	Y	Y
Schoop, U. *et al.*, 2007	N	N	Y	Y	N	Y	Y
Soukos, N. S. *et al.*, 2006	N	N	Y	Y	N	Y	Y
Vezzani, M. S. *et al.*, 2006	N	N	Y	Y	N	Y	Y
Perin, F. M. *et al.*, 2004	N	N	Y	Y	N	Y	Y
Kreisler, M. *et al.*, 2003	N	N	Y	Y	N	Y	Y
Dostαlovα, T. *et al.*, 2002	N	N	Y	Y	N	Y	Y
Schoop, U. *et al.*, 2002	N	N	Y	Y	N	Y	Y
Moritz, A. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Ramskold, L. O. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Gutknecht, N. *et al.*, 1997	N	N	Y	Y	N	Y	Y
Moshonov, J. *et al.*, 1995	N	N	Y	Y	N	Y	Y
Fegan, S. E. *et al.*, 1995	N	N	Y	Y	N	Y	Y
Hardee, M. W. *et al.*, 1994	N	N	Y	Y	N	Y	Y

A) Analysis of the Results of Studies with Specific Bacteria

- ***Enterococcus faecalis***

Enterococcus faecalis survival was examined 19 times by 5 different groups of researchers.

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Table 14. Analysis of treatment protocols.

REFERENCES/ BACTERIA	STORAGE MATERIAL	IRRIGATION SOLUTION	INSTRUMENTATION	LASER	DURATION	NUMBER OF TEETH
De Souza E. B. *et al.* (2008). ***E. faecalis*** ($3*10^8$ cells/ml)	1% NaOCl	G1, G2: 0.5% NaOCl + urea peroxide cream + 17% EDTA-T + saline solution G3: Saline solution	G1, G2,: Rotary instrumentation (Crown down), No 50.	G1: diode laser (830 nm/3 W) G2: no laser irradiation G3: no laser irradiation (control)	2 days	N = 30 G1 = 10 G2 = 10 G3 = 10
Fimple J. L. *et al.* (2008) ***A. israelii*** ($2.5*10^8$ cells/ml) ***F. nucleatum*** ($2.5*10^8$ cells/ml) ***P. gingivalis*** ($2.5*10^8$ cells/ml) ***P. intermedia*** ($2.5*10^8$ cells/ml)	0.5% NaOCl for 2 - 4 weeks.	G1 - G4: RcPrep + 6% NaOCl + 17% EDTA + 6% NaOCl	G1 - G4: Rotary instrumentation (Crown-down) MAF: 0.465	First set of experiments (n = 72, BHI broth): G1: No laser/No MB G2: MB only G3: Diode laser (665 nm/1 W) G4: Diode laser (665 nm/1 W) and MB Second set of experiments (n = 39, PBS broth): G1: No laser/No MB G2: MB only G3: Diode laser (665 nm/1 W) and MB	7 days	First set of experiments (n = 72): G1 = 19 G2 = 18 G3 = 17 G4 = 18 Second set of experiments (n = 39): G1 = 13 G2 = 13 G3 = 13
Bergmans L. *et al.* (2008) ***S. anginosus*** ($4*10^8$ cells/ml)	0.5% chloramine in water at 4˚C	Ga - Gd: 2.5% NaOCl + 17% EDTA + tap water	Ga - Gd: Rotary instrumentation (Crown down), No 30.	Ga: diode laser(635 nm/0.1 W) + TBO + RTF Gb: diode laser(635 nm/0.1 W) + RTF Gc: TBO + RTF Gd: RTF (positive control)	2 days	N = 12 Ga = 3 Gb = 3 Gc = 3 Gd = 3
***E. faecalis*:** ($4*10^8$ cells/ml)	0.5% chloramine in water at 4˚C	Ga - Gd: 2.5% NaOCl + 17% EDTA + tap water	Ga - Gd: Rotary instrumentation (Crown down), No 30.	Ga: diode laser (635 nm/0.1 W) + TBO + RTF Gb: diode laser (635 nm/0.1 W) + RTF Gc: TBO + RTF Gd: RTF (positive control)	2 days	N = 12 Ga = 3 Gb = 3 Gc = 3 Gd = 3
F. nucleatum ($4*10^8$ cells/ml)	0.5% chloramine in water at 4˚C.	Ga - Gd: 2.5% NaOCl + 17% EDTA + tap water	Ga - Gd: Rotary instrumentation (Crown down), No 30.	Ga: diode laser (635 nm/0.1 W) + TBO + RTF Gb: diode laser (635 nm/0.1 W) + RTF Gc: TBO + RTF Gd: RTF (positive control)	2 days	N = 12 Ga = 3 Gb = 3 Gc = 3 Gd = 3
Negative group (uninfected)	0.5% chloramine in water at 4˚C	Ga - Gd: 2.5% NaOCl + 17% EDTA + tap water	Ga - Gd: Rotary instrumentation (Crown down), No 30.	No laser	2 days	N = 2
Foschi F., *et al.* (2007) **E. faecalis** ($5*10^8$ cells)	0.5% NaOCl for 2 weeks.	G1 - G4 6% NaOCl: 17% EDTA deactivated with 6% NaOCl for 3 min.	G1 - G4 Rotary instrumentation (Crown down), MAF: 0.465	G1: no laser/no MB G2: MB G3: Diode laser (665 nm/1 W) G4: Diode laser (665 nm/1 W) and MB	6 days	N = 64 G1 = 15 G2 = 15 G3 = 15 G4 = 15
Wang Q. *et al.* (2007) ***E. faecalis*** (>10^8 CFU/ml)	physiological saline solution at 4˚C	G1 - G4: 5.25% NaOCl+ physiological saline G5: 2.5% NaOCl + 5.25% NaHS + physiological saline G6: None	G1 - G6: Hand instrumentation (step-back), No 50	G1: Er,Cr:YSGG (2780 nm/1 W) G2: Er,Cr:YSGG (2780 nm/1.5 W) G3: Nd:YAG (1064 nm/1 W) G4: Nd:YAG (1064 nm/1.5W) G5: no laser irradiation (positive control) G6: no laser irradiation (negative control)	1 day	N = 60 G1 = 10 G2 = 10 G3 = 10 G 4 = 10 G5 = 10 G6 = 10

