10.5005/jp-journals-10024-1506

ORIGINAL RESEARCH

A Comparative Antimicrobial Analysis of Various Root Canal Irrigating Solutions on Endodontic Pathogens: An *in vitro* Study

[1]Abhinav Misuriya, [2]Archana Bhardwaj, [3]Abhishek Bhardwaj, [4]Sanjay Aggrawal
[5]PS Pawan Kumar, [6]Satyanarayana Gajjarepu

ABSTRACT

Background and objectives: Evolution in understanding the poly-microbial environment of both endodontic infections and that of failed root canal treatments has been debatable over the years. The present study was designed to compare and analyze the effect of various root canal irrigation solutions on certain endodontic pathogens in vitro.

Materials and methods: To analyze *in vitro* the zone of inhibition of the micro-organisms the following irrigating solutions were employed:

a. Sodium hypochlorite (NaOCl) 5%
b. Sodium hypochlorite (NaOCl) 3%
c. Chlorhexidine 2%
d. Chlorhexidine (CHX) 0.12%
e. Doxycycline 0.01%
f. Doxycycline 0.005%
g. MTAD.

An agar culture plate inoculated with four endodontic pathogens was used namely

1. *Enterococcus faecalis* (MTCC-439)
2. *Candida albicans* (MTCC-183)
3. *Fusobacterium nucleatum* (ATCC-25586)
4. *Peptostreptococcus anaerobius* (ATCC-27337)

The inoculums were streaked out on the trypticase soy agar plate for discrete colonies with a wire loop using standard method. The inoculated plates carrying the antibiotic disks were incubated in an anaerobic jar in the following way–Anaerobic incubation–the following procedure was used to provide anaerobiosis with an increased concentration of carbon dioxide.

By this method the zone of inhibition obtained by different irrigating solutions against different pathogens could be compared.

Results: MTAD showed maximum antibacterial activity. In case of C. albicans MTAD was less effective than 5% NaOCl, 3% NaOCl and 2% CHX, 0.12% CHX. However, it was more effective against E. faecalis, F. nuleatum and P. anaerobicus. In any case, antimicrobial activity is not the only prerequisite for an endodontic irrigant. E. faecalis strain used in this study showed resistance to doxycycline; also doxycycline was ineffective against C. albicans at 0.01% and 0.005% concentrations.

Conclusion: It was found that MTAD was more antimicrobial than 5% NaOCl for some of the test micro-organisms; however the ability of MTAD to dissolve pulp tissue is not comparable to 5% NaOCl. In addition, 5 and 3% NaOCl showed significant antimicrobial activity against all test micro-organisms. The best option for a primary endodontic irrigant therefore is 5% NaOCl.

Keywords: Antimicrobial activity, Endodontic irrigants, MTAD.

How to cite this article: Misuriya A, Bhardwaj A, Bhardwaj A, Aggrawal S, Kumar PSP, Gajjarepu S. A Comparative Antimicrobial Analysis of Various Root Canal Irrigating Solutions on Endodontic Pathogens: An *In Vitro* Study. J Contemp Dent Pract 2014;15(2):153-160.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

The role of micro-organisms in infected root canals has been well documented. The harmful effect of micro-organisms present in the pulp and periapical pathologies has been studied since 1894 when Miller proved the presence of bacteria in the interior of infected root canals, which was further confirmed by other studies.[1]

Since then there has been much evolution in understanding the intricate poly-microbial environment of both primary endodontic infections and in that of failed root canal treatments with adequate biomechanical preparation and accepted obturations.

A momentous breakthrough in microbial identification occurred in 1990s with the advances in molecular genetics methodologies. Since then several new endodontic pathogens have been disclosed which have been intimately associated with periradicular infection. It is anticipated that approximately 40% of these microbiota are composed of an as yet uncultivable species of phylotypes[2] making endodontic microbiota far more multifarious than expected. Also some of these micro-organisms may reach up to 500 μm in the dentin.

