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Evaluation the Antibacterial Effect of Sodium Hypochlorite with EDTA, Sodium Hypochlorite with Citric Acid and MTAD Irrigant Against *Enterococcus Faecalis* in Root Canals of Primary Teeth
(An in Vitro Study)

A Thesis
Submitted to the Council of the College of Dentistry at Baghdad University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pediatric Dentistry

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Certification of the Supervisor

I certify that this thesis entitled "**Evaluation the antimicrobial effect of sodium hypochlorite with EDTA and citric acid and MTAD irrigant against Enterococcus faecales in root canals of primary teeth (an in vitro study) "** was prepared by **Sarah Tawfeeq Jaber** under my supervision at the College of Dentistry/ University of Baghdad in partial fulfillment of the requirements for the degree of Master of Science in Oral Medicine.

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Dedication

To

My lovely husband Sarmad who supported and helped me all the time until finished this study

My children Abbas, Hussien and Ruqayah. This work took a lot of your time and without you this work would not have been completed.

To my wonder full family. I love you and thank you for everything God bless you.

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Abstract

Background: Microorganisms are responsible for causing of pulp disease and periradicular lesion. Successful endodontic treatment depends on effective shaping, disinfection and obturation. Irrigation step is very important in root canal treatment as it responsible for disinfection of the canal system. Complete cleaning of root canal is very difficult due to complex anatomy of the canal. As well as, microorganisms can survive even in the environment with scant nutrition with minimal commensality with other bacteria. One of the most common bacteria that had been detected in root canal failure is *Enterococcus faecalis*, which able to survive in harsh condition by formation of biofilm and resist many irrigants solutions and medications.

Aim of the study: the present study aimd to compare the antibacterial effect of 3%Sodium Hypochlorite with 17% Ethylenediamintetraacetic acid, MTAD and 3%Sodium Hypochlorite with 10% Citric Acid irrigants against *Enterococcus Faecalis* in root canals of primary teeth.

Material and method: In this study, 45 single rooted human primary teeth collected from children aged 4-6 years. The crowns of the samples were cut followed by root instrumentation and sterilization. Roots canal samples were collected from children with necrotic pulp for *Enterococcus faecalis* isolation. After the collection, theses samples were subjected to three different microbiological test for bacterial detection. First, gram stain then cultured in the blood agar, bile esculin agar (selective media for *E. faecalis*) and finally by vitek2 system to diagnose the bacteria. After obtaining pure culture of *E.faecalis*, inoculation of the bacterial suspension inside root canals of specimens was done and left it in brain heart infusion for 2 weeks. Before the irrigation procedure, sterile paper point used to take pre-treatment samples from root canal to ensure the presence of bacteria and count the number of bacterial colony in the root

canal. The samples then randomly divided in to 3 groups (15 per each group). First group irrigated with 3% sodium hypochlorite_17% Ethylenediamintetraacetic acid, second group irrigated with MTAD and the third group irrigated with 3% sodium hypochlorite -10% citric acid. The irrigation was performed following the instruction and protocol sequence of each irrigant material. Post-irrigated samples from the canals were taken for comparison with the pre samples. bacterial counting by cfu were done.

Result: Statistical analysis of the data showed no significant difference in the bacterial eradication between 3% sodium hypochlorite_- 17% Ethylenediamintetraacetic acid and MTAD irrigation methods (p value 0.161). Mean percentage of bacterial eradication for the 3% NaOCL-17% EDTA was 99.187, MTAD 98.037 and 3% NaOCL- 10% citric acid was 74.481. The last group demonstrate least antimicrobial effect among other groups. Statistical analysis of data revealed highly significant difference between 3% NaOCL- 10% citric acid with the other two groups (p value .000).

Conclusion: both 3%NaOCL- 17% EDTA and MTAD irrigation methods were equally effective against *E. faecalis* in the root canal of primary teeth. MTAD was an effective irrigant solution and could be used as an alternative rinsing for sodium hypochlorite. 3% NaOCL- 10% citric acid irrigation method showed least antibacterial effect against the same isolated bacteria.

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List of abbreviations

Abbreviation	Complete word
µm	Micrometer
BHI	Brain heart infusion broth
cfu	Colony forming unit
Df	Degree of freedom
E.faecalis	*Enterococcus faecalis*
EDTA	Ethylenediaminetetraacetic acid
HS	High significant
IB	International Baccalaureate
ml	Mill letter
mm	Millimeter
MTA	Mineral trioxide aggregate
MTAD	Mixture of doxycycline ctric acid detergent
NaOCL	Sodium hypochlorite
PH	Potential hydrogen
Sig	Significance

Introduction

Microorganisms are the primary etiological factor of the pulp diseases and periradicular lesion successful root canal therapy depends on three phases, instrumentation, disinfection and finally obturation. Disinfection step is very critical and it is attributed to complete healing of the root canal infection and peri radicular disease (Nara, *et al.,* 2010). Chemo mechanical preparations of root canal with the using of an effective antimicrobial agent is very helpful in elimination of the microorganisms (Siqueira *et al.,* 2000).

Many facultative anaerobic microorganisms persistently detected in the root canal and lead to failure of endodontic treatment, one of the most common bacteria is *Enterococcus faecalis* (Rocas *et al.,* 2004).

It was explained experimentally that *E.faecalis* found in 63% of necrotic pulp of primary teeth. *E.faecalis* can withstand poor nutrient state and resistance to many medications (Hancock *et al.,* 2001).

Sodium hypochlorite has a broad-antibacterial activity and has an excellen lubrication property. However, it possesses disadvantages, such as bad taste and unpleasant odor; in addition to that, NaOCL can cause severe complications and tissue toxicity when it extruded in periapical tissue (Chang *et al.,* 2001).

Sodium hypochlorite lack the ability to dissolve the inorganic particles of dentin and prevent smear layer formation during instrumentation, hence, it is also unable to remove the smear layer when formed, where many microorganisms embedded with it (Hulsmann., 2003).

Therefore, demineralizing agent like Ethylenediamintetraacetic acid or citric acid had been recommended as an adjuncts in root canal irrigation (Ayad, 2001).

MTAD is an endodontic irrigant agent, which is a mixture of 3% doxycycline, 4.25% citric acid and detergent (tween 80). MTAD is an effective

solution in the elimination of *E.faecalis* and removal smear layer (Torabinejad *et al.,* 2003), as well as, MTAD did not cause any post-operative discomfort when used as a final irrigant (Torabinejad *et al.,* 2005).

From all the controversial and differences in the view of the mode of action of these materials that mentioned above, the present study was designed to help the practitioner in choosing the irrigant material for the infected primary teeth.

Aim of the study

This study was aimed to compare the antibacterial effect of :
3% NaOCL- 17% EDTA, MTAD and 3%NaOCL- 10% citric acid against *E.faecalis* in the root canal of primary teeth.