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Bergmans L., *et al.* (2006) ***E. faecalis*** ($4*10^8$ CFU/ml)	0.5% chloramine in water at 4˚C	G1, G2: 2.5% NaOCl+ 17% EDTA+ tap water + 0.9% sterile saline G3: 2.5% NaOCl + 17% EDTA+ tap water	G1 - G3: Rotary instrumentation (Crown down), No 30	G1: Nd:YAG laser (1064 nm/1.5 W/15 Hz) + RTF G2: RTF, no laser irradiation, Infected (positive control) G3: RTF, no laser irradiation, (uninfected, negative control)	2 days	N = 8 G1 = 3 G2 = 3 G3 = 2
Piccolomini R., *et al.* (2002) ***A. naeslundii*** ($1.8*10^8$ CFU/ml)	none	SubA, SubB1, SubB2 Physiological saline + EDTA SubC: physiological saline + EDTA + 5.25% NaOCl	All groups: Hand instrumentation (Crown-down).	SubA: no laser irradiation (control) SubB1: Nd:YAG laser (1064 nm/5 Hz) Sub B2: Nd:YAG laser (1064 nm/10 Hz) SubC: no laser irradiation	1 day.	N = 60 SubA = 5 SubB1 = 10 SubB2 = 10 SubC = 5
P. aeruginosa ($1.8*10^8$ CFU/ml)	none	SubA, SubB1, SubB2 Physiological saline + EDTA SubC: physiological saline+ EDTA+ 5.25% NaOCl	All groups: Hand instrumentation (Crown-down).	SubA: no laser irradiation (control) SubB1:Nd:YAG laser (1064 nm/5 Hz) Sub B2: Nd:YAG laser (1064 nm/10 Hz) SubC: no laser irradiation	1 day.	SubA = 5 SubB1 = 10 SubB2 = 10 SubC = 5
Folwaczny M., *et al.* (2002) ***Escherichia Coli*** ($8.67*10^6$ CFU/ml)	Sterile saline solution	G1a-G1d: saline solution G1d; saline solution+ 1% NaOCl	G1a - G1d: Hand instrumentation, No 40.	G1a:No laser irradiation (positive control) G1b: Nd:YAG laser (1064 nm/0.005 W) G1c: Nd:YAG laser (1064 nm/0.01 W) G1d: No laser irradiation	1 day	N = 114 G1a = 13 G1b = 13 G1c = 13 G1d = 13
Folwaczny M., *et al.* (2002) ***S. aureus*** ($1.44*10^6$ CFU/ml)	Sterile saline solution	G2a - G2d: saline solution G2d; saline solution+ 1% NaOCl	G2a-G2d: Hand instrumentation, No 40.	G2a:No laser irradiation (positive control) G2b: Nd:YAG laser (1064 nm /0.005 W) G2c: Nd:YAG laser (1064 nm/ 0.01 W) G2d: No laser irradiation	1 day	G2a = 13 G2b = 13 G2c = 13 G2d = 13
Negative control (uninfected)	Sterile saline solution	saline solution	Hand instrumentation, No 40.	G3: no laser irradiation (negative control)	1 day	G3 = 10
Moritz A., *et al.* (1999) ***Escherichia coli* and *Enterococcus faecalis*** ($5*10^5$ CFU/mL)	Physiological saline solution	All groups: EDTA+ physiological saline solution	All groups: Rotary instrumentation, (Step-back)	G1: Positive control (untreated) G2: Nd:YAG laser (0.8 W/1064 nm) G3: Nd:YAG laser (1.5 W/1064 nm) G4: Ho: YAG laser (0.8 W/2130 nm) G5: Ho:YAG laser, (1.5 W/2130 nm) G6: Er:YAG laser, (0.8 W/2940 nm) G7: Er:YAG laser, (1.5 W/2940 nm)	1 day	N = 40 G1: 10 G2: 5 G3: 5 G4: 5 G5: 5 G6: 5 G7: 5
Mehl A., *et al.* (1999) ***Escherichia coli*** ($1.1*10^6$ CFU/ml)	none	Ge1-Ge4: sterile saline solution Ge4: 1.25% NaOCl	Hand instrumentation, No 40.	Gs1: no laser irradiation (positive control) Gs2: Er:YAG (2940 nm/0.003 W/15 sec/50 mJ) Gs3: Er:YAG (2940 nm/0.001 W/60 sec/50 mJ) Gs4: no laser irradiation (1.25% NaOCl)	1 day	N = 90 Ge1: 10 Ge2: 10 Ge3: 10 Ge4: 10