[1,2,6]Reader, [3,5]Senior Lecturer, [4]Professor

[1,2,4-6]Department of Endodontics, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India

[3]Department of Oral and Maxillofacial Surgery, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India

Corresponding Author: Abhinav Misuriya, Reader Department of Endodontics, Vananchal Dental College and Hospital, Garhwa, Jharkhand, e-mail: abhinav.misuriya@gmail.com

It is now an established fact that only mechanical strokes of instruments are incapable of promoting satisfactory cleansing due to the complex internal dental anatomy, as seen from the early work of Hess and Zurcher (1999) to more recent studies demonstrating the anatomic complexities of the root canal system, that include multiple foramina, fins, deltas, loops, etc.[3] Hence the collective events of physical and chemical actions become indispensable. Also currently used methods of instrumentation especially rotary instruments techniques produce a smear layer that is 1 to 2 μm thick which covers the root canal walls and the openings of the dentinal tubules. Presence of this smear layer prevents penetration of irrigants, intracanal medicaments and sealers into the irregularities of the root canal system and the dentinal tubules. It also prevents absolute adaptation of obturation materials to the prepared root canal surface.[4]

A biofilm has been defined as — a microbial community characterized by cells that are attached to a substratum, and are in a matrix of extracellular polymeric substance (EPS), and exhibit altered growth phenotypes. Some antibiotics like aminoglycosides are more effective against bacteria growing in aerobic conditions than the same micro-organism growing in anaerobic conditions; therefore, not all cells within the biofilm will be affected in the same way.[5] Finally, it has been speculated that a sub-population of micro-organisms exists known as *Persisters* (Rocas I N, 2004). All the above events make complete eradication of micro-organisms a difficult task.[6]

Many solutions have been tested with the intent of encountering these micro-organisms. An ideal irrigant or medication should be able to disinfect the dentin and it's tubules in one visit. In addition, it should have sustained antimicrobial effect after it is used and it should be bio-compatible with live host tissues.

It is an established fact that use of irrigation during chemico-mechanical preparation of the root canal is imperative for reasonable cleaning of the root canal system.[7]

Amongst these solutions, sodium hypochlorite has been used frequently; its therapeutic consequence in endodontics was initially suggested by Walkerin 1936, promoting a double action. The dissolution of necrotic tissues contributes to its high pH and its germicide property, which is related to the formation of hypochlorous acid by the liberation of chloride ions from its solution. The antibacterial action of hypochlorous acid occurs by the oxidation of bacterial enzymes which lead to the disorganization of their metabolism.[8,9]

Chlorhexidine gluconate is acknowledged as a wide spectrum antimicrobial agent. It is unique in its ability to bind to oral tissues for extended periods from which it is released slowly; this property is called 'Substantivity'. It is relatively nontoxic but does not have the capacity to dissolve tissues (Kuruvilla Jr, 1998).[7-9]

Doxycycline, a broad spectrum antibiotic and a hydroxy derivative of tetracycline, is the most potent anti-collagenase antibiotic. A further benefit is Doxycycline hydrochloride is an effective acid conditioner (pH-2) which aids in smear layer removal.[10] It displays substantivity too without decrease in antimicrobial activity. It is also one of the components of MTAD.[11]

Studies have shown that MTAD (a mixture of a tetracycline isomer, an acid and a detergent) in addition to disinfecting, when used as a final rinse, is capable of removing the smear layer with minimal erosive changes on the surface of dentin.[12]

The clinician may place the etiologic factors of 'post-treatment disease' (PTD) Friedman, 1999 into four groups:

1. Persistent or reintroduced intraradicular micro-organisms
2. Extraradicular infection
3. Foreign body reaction
4. True cyst.[13]

The reduction and elimination of bacteria and their byproducts should be given paramount importance towards achieving a successful endodontic therapy. Even after complying with the best of the treatment regime, root canal therapy can fail due to the presence of certain resistant/ residual organism.

Primary treatment of such cases requires suitable internal irrigates and medicaments which will eliminate these resistant micro-organisms. Cultures from failed root filled teeth are predominantly Gram-positive anaerobes. A very commonly isolated species is *Enterococcus faecalis*, which has been shown to be very resistant to canal disinfection regimens. Using broad range PCR; clones releated to genera *Capnocytophaga, Dilister, Eubacterium, Fusobacterium, Gemella, Mogibacterium, Peptostreptococcus, Prevotella, Propionibacterium, Selenomonas, Solobacterium, Streptococcus*, and *Veillonella* have also been produced from samples of failed root canal cases.[13,14]

Although post-treatment disease has been primarily blamed on bacteria, fungi notably *Candida albicans* are found frequently in persistent endodontic infections and may be responsible for recalcitrant lesions.[10]

The purpose of this study was to compare the antimicrobial actions of sodium hypochlorite, Chlorhexidine, Doxycycline and MTAD on certain endodontic pathogens found in 'post treatment disease' (PTD).