Review of literature

1.1 Dynamic of root canal system

In normal healthy state, the root canal is free from infection. Unlike oral cavity, in which there is a commensal microbiota, any detected microorganism in root canal considered as a pathogen (Persoon and Özok, 2017). When these microorganisms enter the root canal system, serious of complications start from simple reversible pulpitis to pulp necrosis and periapical lesion (Glickman and Schweitzer, 2013). Infected root canal can cause severe pain and carry a danger of spread the infection. There are many pathways by which the microorganism gain access to the root canal which are as follows (Bammann and Estrela, 2009).

1.1.1 Dentinal tubules

As a consequence of extensive carious lesion or during dental procedure, the microorganism find their direct way to the pulp through dentinal tubules, these tubules can provide simple pathway for the invading microbes to reach the pulpal tissue. Microorganisms may invade the dentinal tubules in up to 300 µm depth from pulpal end (Horib *et al.,* 1990). Dentinal tubules contain a considerable amount of non-mineralized collagen, it has been showed that bacterial invasion to the dentinal tubules occurred by bacterial adhesion to collagen and by morphological –growth response induced by collagen (Love *et al.,*2001). As well as, the fluid that found in the dentinal tissue provide nourishment to pathogenic bacteria (Love, 2001). Sjögren, *et al*. (1997) and Gomes (2004), stated that the fluid originates from alveolar bone and periodontal ligament that surrounding the root of the tooth act as other source of bacterial nutrient through radicular dentinal tubules or remain in side root canal for bacteria survival.

1.1.2 Thin Dentine

Microorganism might reach the pulp when the thickness of remaining dentin is 0.2 mm or less (Dahlen *et al.,*1992). Dentin –pulp complex is a term used to describe dentin and dental pulp as a one unit (Avery, 1994; Ten Cate, 1998). Stressed pulp is term used to express bad prognosis of the pulp because of previous deep carious lesion, previous prosthesis, old large restoration, occlusal restoration, occlusal trauma and abrasion, all these reason lead to harm the pulp adaptability (Zollner and Gaengler, 2000). The degree of severity of pulp immediate response depend on the remaining dentin thickness (preparation depth), 2 mm thickness consider as a minimum critical factor for pulp protection. Many experimental studies stated that even a very small difference in the remaining dentin thickness has considerable effect on pulpal response. The thickness of the remaining dentin has a direct effect on the permeability of the dentin. Dentin permeability is a membrane ability to allow solvent and solute pass from one side to another (Pashley, 1985). Permeability of dentin plays a major role in allowing the bacteria and chemical to pass across the dentin, and reach the pulp and per radicular tissue the permeability of normal dentin is more than sclerotic dentin. Dentin permeability decrease as the age increase this is due to dentin sclerosis (Tagami *et al.,* 1992). Dentin barrier is very poor this because it contains a large number of dentinal tubules filled with fluid, these tubules have variable effect on the permeability of dentin, as explained by Koutsi *et al.* (1994), which are:

1. Length of dentinal tubule (longer tubule more resistant for penetration than shorter tubules)

2. Radius, number and diameter of tubule effect on the filtration ability, big size and large number in permanent teeth than in primary teeth. So permeability of permanent teeth more than primary. Dentinal tubule thickness 2 μm at dentin-enamel junction and 3-4 μm near the pulp which is wider and denser (Garberoglio and Brannstr6m, 1976). Many studies explain that as the thickness of dentin

decrease it is permeability increase, this is occurring due to increase the density and diameter of (Koutsi *et al.,* 1994).

1.1.3 Periodontal membrane:

The presence of lateral canal or apical foramen creates a pathway for passing microorganisms from gingival sulcus to the root canal (Narayanan and Vaishnavi, 2010)

1.1.4 Faulty restoration

Lack of isolation and contamination of root canals from salivary component during endodontic treatment leads to infection of the periapical area in less than 42 days even after obturation of root canal (Torabinejad *et al.,* 1990).

1.2 Endodontic microbiology:

The microorganisms that are responsible for endodontic infection are widely varied and form mixture of many bacterial species. It has been explained that microorganism species samples isolated from asymptomatic teeth are different from microbiota isolated from symptomatic teeth (Jaya *et al.,* 2015). This complex mixture of microbiota is composed of anaerobic and aerobic microorganisms. Facultative microorganisms found in root canal primary teeth, the most prevalent bacterial species in root canal of primary teeth are *E. faecalis*, *Porphyromonus gingivalis* and *Treponemadenticola* (Cogulu *et al.,* 2008; Rana *et al.,* 2009). Nevertheless, *E.faecalis* is often present in primary root canal infection of permanent teeth. It was explain experimentally that it found in 63% of necrotic pulp of primary teeth (Hancock *et al.,* 2001). Necrotic teeth are commonly seen in children as a consequence of early childhood caries (ECC). This form of caries is widely prevalent in children (Oncag *et al.,* 2006).

- The most common microorganism identified in root canal is *E.faecalis* even after endodontic treatment, *E.faecalis* can survive and may lead to persistent infection and failure of root canal treatment (Love, 2001). *E.feacalis* is Gram-

positive cocci and it is a facultative anaerobe, these bacteria considered as a normal flora of intestinal structure and may be found in the intraoral cavity, especially in gingival sulcus. If the number of these bacteria is small, it can eradicated easily from mouth, but large numbers of *E.faecalis* are difficult to be eliminate. *E.faecalis* has the ability to colonize inside the dentinal tubule, rami, isthmus and accessory canals and it is predominant bacteria that has been implicated in root canal failures and persistent infections (Distel et al., 2002). In post treatment the apical periodontitis, the prevalence ranges from 24% to 77% (Hancock *et al.,* 2001; Stuart *et al.,* 2006). The depth of penetration of *E.faecalis* inside the dentinal tubule is about 1483.33 µm (rich with nutrient suitable for aerobic condition), 1166.33 µm (rich nutrient suitable for anaerobic condition) and 620 µm (nutrient deprived suitable for anaerobic condition) this micro colonies collected to gather create (mushroom shaped) of small colonies (Kowalski *et al.,* 2006). *E.faecalis* considered as a normal flora in the oral cavity. Patient who receiving endodontic treatment has more *E.faecalis* on his oral rinse sample than the patient with no previous endodontic treatment (Sedgley *et al.,* 2004), this means that *E.faecalis* cause second category of infection (secondary infection). Much higher *E.faecalis* found in chronic root canal infection than that of primary infection. Studies showed that the case with failure root canal treatment contain *E.faecalis* nine times more than that in primary infection (Rộcas *et al.,* 2004) .the prevalence of *E.faecalies* occurrence in case of the root canal that previously treated endodontically is relatively high (Gomes *et al.,* 2004). *E.faecalis* has the ability to inhibit the lymphocytes action and this considered as a potential cause of endodontic failure (Lee *et al.,* 2004). According to Stuart *et al.* (2005) explained that *E. faecalis* considered risk factor in root canal treatment because it possesses several survival and virulence factors, which are:

1- Can withstand poor nutrient state.
2- Resistance to several medications like (calcium hydroxide)

3- Resistance to antibiotic.