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S. aureus ($1.53*10^6$ CFU/ml)	none	Gs1-Gs4: sterile saline solution Gs4: 1.25% NaOCl	Hand instrumentation, No 40.	Gs1:no laser irradiation (positive control) Gs2: Er:YAG (2940 nm/0.003 W/15 sec/50 mJ) Gs3:Er:YAG (2940 nm/0.001 W/60 sec/50 mJ) Gs4: no laser irradiation (1.25% NaOCl)	1 day	Gs1: 10 Gs2: 10 Gs3: 10 Gs4: 10
Negative control (uninfected)	none	None	Hand instrumentation, No 40.	Gn: negative (uninfected)	1 day	Gn: 10

Table 15. *Enterococcus faecalis* survival.

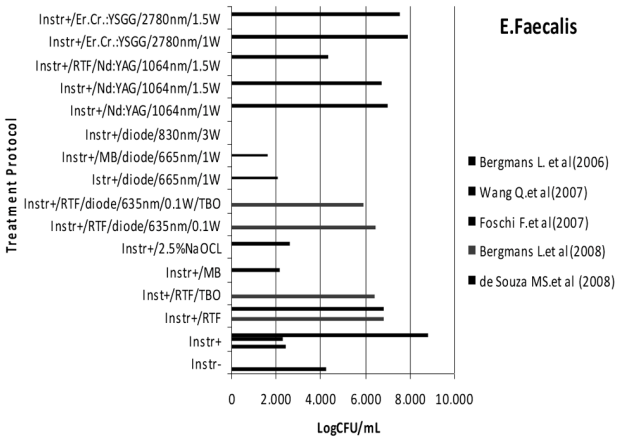


Table 16. *Streptococcus anginosus* survival.