METHODOLOGY

Irrigants

1. Sodium hypochlorite 5%
2. Sodium hypochlorite 3%
3. Chlorhexidine 2%
4. Chlorhexidine 0.12%

Fig. 1: Gas jar used in the study

Fig. 2: Recording measurement with the help of Vernier caliper

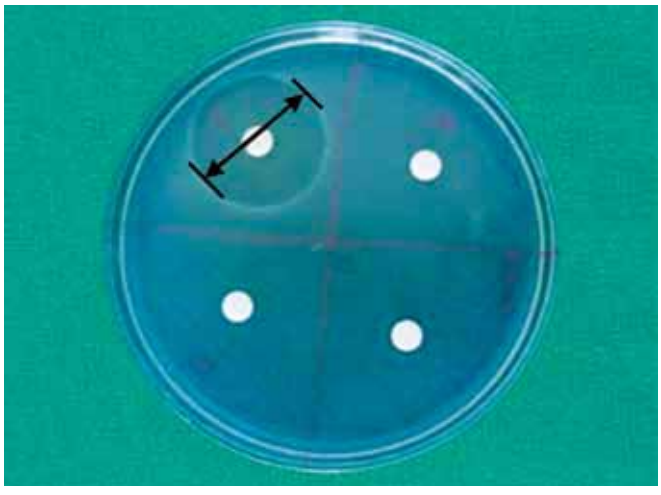


Fig. 3: Antibacterial disk showing zone of inhibition for *E. faecalis*

Fig. 4: Antibacterial disk showing zone of inhibition for *C. albicans*

5. Doxycycline 0.01%
6. Doxycycline 0.005%
7. MTAD

Isolation of Organisms

Two screw-capped test tubes containing 10 ml of Robertson cooked meat medium (RCM) were inoculated with lyophilized pellets of a single species of micro-organism, and incubated at 37°C in an anaerobic jar for 48 hours. Slide was prepared from the culture tube and mounted under oil immersion in microscope to confirm the micro-organisms. The inoculums from the RCM were streaked out on the trypticase soy agar plate for discrete colonies with a wire loop using standard method. The culture plates were incubated at a temperature of 37°C for a period of 48 hours in an anerobic jar (Fig. 1). Identification of bacterial pathogens was made by standard technique.[15]

Preparation of the Inoculum

After obtaining maximum growth activity, few colonies were picked up and dissolved into RCM and incubated for 2 hours anaerobically. The tubes were read in an spectrophotometer at 625 nm. The cultures were diluted in RCM. A Mc Farland standard of 0.5 was prepared.

Preparation of the disks: Each 6 mm sterile disk from Hi Media was impregnated with 50 µml of irrigant under sterile conditions. For testing, plates were divided into sections to receive the 7 irrigants and distilled water. This served as a negative control. To test two concentrations of NaOCl and CHX each agar plate was divided into 5 sections to receive the irrigants and the 5th section had distilled water as a negative control. In the case of *candida* due to overlapping of zone of inhibition, each plate was split into two halves to contain an irrigant and a negative control. Similarly to test concentrations of Doxy and MTAD each agar plate was divided into 4 sections to receive the irrigants and the 4th section had distilled water (Figs 2-4).

ANTIBIOTIC SENSITIVITY TESTING

Isolates were tested for antibiotic susceptibility test by Kirby – Bauer disk diffusion method on trypticase soy agar plate using standard NCCLS guidelines.[16] The inoculated plates

carrying the antibiotic disks were incubated in an anaerobic jar in the following way–Anaerobic incubation–the following procedure was used (Collee et al, 1971) to provide anaerobiosis with an increased concentration of carbon dioxide. Increased concentration of CO_2 enhances growth of many clinically important anaerobes on solid media (Watt 1973).[17] Petri dishes with the medium uppermost and lid downwards are placed inside the jar. After putting the plates into the jar, the lid is clamped on. Approximately air pressure was reduced to 100 mm Hg as monitored on the gauge. The pump was disconnected and the reduced pressure was brought upto 160 mm Hg with CO_2 and then to 760 mm Hg (i.e. atmospheric) with hydrogen from a separate valve. A 90% H_2 + 10% CO_2 gas mixture was used. The inlet valve was now closed and jar was sealed. The jar was then incubated at 37°C for 48 hours.[17]