4- Enter the dentinal tubule and metabolize the fluid with in it.

5- Survive in extreme low pH and high temperature.

6- Survive in long period of starvation and metabolize the fluid from periodontal ligament.

E.faecalis don't depend only on their virulence factors but participate the virulence factors with other species, so that it enforces their survival process and potentiates it is ability to cause disease (Jett *et al.,* 1994). The small size of *E.faecalis* enable it to invade and live professionally inside the dentinal tubule (Love, 2001). *E.faecalis* bacteria that grow as biofilm able to altered metabolic and genetic processes of bacteria with its matrix, by this way prevent the penetration and action of antimicrobial agent (**P**rabhakar *et al.,* 2010). *E.faecalis* that found in the dentinal tubule can resist the calicium hydroxide medicament that applied inside the canal for more than 10 days (Haapasalo and Orstavik, 1987). The reason for that as were explained by Stuart *et al.* (2005)as follow:

E.faecalis can maintain pH hemostasis passively (this due to it is penetrating ability of ions through cell membrane and buffering capacity of cytoplasm). *E.faecalis* maintains the pH by proton pump process, that enable it to pump the protons in the cell with lower internal Ph. *Efaecalis* loss it is ability to survive in about more than 11.5 pH (McHugh *et al.,* 2004). However, this pH (pH of calcium hydroxide 11.5) is difficult to maintain in the dentinal tubule due to dentin buffering capacity. Studies examined the dentin found that it has suppressing effect to different concentration of root canal medicament involved: calcium hydroxide, chlorhexiden, sodium hypochlorite and iodine potassium iodide (Portenter *et al.,* 2001).

Many researches approach aim to find an effective method and material to prevent or eradicate *E.faecalis*, these bacteria can invade the root canal system during the first visit of treatment, between visit or even after finish the treatment (Rộcas *et al.,* 2004), therefore it is necessary to direct the aim of treatment to

prevent *E.faecalis* infection during all phases of therapy. In the first stage of treatment during preparation procedure, it is preferable to prepare the apical part of the root canal to larger size by instrument. This facilitate elimination of interacanal microorganisms through reach area that is not accessible to smaller size master apical file. Beside that enlarge the apical portion of the root canal help in eliminate the inner most (pulp) dentin. This potentially facilitate removal of intertubular microorganisms and allow antimicrobial agent to effectively penetrate the tubule (Card *et al.,* 2002).

1.3 Irrigation

1.3.1 Rational of Irrigation in endodontic:

The aim of root canal treatment is to eradicate the pathogenic bacteria which are the main cause of infection of the root canal and effect healing in periapical area (Sjogren *et al.,* 1997). Complete elimination of microorganisms is extremely difficult in endodontic therapy due to complex anatomy of root canal structure, success of endodontic treatment depends on many factors: instrumentation, disinfection and well fit root canal filling (Orstavik *et al.,* 2004). There are many of irrigant compounds have been modified chemically and also several mechanical devices have been developed to help the irrigant solution to work probably. All these modifications and systems try to improve the effectiveness and penetration ability of irrigant products. It has been showed that the rate of endodontic success is (50%-70%) for secondary treatment in previously failed endodontic treated teeth. But with recent development and promising therapy for regeneration of pulp involving isolation of stem cell from pulp, complete and partial pulp regeneration is very helpful (Nakashima and lohara, 2014).

As explain before due to complex anatomic structure of root canal system, many teeth contain one or more accessory or lateral canals, that are too fine to be

seen in radiograph and because of it is small size it is very difficult to be reached by instrument. Therefore, even with proper preparation and thorough instrumentation, some organic tissue (e.g. pulp) and inorganic tissue (e.g. remnant of previous filling) will be remain in the root canal. Besides that, residual microorganism and bacteria still inside the dentinal tubule of root canal. Bacterial biofilm that form safe habitat against several antibiotic add another challenge in decreasing the efficacy of disinfection and cleaning the canal. All these factors make difficult challenging in complete cleaning of root canal system by mechanical instrumentation alone. It has been investigated experimentally that even with complete disinfection of root canal and finish endodontic treatment recurrence of infection still possible (Jaya *et al.,* 2015).

1.3.2 Essential properties of root canal irrigant material

Root canal irrigants should have several properties according to (Zehnder, 2006; Haapasalo *et al.,* 2010; Basrani and Haapasalo, 2012; Agrawal Vineet *et al.,* 2014) these are:

1- Broad spectrum –antibiotic.

2- Highly effective against facultative and anaerobic microorganisms that detected in biofilm dissolvent.

3- Able to dissolve the remnant of necrotic pulp.

4- Non-cariogenic properties.

5- It should nontoxic systematically and safe if it reaches the vital tissue and periodontal tissue.

6- Does not cause any anaphylactic reaction.

8- Effective against germ and fungi.

9- It should have prolonged antibacterial effect and sustained antimicrobial effect after use.

10- Maintain its activity in the presence of serum, blood and protein derivative from tissue.

12- Does not effect on the healing of periapical tissue.

13- Free from staining component on tooth structure.

14- Does not mediated any immune response.

15- Does not effect on the physical trait of dentin, sealing properties of restorative material.

16- Simply to use and not expensive.

17- Low surface tension

18. Solubility ability

19- No weakening of tooth structure

20- No negative effect on sealing abilities of sealer.

21- Long shelf life

22- Ease of application and low cost

23- No negative effect on the physical properties of the tooth.

24- Irrigation solution should have the ability to eradicate the endotoxin of bacteria from the root canals. Endotoxin present in all necrotic teeth with periradicular lesion. And this left endotoxin has the ability to induce inflammatory response. Without regard the presence or absence of the variable bacteria, therefore, if the endotoxin resides in the canal after complete endodontic treatment could be a potent cause for failure of treatment.

25- Smear layer removal:

Instrumentation of root canal lead to formation smear layer on the canal's walls; this layer composed of organic and inorganic substances. Ability of irrigant to remove smear layer is very important as it can attach to bacteria and able to protect bacterial biofilm that formed on the walls of the canal (Sen'bh, 1999). Additionally, smear layer can interfere with adherent accessibility of the sealer and dentin's walls of root canal, Therefore, smear layer should be eliminated to allow good adherence of sealer to the dentine's walls.

When specific irrigant unable to remove smear layer micro leakage may be promoted (Caron et al., 2010).