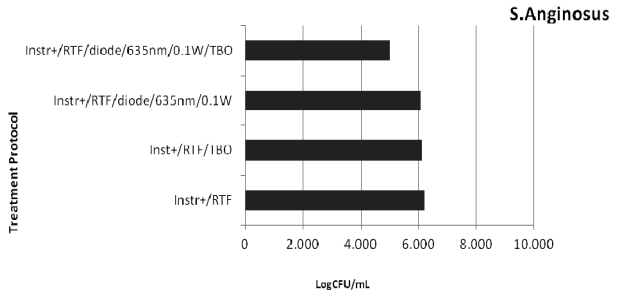
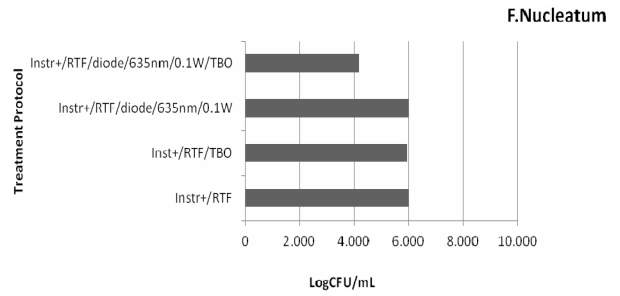


Table 17. *Fusobacterium nucleatum* survival.



In the first place, they contaminated the root canals with *E. faecalis*. In the second place they performed chemo-mechanical preparation and/or laser irradiation with or without specific solutions. The logCFU/ml ranges from 1.636 to 8.818 (**Table 15**).

Table 18. *Escherichia coli* survival.

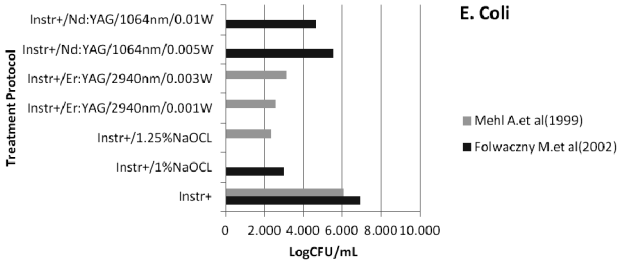


Table 19. *Actinomyces naeslundii* survival.

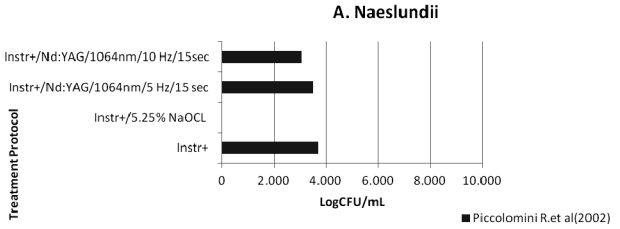


Table 20. Staphylococcus aureus survival.

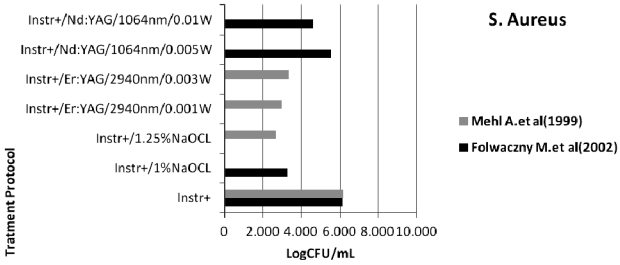
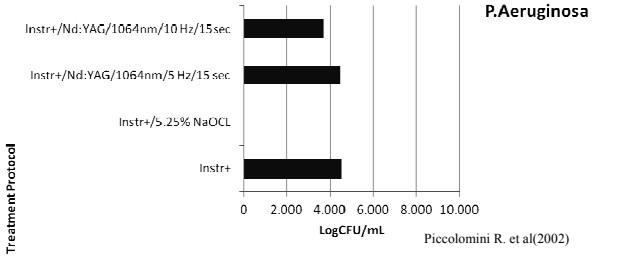


Table 21. *Pseudomonas aeruginosa* survival.



The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal and diode laser/665 nanometer/1 Watt irradiation with the combined effect

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Table 22. Polymicrobial survival.