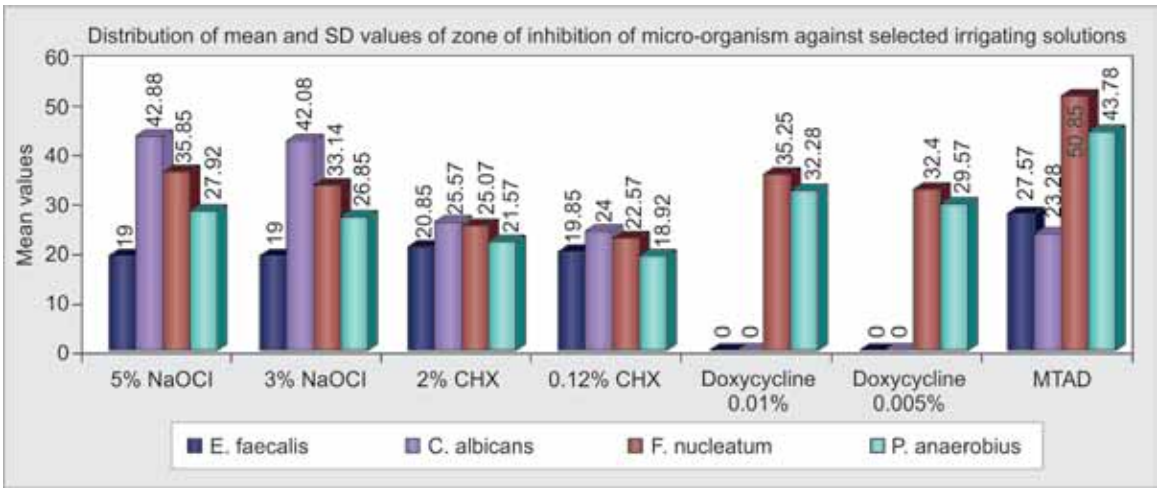
MEASUREMENT

The diameter of the zone of inhibition around each irrigant disk was measured and readings were then taken with the help of a divider and a vernier caliper. This method was repeated for each of the micro-organisms tested (Figs 2-4).

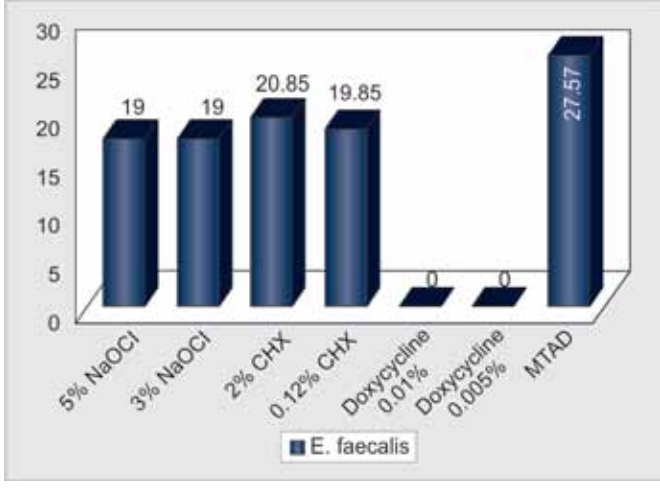
Data was analyzed by applying one way analysis of variance (ANOVA) test at 5% level of significance for comparison of the test groups for each of the micro-organisms. A significant difference by ANOVA ('p' value < 0.05) was further analyzed by students Newman Keuls test for comparison amongst each of there test solutions. Various tables and graphs were constructed to present the interpretation in the study. (Tables 1 and 2, Graphs: 1 to 5).

All work was done, taking adequate bio safety work precautions and the used material was discarded in appropriate disinfectant solution (2% Sodium hypochlorite).