26. lubrication:

One of the important properties of irrigant product is lubrication, irrigant material used to lubricant the root canal space, which help in better and smoother facilitating of instrumentation. Because of flushing action of irrigant and removal of debris it will help in prevent the blockage of the root canal. Lubrication help in lowering the frictional resistance of the tooth structure and by this way reduce the mechanical stress on the endodontic instruments, in result reduce the risk of fracture instrument inside the canal (Basrani and Haapasalo, 2012).

1.3.3 Classification of irrigant solution

Several classifications were drawn regarding irrigant solutions, Agrawal Vineet *et al.,* 2014 classify them as follows:

a. Auxiliary substance to instrumentation (these material don't need any special physical properties, just chemical one):

1. NaOCL (sodium hypochlorite).
2. ChX (chlorhexidine).
3. EDTA (ethylene diamine tetra acetic acid)
4. QMIX. (mixture of bisbigunide antimicrobial agent, polyaminocarboxylic acid calcium-chelating agent, and surfactant).

b. Irrigant material: These irrigants are used during irrigation aspiration and need special physical properties like lower viscosity and lower surface tension.

1- NaOCL
2- Distilled water
3- Normal saline
4- MTDA (doxycycline, acid and detergent)
5- QMIX
6- Tetracycline
7- Green tea, triphala

8- Herbal derivative.

Another classification according to origin of mechanism of action (Kandaswamy and Venkateshbabu, 2010) (figure 1.1).

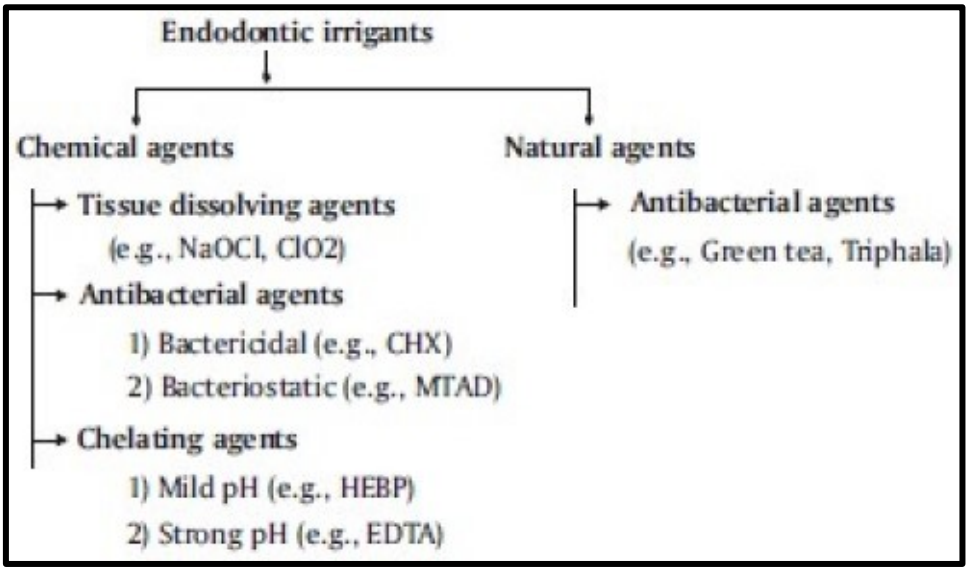

Figure 1-1: Classification of irrigants solution according to origin of mechanism of action (Kandaswamy and Venkateshbabu, 2010).

However, there is no unique irrigant solution that meets all the requirements for an optimal irrigating solution (Agrawal Vineet *et al.,* 2014). Using a combination of irrigation products in the right irrigation sequence and techniques result in successful endodontic treatment outcomes.

1.4 Sodium hypochlorite:

Sodium hypochlorite introduced to be used as irrigant material since 1920. Nowadays. It is the most popular and effective irrigant solution. Because it has majority of the requirements of root canal irrigant material than others known substances have. NaOCL has broad-antibacterial activity, excellent lubrication property, not expensive, widely available and it has acceptable shelf life. However, NaOCL should be used with caution due to it is caustic to tissue, Precaution is very important for patient and dentist (Faras *et al*., 2016).

1.4.1 History:

Sodium hypochlorite (NaOCL) has considerable history in medical and dental field and it is popularity continuous till today. During the first world war, the surgeon Alexis Carrel and chemist Henry Drysdale extended the use of buffered solution 0.5% of NaOCL to infected wound irrigation. Dakin introduce chlorin-realesing agent in 1936, during second world war, waker had first suggestion to used NaOCL in endodontic treatment. in 1941, Grossman utilized it as a medication inside the root canal (Kandaswamy and Venkateshbabu, 2010).

1.4.2 Mechanism of action:

Pecora *et al.* (1999) explained that NaOCL demonstrates a dynamic balance as is shown by the reaction:

($NaOCL + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$)

The chemical reactions between NaOCL and organic tissue are shown in (figure 1.2) (Pecora *et al.,* 1999; Spanσ *et al.,* 2001).

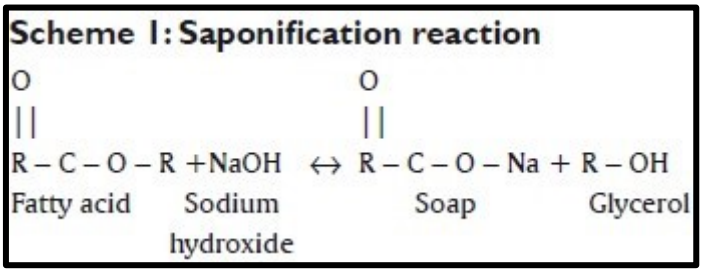

Figure 1.2: Sponification reaction of NaOCL (Pecora *et al.*, 1999; Spanσ *et al.*, 2001)

Sodium hypochlorite considers as a solvent for fat and organic compound, breaking the fatty acids and converted them in to fatty acid salt (soap) and glycerol (alcohol), which decrease the surface tension of solution (figure: 1.3) (Esterla *et al.,*2002).

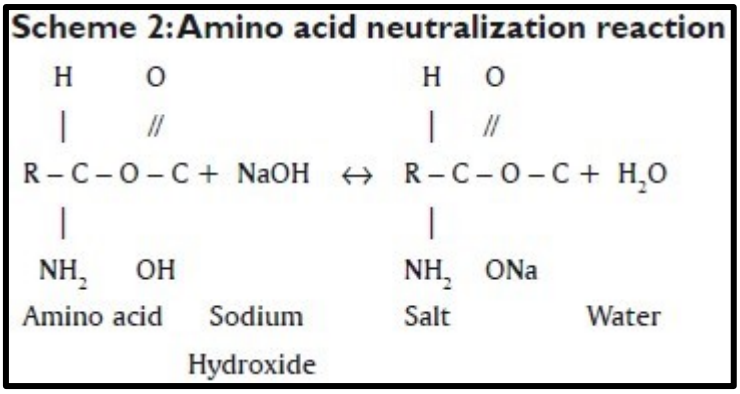

Figure 1.3: neutrilization reaction of NaOCL (Esterla *et al.*, 2002).