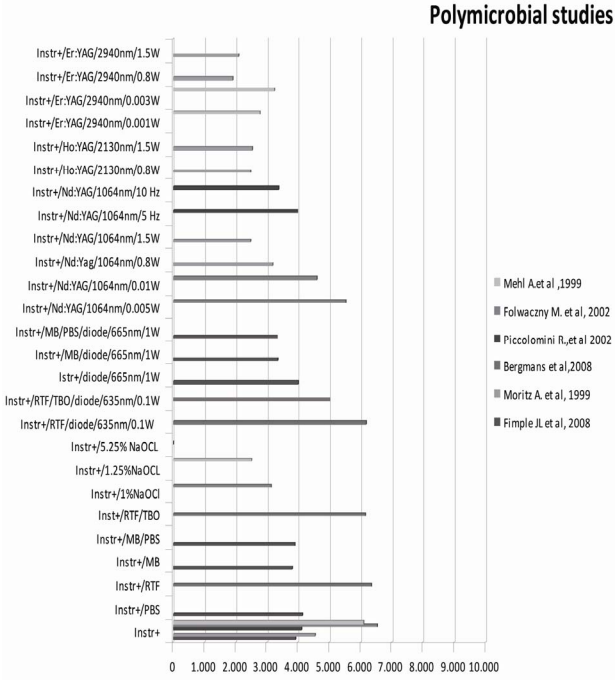
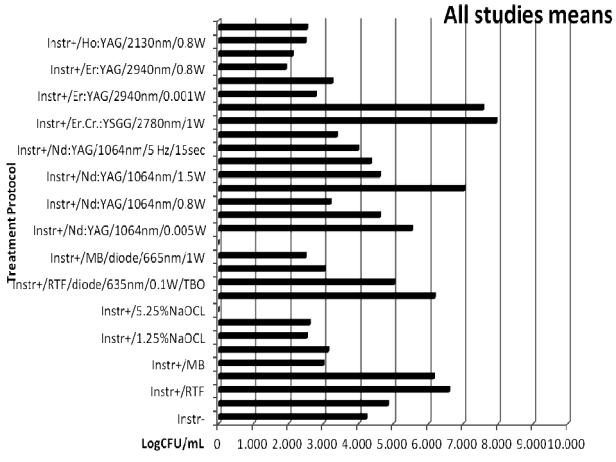


Table 23. Mean microbial survival.



of Methylene Blue (MB) as photosensitizing agent (logCFU/ml = 1.636) [33].

Instrumentation of the root canal and diode laser/665 nm/1 W irradiation

(logCFU/ml = 2.061) [33].

Instrumentation of the root canal combined with MB as photosensitizing agent (logCFU/ml = 2.190) [33].

Instrumentation followed by irrigation with 2.5% NaOCl (sodium hypochloride)

(logCFU/ml = 2.602) [31].

When instrumentation and diode laser/830 nm/3 W were used, no CFU/ml was found. However, as the author explained, this result is due to the fact that the level of sensitivity of the methodology used, was insufficient for detecting viable cells in low concentrations (contact with the author via e-mail) [32].

- ***Streptococcus anginosus***

Streptococcus anginosus survival was examined 4 times by 1 group of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with *S. anginosus*. Consequently, they completed root canal preparation and either used or not laser irradiation with specific solutions. The logCFU/ml ranges from 5.000 to 6.204 (**Table 16**) [30].

These results demonstrate no satisfying reduction of S. anginosus after diode laser/635 nm/0.1 W irradiation combined with RTF (reduced transferred fluid) and/or TBO (toluidine blue), nor after instrumentation and irrigation with RTF and/or TBO (logCFU/ml > 5.000).

- ***Fusobacterium nucleatum***

Fusobacterium nucleatum survival was examined 4 times by 1 group of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with *F. nucleatum*. Consequently, they completed root canal preparation and either used or not laser irradiation with specific solutions. The logCFU/ml ranges from 4.176 to 6.000 (**Table 17**) [30].

These results demonstrate no satisfying reduction of F.nucleatum after diode laser/635 nm/0.1 W irradiation combined with RTF (reduced transferred fluid) and/or TBO (toluidine blue), nor after instrumentation and irrigation with RTF and /or TBO (logCFU/ml > 4.176).

- ***Escherichia coli***

Escherichia coli survival was examined 8 times by 2 different groups of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with *E. coli*. Consequently, they completed root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 2.342 to 6.938 (**Table 18**).

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl

(logCFU/ml = 2.342) [28].

Instrumentation of the root canal and Er:YAG laser/2,940 nm/0.001 W irradiation

(logCFU/ml = 2.572) [28].

Instrumentation of the root canal followed by irrigation with 1% NaOCl

(logCFU/ml = 3.012) [25].

Instrumentation of the root canal and Er:YAG laser/2940 nm/0.003 W irradiation (logCFU/ml = 3.155) [28].