RESULTS



Graph 1: X-axis represents irrigating solution and bacteria's. Y-axis average zone of inhibition in mm



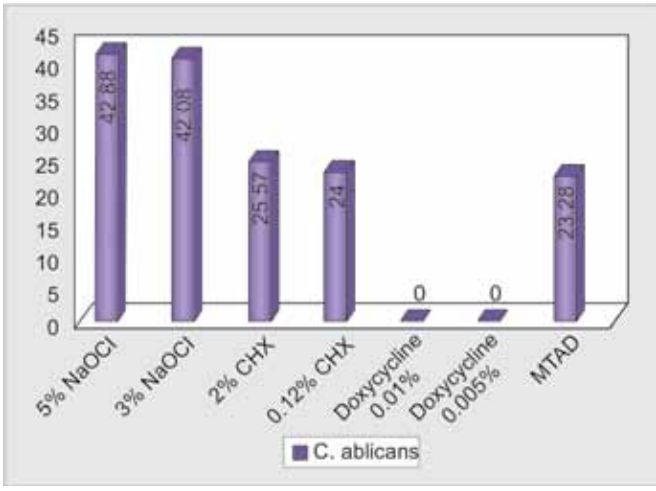
Graph 2: X-axis irrigating solution against individual bacteria. Y-axis represents average zone of inhibition in mm

One-way Analysis of Variance. (ANOVA). 'P-value' was <0.001(HS)

Since ANOVA was significant, a second test was applied to elicit significant difference between individual groups using 'Student-Newman-Kuel Test' (a p-value of < 0.05 is considered as significant).

The following inference can be drawn.

1. MTAD > 2% CHX ~ 0.12% CHX ~ 5% NaOCl ~ 3% NaOCL.
2. 2% CHX > 5% NaOCl and 3% NaOCl.
3. Both 0.01% and 0.005% Doxycycline did not show any zone of inhibition.

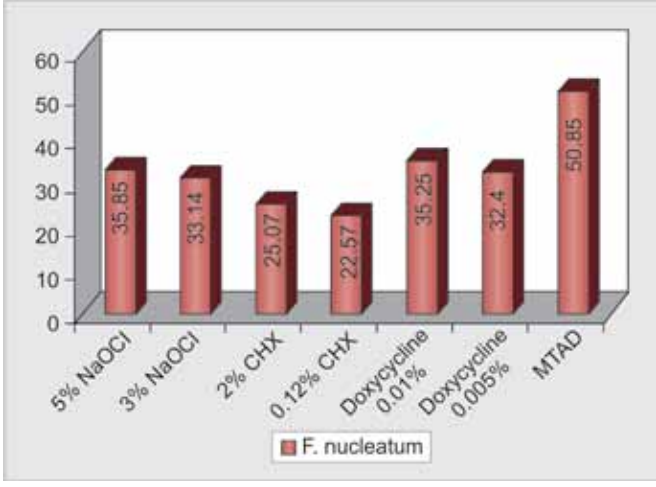


Graph 3: X-axis irrigating solution against individual bacteria. Y-axis represents average zone of inhibition in mm

One-way Analysis of Variance. (ANOVA). 'P-value' was <0.001(HS)

The following inference was drawn.

1. 5% NaOCl ~ 3% NaOCl > 2% CHX > 0.12% CHX ~ MTAD.
2. Both 0.01% and 0.005% Doxycycline did not show any zone of inhibition.

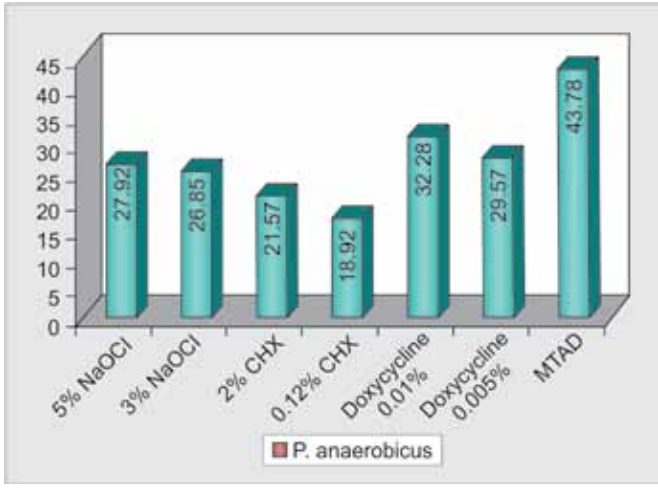


Graph 4: X-axis irrigating solution against individual bacteria. Y-axis represents average zone of inhibition in mm

One-way Analysis of Variance. (ANOVA). 'P-value' was <0.001(HS)

The following inference can be drawn.