NaOCL can neutralize amino acids to form water and salt. (Figure: 1.4). with resales of hydroxyl ions. There is decreasing of PH.

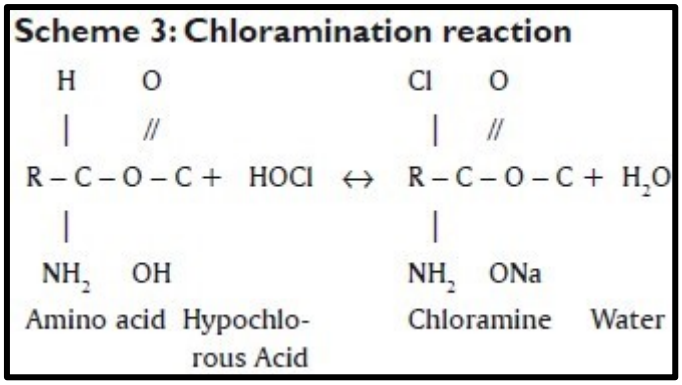

Figure 1.4: cloramination reaction of NaOCL (Esterla *et al.*, 2002).

When hypochlorous acid, compound present in NaOCL solution, come in direct contact with organic tissue, it works as a solvent result in release of chlorine. The later combined with protein amine group for formation chloramine. Hypochlorite ions (ocl-) and hypochlorous acid (hocl-) lead to degradation and hydrolysis of amino acid. The reaction of chloramination between amino group (NH) and chlorin lead to formation chloramines that able to interference with cell metabolism. Chlorin compound (as a strong oxidant) act as antimicrobial agent that inhibit the enzyme of bacteria. And result in an irreversible oxidation of sulphdyl groups of bacterial essential enzyme.

Thus, all these reactions include saponification reaction, neutralization of amino acid and reaction of chloramination that occur in the presence of organic

tissue and microorganism lead to antimicrobial action and responsible for tissue dissolution process. (Esterla *et al.,* 2002).

1.4.3 Concentration of sodium hypochlorite:

There is large controversy about the suitable concentration of sodium hypochlorite that should be used in endodontic treatment, %5 of sodium hypochlorite is designed for treating open wounds. Some authors reported that the most effective regimen for endodontic irrigation is reported to be 5.25% of NaOCL at 40 minute. It has been explained that the restricted area such a root canal required higher concentration of sodium hypochlorite to be more effected in disinfection of complicated root canal system, but important factor should be taken in consideration which is NaOCL toxicity (Siqueira *et al.,* 2000). Most of American practitioner utilized full strength of NaOCL because its available and sold as a house healed bleaching solution. However, when NaOCL used in such concentration, sever irritation if it extruded periapically, in Addition, such concentration reduced the flexure strength and elasticity modulus of dentin (Siqueira *et al.,* 2000). According to the previously available researches, there is no rationale for using sodium hypochlorite solutions at concentrations more than 3 % (Nara *et al.,* 2010). 3% sodium hypochlorite with adequate amount has ability to kill *E.faecalis* inside the root canal (Siqueira *et al.,* 1997). Sodium hypochlorite is a potent irrigant in destroying all form of *E.faecalis* even biofilm (Abdullah *et al.,* 2005).

1.4.4 Effect on biofilm:

According to Bryce *et al.* (2009) NaOCL can effect on the biofilm by several ways:

1. NaOCL can cause completely dissolution cells with no visual evidence can be detected.
2. Disruption of bacterial cell and detached it from the biofilm and make it nonviable.
3. Bacterial cells may remain attached to biofilm but are not viable.
4. Disruption of bacteria and detached the cells from biofilm but are remain viable.
5. Bacterial cells still attached within the biofilm and are viable bacteria.

1.4.5 Complication of sodium hypochlorite:

In addition to toxic effect of NaOCL on vital tissue, it has other complication that result from improper irrigation with NaOCL.

1.4.5.1 Damaging the clothes: this is common complication in endodontic irrigation, even when the NaOCL is used in small amount it may result in great damage, especially when ultrasonic device activator is used, during irrigation aerosol droplet may cause damage to the clothing (Spencer *et al.,* 2007).

1.4.5.2 Eye damage: when NaOCL touch patient's eye or operator's eye sudden pain burning, water perfusion and erythema have been recorded. NaOCL can react with the lipid of corneal epithelial cells, result in formation a soap bubble that able to penetrates the corneal stroma and then enters into the anterior chamber causing tissue necrosis. Lead to endophthalmitis and loss of eye (Spencer *et al.,* 2007).

1.4.5.3 Damage to oral mucosa

Accidental extrusion of NaOCL in the subcutaneous tissue result in rection between sodium hypochlorite with protiens and fats of oral mucosa which might

lead to secondary infections facial atrophy and tissue damage. (Spencer *et al.,* 2007).

1.4.5.4 Extrusion of sodium hypochlorite beyond the apical foramen:

a. Chemical burn and tissue necrosis

Accidental leaking of sodium hypochlorite in the apical area most commonly develop when the apical foramen is wide, damaging to apical constriction of the root canal during preparation or root resorption. Other causes such as aggressive pressure due to injection or bending needle tip of irrigation syringe result in releasing large amount of irrigant substance in the periapical area. Because of excellent ability of NaOCL in dissolution vital tissue, necrosis and chemical burn may result. Sever inflammation in the affected area develops later, followed by swelling in the intraoral side as well as extra orally involving skin and subcutaneous tissue. Edema, hemorrhage or both of them may noticed (Mehra *et al.,* 2000).

Extension of swelling to involved large area may give expectation of acute infection in the effected tooth (Joffe, 1991). The hall mark of damaging to the tissue is recognize by sudden onset of severe pain either immediately or delay for few minute or hours (Witton *et al.,* 2005).

b. Neurological complication:

Extrusion of NaOCL in periapical area Cause anesthesia or paresthesia in the adjacent nerve especially Inferior dental nerve, mental nerve and infraorbital branch of tinning nerve. Complete healing of nerve and return to normal sensation may take several months (Serper *et al.,* 2004).

c. Upper air way obstruction:

Improper isolation during irrigation with NaOCL may cause leaking of sodium hypochlorite solution inside the oral cavity lead to ingested or inhaled by the patient that cause irritation to the throat and in more severe cases compromised the upper air way may have occurred (Becking, 1991)

1.4.6 Allergy to sodium hypochlorite:

Some cases have been reported with hypersensitivity reaction to NaOCL, Kaufman and Keila in 1989 diagnosed a case with hypersensitivity before starting endodontic therapy and the patient recorded as allergist. It is preferable to avoid NaOCL in endodontic irrigation and replaced it by solvidont so the procedure will be completed safely. Another case was reported in 1994 when 1% of sodium hypochlorite that used in irrigation of upper central incisor with horizontal fracture in the middle part of the root. Immediately, the patient felt sever pain and sensation of burn, followed by swelling the upper lip, check and infraorbital area. This sign corresponding with ecchymosis and hemorrhage perfusion from root canal after few minute the pain decrease but the patient still complains of difficulty in breathing (Calişkan et al., 1994).