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- ***Actinomyces naeslundii***

Actinomyces naeslundii survival was examined 4 times by 1 group of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with A. naeslundii. Consequently, they completed root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 0 to 3.698 (**Table 19**) [29].

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl (logCFU/ml = 0).

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/10 Hz/15 sec irradiation (logCFU/ml = 3.053).

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/5 Hz/15 sec irradiation (logCFU/ml = 3.518).

Instrumentation of the root canal solely (logCFU/ml = 3.698).

- ***Staphylococcus aureus***

Staphylococcus aureus survival was examined 8 times by 2 groups of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with S.aureus. Consequently, they completed root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 2.703 to 6.184 (**Table 20**).

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl

(logCFU/ml = 2.703) [28].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.001 W irradiation (logCFU/ml = 2.973) [28].

Instrumentation of the root canal followed by irrigation with 1% NaOCl

(logCFU/ml = 3.246) [25].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.003 W irradiation (logCFU/ml = 3.348) [28].

- ***Pseudomonas aeruginosa***

Pseudomonas aeruginosa survival was examined 4 times by 1 group of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with *P. aeruginosa*. Consequently, they completed root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 0 to 4.544 (**Table 21**) [29].

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl (logCFU/ml = 0).

Instrumentation of the root canal and Nd: YAG laser/ 1064 nm/10 Hz/15 sec irradiation (logCFU/ml = 3.695).

B) Analysis of the Polymicrobial Studies Results

Microbial survival after polymicrobial infection of root canals was examined 30 times by 6 groups of researchers. In the first place they performed chemo-mechanical preparation and in the second place they contaminated the root canals with some of the following microorganisms; *E. coli*, *S. aureus*, *A. naeslundii*, *P. aeruginosa*, *S. anginosus*, *E. faecalis*, *F. nucleatum*, *A. israelii*, *P. gingivalis* and *P. intermedia*. Consequently, they completed root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 0 to 6.548 (**Table 22**).

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl (logCFU/ml = 0) [29].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.8 W irradiation

(logCFU/ml = 1.924) [27].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/1.5 W irradiation

(logCFU/ml = 2.113) [27].

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/1.5 W irradiation

(logCFU/ml = 2.477) [27].

Instrumentation of the root canal and Ho:YAG laser/ 2130 nm/0.8 W irradiation

(logCFU/ml = 2.491) [27].

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl (logCFU/ml = 2.522) [28].

Instrumentation of the root canal and Ho:YAG laser/ 2130 nm/1.5 W irradiation

(logCFU/ml = 2.531) [27].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.001 W irradiation

(logCFU/ml = 2.772) [28].

Instrumentation of the root canal followed by irrigation with 1% NaOCl (logCFU/ml = 3.138) [25].

Instrumentation of the root canal and Nd: YAG laser/ 1064 nm/0.8 W irradiation

(logCFU/ml = 3.204) [27].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.003 W irradiation

(logCFU/ml = 3.251) [28].

Instrumentation of the root canal and diode laser/665 nm/1 W irradiation combined with MB as photosensitizing agent and PBS (Phosphate-Buffered Saline) broth

(logCFU/ml = 3.341) [34].

Instrumentation of the root canal and diode laser/665 nm/1 W irradiation combined with MB as photosensitizing agent (logCFU/ml = 3.344) [34].

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/10 Hz/15 sec irradiation(logCFU/ml = 3.374)

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[29].

Instrumentation of the root canal combined with MB as photosensitizing agent

(logCFU/ml = 3.814) [34].

Instrumentation of the root canal combined with MB as photosensitizing agent and PBS (Phosphate-Buffered Saline) broth (logCFU/ml = 3.906) [34].

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/5 Hz/15 sec irradiation (logCFU/ml = 3.993) [29].

C) Analysis of the Mean Averages of Microbial Survival in General

The survival of 10 microorganisms well connected with endodontic problems: *E. faecalis*, *S. anginosus*, *F. nucleatum*, *A. israelii*, *P. gingivalis*, *P. intermedia*, *A. naeslundii*, *P. aeruginosa*, *E. coli*, and *S. aureus* was examined by 10 groups of researchers regarding the bactericidal effect of 30 different treatment protocols on them. In the first place the majority of them performed chemomechanical preparation and in the second place they contaminated the root canals with one or more microorganisms. Then, they completed the root canal preparation and either used or not laser irradiation with or without specific solutions. The logCFU/ml ranges from 0 to 7.940 (**Table 23**).