1. MTAD > 5% NaOCl ~ 3% NaOCl ~ 0.01% Doxy ~ 0.005% Doxy > 2% CHX > 0.12% CHX.



Graph 5: X-axis irrigating solution against individual bacteria. Y-axis represents average zone of inhibition in mm

One-way Analysis of Variance. (ANOVA). 'P-value' was <0.001(HS)

The following inference can be drawn.

1. MTAD > 0.01% Doxy ~ 0.005% Doxy ~ 5% NaOCl ~ 3% NaOCl > 2% CHX ~ 0.12% CHX.

The result is summarized as follows:

1. Against individual test micro-organism.

DISCUSSION

The reasons for disease persistence in well-treated root-filled teeth were characterized using block biopsy material from non-healed periapical tissues including apices of the root-filled teeth. Analysis by correlative light and electron microscopy has shown that there are four factors that may contribute to persistence of periapical radiolucency after adequate treatment.

These factors are:

i. Intraradicular infection,
ii. Extraradicular,
iii. Foreign body reaction, and
iv. True cysts.

Of all these factors, the major cause of persistent disease after root canal treatment is the persistence of micro-organisms in the apical part of root-filled teeth (Table 3).[6,13]

Endodontic *Post-treatment disease* (PTD), or *Apical Periodontitis* associated with a root-filled tooth is an infectious disease caused by micro-organisms colonizing the root canal system. For an optimal outcome of the endodontic treatment to be achieved, bacterial populations within the root canal should be ideally eliminated or at least significantly reduced to levels that are compatible with periradicular tissue healing. If bacteria persist after chemo-mechanical preparation supplemented with/without an intracanal medication, there is an increased risk of adverse outcome of the endodontic treatment.[6,13]

Table 1: Represents average zone of inhibition of bacteria (mm)

Irrigating solutions	*E. faecalis (n = 7)*	*C. albicans (n = 7)*	*F. nucleatum (n = 7)*	*P. anaerobius (n = 7)*
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
5% NaOCl	19.0 ± 1.31	42.88 ± 0.5	35.85 ± 0.87	27.92 ± 2.66
3% NaOCl	19.0 ± 0.92	42.08 ± 0.18	33.14 ± 0.81	26.85 ± 2.29
2% CHX	20.85 ± 0.83	25.57 ± 1.60	25.07 ± 0.87	21.57 ± 0.49
0.12% CHX	19.85 ± 0.83	24.0 ± 1.51	22.57 ± 0.67	18.92 ± 1.29
Doxycycline 0.01%	0 ± 0	0 ± 0	35.25 ± 3.58	32.28 ± 4.46
Doxycycline 0.005%	0 ± 0	0 ± 0	32.4 ± 2.70	29.57 ± 3.11
MTAD	27.57 ± 1.06	23.28 ± 1.75	50.85 ± 2.09	43.78 ± 1.19
One way ANOVA $p < 0.001$	HS	HS	HS	HS

Table 2: Maximum zone of inhibition shown by an irrigant as per decreasing order against individual micro-organism

Sl. No.	*Micro-organism*	*Test Agents*
1.	*E. faecalis*	1. MTAD > 2% CHX~ 0.12% CHX~5% NaOCl ~3% NaOCl
		2. 2% CHX > 5% NaOCl & 3% NaOCl
		3. 0.01% & 0.005% Doxy did not show any zone of inhibition
2.	*C. albicans*	1. 5% NaOCl~3% NaOCl > 2% CHX > 0.12% CHX ~MTAD
		2. 0.01% & 0.005% Doxy did not show any zone of inhibition
3.	*F. nucleatum*	MTAD > 5% NaOCl~3% NaOCl~0.01% Doxy~0.005% Doxy > 2% CHX > 0.12%CHX
4.	*P. anaerobicus*	MTAD > 0.01% Doxy~0.005% Doxy~5% NaOCl~3% NaOCl > 2% CHX~0.12% CHX

Table 3: Clinician versus bacteria: The bacterial way of deceiving treatment[6]