1.4.7 Toxicity of sodium hypochlorite:

The pH of sodium hypochlorite is 11.2, so when NaOCL come in direct contact with human tissue protein and nitrogen caused formation of acetaldehyde and formaldehyde by breaking the peptide link and protein dissolution (Hauman and Love, 2003), This procedure involved replacement of hydrogen in amine groups (-NH-) by chlorin (-NCL-) so the chloramin was formed. The later compound is responsible for antimicrobial activity. Dissolution of pus and necrotic tissue facilitate the action of antibacterial agent to clean the infected area. A rise in temperature of NaOCL improve its antimicrobial effectiveness and ability tissue lysis. As the concentration of NaOCL increased its toxicity and tissue irritation increased (Hauman and Love, 2003). It was reported that the application of 10 ml of 3.12, 5.25% of NaOCL on a dog esophagus over a period, the effect is different when NaOCL applied on mucosal surface from that introduced interstitially of 5-minute cause acoustic burn. Therefore, the contact period should be as least as possible, the results of this experimental study stated

that untoward effect is different when NaOCL applied on mucosal surface from that introduced interstitially (Yarington, 1970). Another supporting study by Weeks and Ravitch in 1971 reported ulceration, necrosis area of hemorrhage, sever edema also stricture formation in the cat's esophagus as a result of undiluted household NaOCL come in direct contact with mucosa. An in vitro Study observed the cytotoxicity of sodium hypochlorite in 3 independent biological model, the result of this study suggested that concentration as minimum as 1:1000 (v.v) NaOCL in normal saline can be able to completely hemolysis the red blood cell, because of the solution utilized in this study was isotonic solution this means exclude the osmotic pressure gradient, so that the result of hemolysis and loss protein of the cell was due to oxidation reaction of NaOCL in cell membrane.

Heggers *et al.* in 1991examined bactericidal properties of NaOCL when used as irrigant for wound healing *in vitro* and *in vivo* study, they found that the safest concentration of NaOCL is 0.025% by using this concentration the NaOCL has good bactericidal effect without tissue toxicity. Zhang *et al.* (2003) examined the cytotoxic properties of different four concentrations of NaOCL (0.66%, 1.31%, 2.63%, 5.25%) 3%H_2O_2, eugenol, ca$(OH)_2$ past and MTAD. Result of this study indicate that toxicity of NaOCL depend on the dose. Barnhart *et al.* in 2005 Used the CyQuant assay procedure on culture gingivalis fibroblast to measure the cytotoxic effect of several root canal agents, result of this measurement was NaOCL significantly more cytotoxic than iodine-potassium iodide and $(CaoH)_2$.

1.5 Ethylenediamintetraacetic acid

Ferdinand Munz who first described the EDTA compound in 1935, he prepared it from Ethylene Diamine and Chloroacetic Acid (Cagnasso et al., 2007). Ethylenediamintetraacetic acid (EDTA) is classified as chelating agent and its formula is (HO2CCH2) 2NCH2CH2N (CH2CO2H) 2. EDTA is polyamine carboxylic acid that commonly used to sequester divalent and trivalent

ions of metal. EDTA able to bind the metal through four carboxylate groups and two amine groups. EDTA can bind with Cu (II), Mn (II) Co (III) and Fe (III) to form strong complexes. EDTA most commonly prepared by mixing 1,2-diaminoethane (ethylene diamine), formaldehyde, sodium cyanide and water (Holleman and Wiberg, 2001; Spencer *et al.,* 2011), This result in formation tetra sodium salt. Which can be changed to acidic form by process of acidification. EDTA is color less (water-soluble solid polyaminocarboxylic acid). It is commonly utilized in dissolution Lime scale. It is very useful because able to hexadenal ligand as well as chelating agent it can sequester metal ions such as Ca^{2+} and Fe^{3+}. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentin to a depth of 20-30 μm in 5 min. (Mohammadi *et al.,* 2013)

After ions bound by EDTA, they remain in solution, but show diminished reactivity. Stay in solution with decrease its reactivity (Harris, 2006) EDTA is available as several salt mostly calcium- disodium EDTA and disodium EDTA.

1.5.1 Removal of smear layer:

Reaction between EDTA and calcium ions in dentin result in formation calcium chelates. EDTA able to dentin decalcification to the depth of 20 -30 mm in about 5 min (Doumani *et al.,* 2017). The liquid type of EDTA is more effective in smear layer removal than paste type. Normally EDTA used in concentration 17% and in this concentration it can remove smear layer in less than 1 min after direct contact with root canal (Doumani *et al.,* 2017). Addition of surfactant to EDTA did not affect its ability to smear layer removal (Lui *et al.,* 2007). Di Lenarda *et al.* (2000) accreted that EDTA is very effective in removal the smear layer...

According to Teixeira *et al.* (2005) one-minute irrigation with 17% EDTA is more effective than irrigation for 30 second in smear layer removal. Perez and Rouqueyrol-Pourcel in 2005 explained that the ability of 8% of EDTA was as effective as 1 min irrigation with 15% EDTA. The effectiveness of EDTA in

smear layer removal was proved by da Silva *et al.* (2008) as well as by Mancini *et al.* (2009)

Caron G, et al in 2009 confirm that 17% of EDTA and 3% of NaOCL combination is effective n smear layer removal, ultrasonic activation can improve the effectiveness of this combination. Sen *et al.* (2009) confirmed that there was difference between various concentration of EDTA (1%, 5%, 10%, 15%) in ability to smear layer removal.

Rödig *et al.* (2010) affirm the effectiveness of EDTA in smear layer removal. Adigğzel *et al.* (2011) demonstrated that continuous irrigation with EDTA with self-adjusted file procedure result in canal walls without smear layer in 85%, 60% and 50% and free of debris in 95%, 90% and 85% in cervical third, middle third and apical third respectively. Prado *et al.* in 2011 showed that EDTA was comparable with phosphoric acid in ability to smear layer removal. Wu *et al.* (2012) stated that the ability of 17% EDTA in removing smear layer is much more significant than 20% of citric acid and MTDA

1.5.2 Antimicrobial activity of Ethylenediamintetraacetic acid:

Patterson in 1963 Revealed that the antibacterial activity of EDTA is limited. In the other hand, EDTA had no antimicrobial effect against *E.faecalis* even after contact for 60 min (Arias-Moliz *et al.,*2008). According to Orstavik and Haapasalo (1990) the antibacterial activity of 17% EDTA is questionable. It appears that EDTA exhibit antibacterial action by chelation of certain form the bacterial outer membrane (Mohammadi *et al.,* 2013). According to Russell in 2003 10% of EDTA produced inhibition zone of bacterial growth similar to creosote. However, when decrease the concentration of EDTA, little or no inhibition zone has been appeared. Ordinola-Zapata *et al.* in 2012 showed that that the effect of EDTA against *E.faecalis* same as maleic acid.