The best treatment protocols for root canal disinfection in descending order are as follows:

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl (logCFU/ml = 0) [29].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.8 W irradiation

(logCFU ml = 1.924) [27].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/1.5 W irradiation

(logCFU/ml = 2.133) [27].

Instrumentation of the root canal and diode laser/665 nm/1 W irradiation with the combined effect of Methylene Blue (MB) as photosensitizing agent (logCFU/ml = 2.490) [33,34].

Instrumentation of the root canal and Ho:YAG laser/ 2130 nm/0.8 W irradiation

(logCFU/ml = 2.491) [27].

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl (logCFU/ml = 2.522) [28].

Instrumentation of the root canal and Ho: YAG laser/ 2130 nm/1.5 W irradiation

(logCFU/ml = 2.531) [27].

Instrumentation followed by irrigation with 2.5% NaOCl (logCFU/ml = 2.602) [31].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.001 W irradiation

(logCFU/ml = 2.772) [28].

Instrumentation of the root canal with the combined effect of Methylene Blue (MB) as photosensitizing agent (logCFU/ml = 3.002) [33,34].

Instrumentation of the root canal and diode laser/665 nm/1 W irradiation

(logCFU/ml = 3.033) [33,34].

Instrumentation followed by irrigation with 1% NaOCl (logCFU/ml = 3.138) [25].

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm 0. W irradiation

(logCFU/ml = 3.204) [27].

Instrumentation of the root canal and Er:YAG laser/ 2940 nm/0.03 W irradiation

(logCFU/ml = 3.251) [28].

Instrumentation of the root canal and Nd:YAG laser/ 1064 nm/10 Hz/15 sec irradiation (logCFU/ml = 3.374) [29].

Instrumentation of the root canal and Nd:YAG laser / 1064 nm/5 Hz/15 sec irradiation (logCFU/ml = 3.993) [29].

4. DISCUSSION

Generally, it is well known that certain microorganisms are related to specific pathological situations in endodontics. Being aware of them is necessary so as to be able to consider the importance of the disinfection capacity of each treatment plan. Consequently, it has been proved that:

1) With symptomatic endodontic disease and apical bone resorption *T. denticola* is associated [1].
2) In endodontically infected teeth without a sinus tract *E. faecalis* and *Strept. anginosus* were mostly found [2].
3) In teeth with necrotic pulp *P. gingivalis*, *P. endodontalis*, *P. intermedia*, and *P. nigrescens* were identified more frequently [4].
4) With root canal treatment failures *E. faecalis* is associated [1,3].
5) In endodontically infected teeth with sinus tracts *P. gingivalis* and *F. nucleatum* were mostly identified [2].

This systematic review identified 10 *in vitro* studies that examined the effectiveness of treatment protocols, among classical methods and/or various laser applications in root canal disinfection. The results of these 10 studies indicated that the treatments which provide the best bactericidal ability regarding each bacterial solely and all of them were, in descending order of efficacy:

4.1. *Enterococcus faecalis*

Instrumentation of the root canal and diode laser/665 nm/ 1 W irradiation with the combined effect of Methylene Blue (MB) as photosensitizing agent seemed to be better in root canal disinfection than instrumentation of the root

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canal and diode laser/665 nm/1 W irradiation. In addition, the last method was slightly better than instrumentation of the root canal combined with MB as photosensitizing agent and significantly better than instrumentation followed by irrigation with 2.5% NaOCl (sodium hypochloride).

Regarding the instrumentation and diode laser/830 nm/3 W irradiation where no CFU/ml was found, this is due to the lack of sensitivity of the methodology used to detect low concentrations of viable cells (contact with author).

4.2. *Streptococcus anginosus*

Instrumentation of the root canal and diode laser/635 nm/0.1 W irradiation combined with RTF (reduced transferred fluid) and/or TBO (toluidine blue), as well as instrumentation with RTF and/or TBO demonstrated neither accepted nor satisfying reduction of *S. anginosus* (logCFU/ml = 5.000, logCFU/ml = 6.079, logCFU/ml = 6.113 and logCFU/ml = 6.204, respectively).