What treatment does	*What bacteria have to do to survive*
Mechanical effect: Removal by instruments	Colonize areas distant from the main canal (e.g. isthmus, ramifications, and dentinal tubules). Adhesion
Chemical effect: Irrigation	Colonize areas distant from the main canal.
	Be protected by tissue remnants, dentin, serum or dead cells, all of which have the ability to inactivate or reduce the efficacy of antimicrobial agents.
	Be intrinsically resistant to the antimicrobial agent.
	Form biofilm structures enclosed by a protective polysaccharide matrix.
Ecological effect: Killing of key species	Adapt to the new environment, turning on survival genes and alternative metabolic pathways;
	Form new pairs and partnerships
Ecological effect: Nutrient deprivation	Adapt to the new environment, turning on survival genes and alternative metabolic pathways.
	Enter a viable but non cultivable state.
	Be located in areas where nutrient sources were relatively unaffected (very apical part of the canal near the foramen, ramifications).

About 100 species/phylotypes have already been detected in postinstrumentation and/or postmedication samples, of these Gram-positive bacteria are the most dominant. It is important to understand some aspects related to the significance of bacteria found in post-treatment samples.

In this context, one should be aware of the time that bacterial '*Persisters*' are detected in treated canals. Studies of the bacteria occurring in the root canal after treatment approaches involve three basic conditions:

1. Postinstrumentation samples (collected immediately after completion of chemo-mechanical procedures),
2. Postmedication samples (collected immediately after the removal of inter-appointment dressings), and
3. Postobturation samples (collected from root canal–treated teeth with associated apical periodontitis lesion at a given time, months to years after treatment).[6]

Studies investigating bacteria remaining in the root canals after chemo-mechanical procedures or intracanal medication serve the purpose to disclose the species that have the potential to influence the treatment outcome (outcome into perspective). On the other hand, studies dealing with the microbiota of root canal–treated teeth evincing apical periodontitis serve to show the association of species with treatment failure because the micro-organisms detected are likely to be participating in the etiology of persistent disease (Bacteria found in postobturation samples of teeth

indicated for retreatment because of post-treatment disease are conceivably adapted to the new environment and are remainders of a primary infection that resisted treatment procedures or penetrated in the root canal after restoration via coronal leakage (reinfection). In these cases, failure is already established, and the bacterial species/phylotypes found in the root canals are arguably the ones to blame.[6]

Microbiology of Canals with Persistent Infection

Usually one or just a few species are recovered from canals of teeth with persistent disease. These are predominantly gram-positive micro-organisms and there is an equal distribution of facultative and obligate anaerobes. This microbial flora is distinctly different from infections in untreated root canals, which typically consists of a polymicrobial mix with approximately equal proportions of Gram-positive and Gram-negative species, dominated by obligate anaerobes. There is some diversity of species isolated from root filled teeth with persistent periapical disease, but there is a consensus amongst most studies that there is a high prevalence of *Enterococci* and *streptococci* (Engstrom 1964). Other species found in higher proportions in individual studies are *lactobacilli, Actinomyces species* and *peptostreptococci* and *P. alactolyticus, P. propionicum, D. pneumosintes, and F. alocis.*[14]

TIME

The longer the irrigant is in contact with the root canal, the greater the antimicrobial action, tissue dissolving capacity and smear layer removal effectiveness will be. The advent of NiTi rotary instruments has proven to be more effective in the tapering design of the root canal space than traditional hand instrumentation. However, the cutting speed of NiTi instrumentation may reduce the time component of contact with the irrigants and under these circumstances it may prove to be disadvantageous for a successful end result. The variables of heat, ultrasonic vibration, and variable irrigant combinations must be factored into the equation to compensate for time adjustment that may be decreased by using NiTi instrument systems.

CONCLUSIONS

Cleaning and shaping of the root canal is the imperative and definitive procedure in the treatment of endodontic diseases. The effects of irrigation on intracanal micro-organisms have augmented activity in this area of research. As none of the elements of endodontics therapy (antibiotic therapy, instrumentation, irrigation, intracanal medicaments, and coronal restoration) can single-handedly assure complete disinfection, it is paramount to aim at the highest possible disinfection in every phase of treatment.