1.6 MTAD

MTDA is an irrigant solution which is a mixture of tetracycline isomer (3% doxycycline) as antimicrobial agent, 4.25% citric acid (demineralizing agent) and a detergent (tween 80) to reduce the surface tension of solution. MTAD showed encouraging results as an effective endodontics irrigant (Torabinejad *et al.,* 2003). Recent studies evaluating the use of MTAD as an irrigant solution have been showed favorable properties in smear layer removal, disinfection of root canal and eradication of *E.faecalis* (Kamberi et al ., 2012; Rizvi *et al.,* 2014; Khawaja, et al., 2015)

1.6.1 Antimicrobial activity of MTAD

Shabahang *et al.* (2003) investigated the antimicrobial activity of MTAD, the investigation showed that MTAD can maintain its bactericidal properties even after diluted it for 200 times. This characteristic of MTAD was in contrast to NaOCL, which lose it is bactericidal properties beyond 32% dilution. In addition to decreased NaOCL's antibacterial activity, diluted NaOCL result in considerable decrease in its dissolution ability of necrotic tissue as showed by (Hand *et al.,* 1978), in spite of that dilution of any irrigant result in reduction in its inhibition zone, But the result of microbiological investigation showed that MTAD can maintain its bactericidal activity more than both NaOCL and EDTA.

According to (Torabinejad *et al.,* 2003) MTAD capable of eradicate *E.faecalis* after 2 or 5 minute of exposure. And this property was not observed when NaOCL or EDTA were used. Shabahang *et al.* in 2003 investigated the antimicrobial activity of NaOCL and MTAD by testing their ability to eradicate the infection of root canal that had been previously contaminated with saliva, investigation's result showed that 40% of root canals remain contaminated when using NaOCL beside that, MTAD had superior antimicrobial activity in comparison with 5.25% NaOCL. Another important finding of their study is that

MTAD can exert antimicrobial activity in brief period of time, this property of MTAD is desirable because during irrigation procedure in clinical practice irrigant solution may reach some areas of root canal system for short time. Addition to that the disinfection ability of MTAD obviated the need for putting intercanal dressing that need multiple visits.

Portenier *et al.* in 2006 evaluate the antibacterial activity of MTAD against two different strain of *E.faecalis* their result showed that both bacterial strain were significantly decreased. Diluting the MTAD to 10% of it is primary concentration decrease the speed of bacterial killing. As MTAD containing doxycycline that binds to dentin, the effect of it remains available for more than 5 minute to the body. This mean that even after removal of MTAD cleanser it has persistence activity in killing bacteria.

1.6.2 Biocompatibility

Zhang *et al.* in 2003 investigated the cytotoxicity of MTAD and compared it with several common irrigants that currently used. According to the test result, it appears that the cytotoxicity of MTAD is less than 3% H2O2, eugenol, CA(OH)2 paste, peroxide 5.25 NAOCL and EDTA. Also indicated that MTAD is more cytotoxic than 2.63%, 1,3% and 0.66% of NaOCL. Torabinejad *et al.* in 2005 compared between two protocols of smear layer removal regarding to levels of discomfort post-opratively (6 hours to 7 days), the results indicated that there is no significant difference in levels of post-operative discomfort between MTAD cleanser and EDTA solution, another finding of their study is MTAD did not cause any post- operative discomfort when used as a final irrigant.

According to Machnick *et al.* in 2003, MTAD didn't cause undesirable effects on the physical properties of dentin, the result of their study showed that MTAD achieve most of positive requirement of ideal irrigant solution.

1.6.3 Smear layer removal

MTAD is able to remove smear layer without erosion effect on dentin (Barkhordar *et al.,* 1979; Torabinejad *et al.,* 2003). Beltz *et al.* in 2003 compared between various concentration of NaOCL, EDTA and MTAD in relation to amount of tissue loss by exposing the bovine pulp and dentin to these irrigants material, their results indicated that different concentration of NaOCL able to remove organic tissue from dentin and pulp effectively. EDTA showed similar effect to MTAD as it is solubilizing effect, this mean that the difference between these irrigant solutions due to high binding affinity of MTAD's doxycycline to dentin.

Torabinejad *et al.* in 2003 evaluate the effectiveness of MTAD in smear layer removal, ability to open dentinal tubule that facilitating the penetration of antibacterial irrigants to the whole canal system, The results demonstrated that MTAD is an effective irrigants in smear layer removal. Based on the result of this evaluation, it seems that MTAD has less destructive effects on the tooth structure compared with EDTA solution. Deep examination of the structure of dentinal tubule indicated that there is a higher amount of erosion with EDTA group.

1.6.4 Tooth discoloration

The major component of MTAD is doxycycline which is highly adsorbed by tooth structure that cause the tooth to keep its staining, this phenomenon occurs when MTAD cleanser and NaOCL irrigants were used together in the presence of natural light because of photo oxidation process that was exaggerated by the presence of NaOCL as it oxidizing agent, it has been reported that red-purple color on the root dentin that exposure to light when irrigated with 1.3% NaOCL as initial irrigant and MTAD as final irrigant rinse (Tay *et al.,* 2006 a). The product result from photo oxidation possess a high affinity for hydroxyapatite. This discoloration of teeth possibly due to triggering by NaOCL, can be avoided

by application of reducing agent like ascorbic acid previous to MTAD final irrigation (Tay *et al.,* 2006 b).

1.7. Citric acid

Citric acid is unusual irrigant solution, its chelating agent has the ability to react with metal ions and form nonionic soluble chelate. concentrations ranging from 1% to 50% have been used to remove the inorganic substance of the smear layer with a 10% solution being the most common and are used for 2–3 min at the end of instrumentation and after NaOCl irrigation. Citric acid is a weak organic acid with the appearance of white crystalline powder at room temperature. It can exist either in water-free form (anhydrous) or monohydrate form. The water-free form crystallizes from the hot water, whereas the monohydrate forms from the cold water. The latter may be converted to anhydrous form by heating more than 78°C. (Haapasalo *et al.,* 2010).

1.7.1 Antimicrobial activity of citric acid:

The use of 25% citric acid was found to be ineffective in eradication of biofilms of *E.faecalis* after 1, 5, and 10 min of exposure (Arias-Moliz *et al.,* 2009). Its most commonly used as adjunctive solution with another strong antibacterial agent.