4.3. *Fusobacterium nucleatum*

Instrumentation of the root canal and diode laser/635 nm/0.1 W irradiation combined with RTF (reduced transferred fluid) and/or TBO (toluidine blue), as well as instrumentation with RTF and/or TBO showed insufficient disinfection of *F. nucleatum*. (logCFU/ml = 4.176, logCFU/ml = 6, logCFU/ml = 5.939 and logCFU/ml = 5.986, respectively).

4.4. *Escherichia coli*

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl seemed to be better in root canal disinfection than instrumentation of the root canal and Er:YAG laser/2940 nm/0.001 W irradiation. Furthermore, instrumentation of the root canal followed by irrigation with 1% NaOCl was less effective than the previous treatments, but slightly better than instrumentation of the root canal and Er:YAG laser/2940 nm/0.003 W irradiation.

4.5. *Actinomyces naeslundii*

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl seems to be the best in root canal disinfection as with this concentration no viable cells of Actinomyces naeslundii are detected. This method is used and mentioned only by one group of researchers [29].Concerning the other treatments, they are significantly worse and in fact instrumentation of the root canal and Nd:YAG laser/1064 nm/10 Hz/15 sec irradiation is slightly better than the same method but with 5 Hz irradiation or instrumentation solely.

4.6. *Staphylococcus aureus*

Instrumentation of the root canal followed by irrigation with 1.25% NaOCl was better enough than instrumentation and Er:YAG laser/2940 nm/0.001 W irradiation and seemed to be better in root canal disinfection than instrumentation of the root canal followed by irrigation with 1%NaOCl and instrumentation with Er:YAG laser/2940 nm/0.003 W irradiation.

4.7. *Pseudomonas aeruginosa*

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl was the best in root canal disinfection, as no CFU/ml of Pseudomonas aeruginosa was found. This method was conducted by one group of researchers [29]. Regarding instrumentation and Nd:YAG laser/1064 nm/10 Hz/15 sec irradiation, it was moderately worse than the previous one.

4.8. Crobial Studies Results

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl seemed to be the best in root canal disinfection as no viable cells were detected. However, this concentration of NaOCl is not used *in vivo* because it actively attacks living tissue without contributing significantly to treatment [35]. Furthermore, instrumentation and Er:YAG laser/2940 nm/0.8 W irradiation was well successful and slightly better than the same treatment but with 1.5 Watt irradiation, which was also better than instrumentation of the root canal and Nd:YAG laser/1064 nm/1.5 W irradiation. Also, instrumentation of the root canal and Ho: YAG laser/2130 nm/0.8 W irradiation was effective enough, as well as instrumentation of the root canal followed by irrigation with 1.25% NaOCl and instrumentation followed with Ho:YAG laser/2130 nm/1.5 W irradiation. Finally, instrumentation of the root canal and Er:YAG laser/2940 nm/0.001 W irradiation was less effective than the previous methods. All the other methods seemed to be worse in root canal disinfection.

4.9. Microbial Survival in General

Instrumentation of the root canal followed by irrigation with 5.25% NaOCl seemed to be the best in root canal disinfection as no viable cells were detected. Instrumentation and Er:YAG laser/2940 nm/0.8 W irradiation was well successful and slightly better than the same treatment but with 1.5 Watt irradiation. Instrumentation followed with diode laser/665 nm/1 W irradiation with the combined effect of Methylene Blue (MB) as photosensitizing agent showed the same results when compared to instrumentation of the root canal and Ho:YAG laser/2130 nm/0.8 W irradiation. Also good results show instrumentation of the root canal followed by irrigation with 1.25% NaOCl and Ho:YAG laser/2130 nm/1.5 W irradiation which both are slightly better than instru-

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mentation followed by irrigation with 2.5% NaOCl and Er:YAG laser/2940 nm/0.001 W irradiation. All the other methods seemed to be worse in root canal disinfection.

5. CONCLUSIONS

There are treatment protocols with the assistance or not of laser irradiation that can eliminate *E. faecalis*, *E. coli* and *S. aureus* inside the root canal. However, there is a serious number of *S. anginosus*, *F. nucleatum*, *A. naeslundii* and *P. aeruginosa* that remain inside the root canal even after laser irradiation. *In vitro*, NaOCl 5% seems to be the strongest solution in root canal disinfection. Concluding, it seems that new research is needed in order to set a treatment protocol effective in the root canal disinfection from all bacteria mentioned above that are related to endodontic origin pathology.

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