MTAD showed maximum antibacterial activity. In case of *C. albicans* MTAD was less effective than 5% NaOCl, 3% NaOCl and 2% CHX, 0.12% CHX. However, it was more effective against *E. faecalis*, *F. nuleatum* and *P. anaerobicus.* In any case, antimicrobial activity is not the only prerequisite for an endodontic irrigant.

Despite antibacterial properties, neither chlorhexidine nor doxycycline has shown to dissolve tissue in the root canal system. Therefore, chlorhexidine does not possess a key property of an ideal primary endodontic irrigant.

With the possible leakage of irrigating agents out of apical or accessory foramina, the potential exists for toxicity and hypersensitivity reactions or the development of bacterial antibiotic resistance. *E. faecalis* strain used in this study showed resistance to doxycycline; also doxycycline was ineffective against *C. albicans* at 0.01% and 0.005% concentrations. This augments the fact about the misuse and development of resistance to antibiotics (Harrison 1998). This may be a clinical concern regarding routine use of doxycycline as an intracanal irrigant.

It was found that MTAD was more antimicrobial than 5% NaOCl for some of the test micro-organisms; however the ability of MTAD to dissolve pulp tissue is not comparable to 5% NaOCl. In addition, 5 and 3% NaOCl showed significant antimicrobial activity against all test micro-organisms. The best option for a primary endodontic irrigant therefore is 5% NaOCl. Full strength is recommended over lower concentrations because of its superior antimicrobial and tissue dissolution properties.

A possible irrigant for a final rinse may be chlorhexidine gluconate, or MTAD because they possess the ability to sustain antimicrobial activity, which may give the time needed to kill these '*Persisters*'. However chlorhexidine gluconate has the added benefit of avoiding possible negative biological sequel of antibiotics. It also possesses substantivity similar to doxycycline. This should be followed by an acid wash to facilitate intimate contact of sealers to the dentinal walls.

REFERENCES

1. Sundquist G. Bacteriological studies of necrotic dental pulp. Dissertation. University of Umea, Sweaden 1976, International Endodontic Journal 1981;14:69-79.
2. Munsun MA, Pitt Ford. Molecular and cultural analysis of the microflora associated with endodontic infections. JDR 2002; 81:761-766.
3. Frank J. Vertucci Root canal morphology and its relationship to endodontic procedures, endodontic topics 2005;10:3-29.
4. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endodon 1984:10:477-483.
5. Thomas R, Dunavant, Regan JD, Gerald N, Glickman, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. J Endod 2006 June;32(6):527-531.

6. Siqueira JF Jr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures J Endod 2008;34(11):1291-1301.
7. Zehnder M. Root Canal Irrigants J Endod 2006 May 32(5):389-398.
8. Spratt DA, Pratten J, Wilson M, Gulabivala K. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001;34:300-307.
9. Carlos Estrela, Cyntia RA Estrela, Eduardo luis barbin, Júlio césare Spanó, Melissa a Marchesan, Jesus d pécora. Mechanism of action of sodium hypochlorite. Braz Dent J 2002;13(2):113-117.
10. lau H, Ballal V, Shenoy S, Acharya SR. Evaluation of antifungal efficacy of 5% doxycycline hydrochloride, 2.5% sodium hypochlorite, 17% ethylenediamine tetraacetic acid and 0.2% chlorhexidine gluconate against Candida albicans: An in vitro study. Endodontology. 2008 June;20(1).
11. Carson KR, Goodell GG, McClanaban SB. Comparison of the antimicrobial activity of six irrigants on primary endodontic pathogens. J Endod 2005 June;31(6):471-473.
12. Torabinejad M, Cho Y, Khademi AA, Barkland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod 2003 April;29(4):233-239.
13. Cohen: Pathways of pulp, Edition 9th page 945.
14. Figdor. A big role for the very small – understanding the endodontic microbial flora: Australian dental journal supplement 2007;52(1 Suppl):S38-S51.
15. Colle JG, Miles RS, Watt B. Test for the identification of bacteria. In: Colle JG, Duguid JP, Frazer AG, Marmino BP, Simmons A Mackie and McCartney, Practical Medical Microbiology, 14th ed. London: Churchill Livingstone 1996;p.131-145.
16. National committee for clinical laboratory standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests NCCLS document M2-A7, Approved Standard, 7th edition: Wayne, PA: NCCLS 2000.
17. Mackie, Mc Cartney. Practical medical microbiology, 14th edition.