1.7.2 Smear layer removal by citric acid:

Citric acid has the ability to smear layer removal (Haapasalo *et al.,* 2005). Citric acid is available in many concentrations range from 1% -50% (Loel, 1975). Liolios *et al.* (1997) Stated that commercial EDTA is more effective than 50% citric acid in smear layer removal. Takeda *et al.* (1999) showed that irrigation with 17% EDTA, 6% citric acid, 6% phosphoric acid cannot remove whole smear layer from root canal. it had been reported that both EDTA and citric acid able to remove smear layer when used together with NaOCL.

Other researches Di Lenarda *et al.* (2003) and Scelza *et al.* (2000) Reported that there is negligible difference in smear layer removal between citric acid and 15% of EDTA.

1.7.3 Toxicity of citric acid:

By using the 3-(4,5-dimethylthiazol-2-yl0-2.5-diphenyltetra-zolium bromide assay) (MTA), Prado *et al.* (2015) Showed that cell viability of 10% citric acid is higher when compared with other examined irrigant solutions. Marins *et al.,* 2012 Investigate the ability of several irrigant solution to induce cellular death or/and genetic damage in fibroblast (invitro study), The result indicated that citric acid, EDTA and NaOCL cytotoxicity is does dependent and they don't show any Geno toxicity.

Kang *et al.* (2013) Examined the biocompatibility of MTA by mixed it with hydration accelerator such as calcium chloride, citric acid and lactate gluconate, they conclude that best result showed when MTA mixed with 0.1 wt % of citric acid.

Materials and Method

This study was an *in vitro* and comparative study (figure 2.1). it was conducted in the pathologycal department/ College of Dentistry/ University of Kufa from 1.12.2017- 1.8.2018. Samples were collected from Muslim Ibn Aqeel Primary health center at Al-Kufa city.

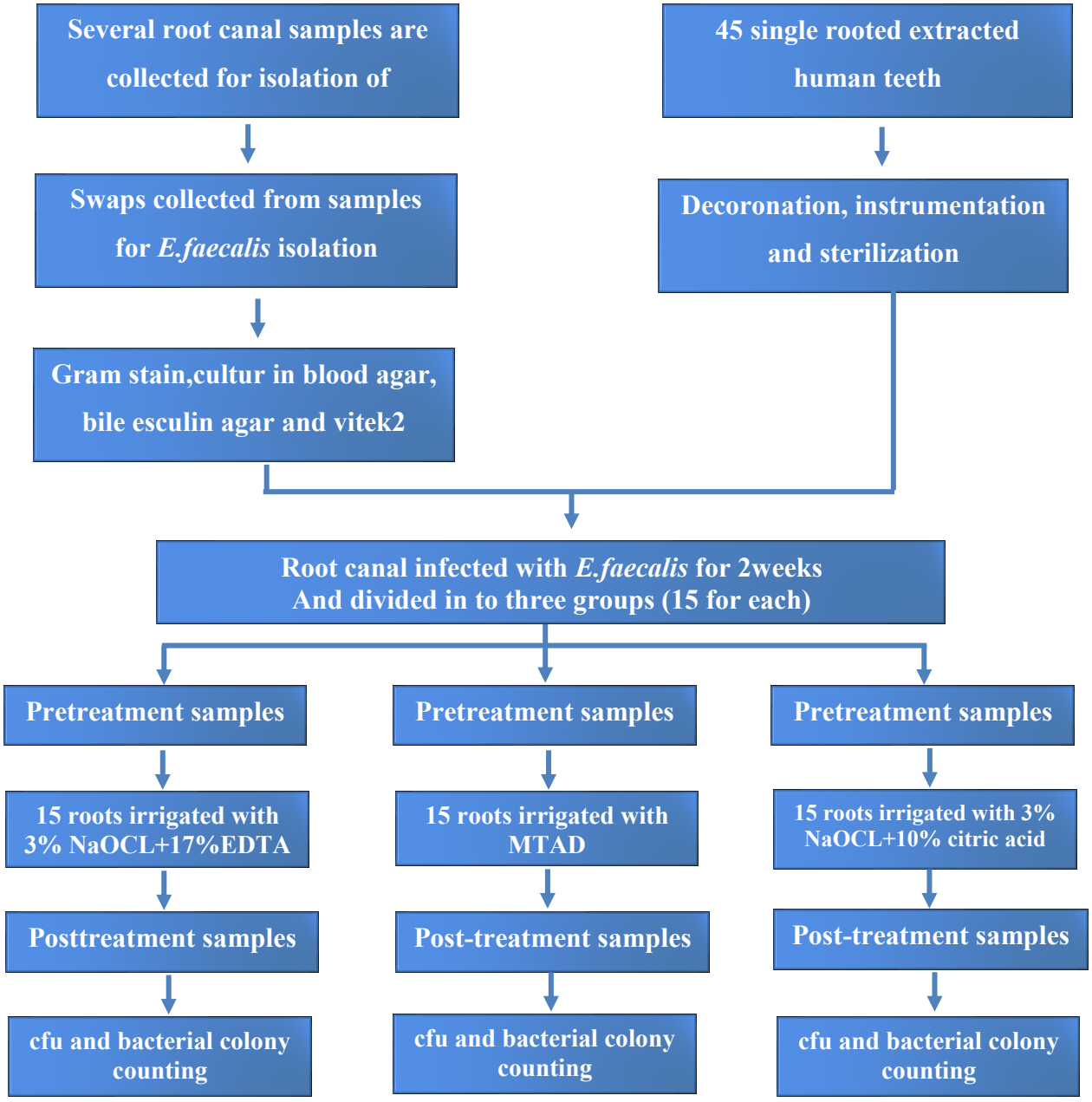

Figure 2.1: Design of the study

2.1 Sample size and source

The sample consist of 45 extracted human primary anterior teeth. This sample size was estimated for comparative evaluation to the amount of the disinfection by microbiological analysis using a formula that was used by the previous studies (Attiguppe *et al.,* 2017). These teeth collected from healthy children aged 4-6 years.

2.1.1 Inclusion criteria:

1. Two third of the root length should be preserved (Goztas *et al.,* 2014).
2. The teeth collected in this study were being extracted due to therapeutic cause such as over retained deciduous tooth another reason when the patient was not willing for pulpatomy or polypectomy procedure.
3. Trauma to the upper anterior teeth.

2.1.2. Exclusion criteria:

1. Patients with systemic diseases or taking medications.
2. Patient with congenital or acquired dental anomalies.
3. Patient with extensive root resorption.

2.2 Preparation of teeth

Scaling and polishing were performed for all the teeth to remove any presented calculus and remnant periodontal tissue (Zhu *et al.,* 2013). Working length was determined clinically by using A #10 Kerr file (dentsply) noticing the file tip through apical foramen (Xhevdet *et al.,* 2014). Preparation and instrumentation of root canal was done by using iso system files till reach size 30 k file (Zhu *et al.,* 2013) (Figure: 2.2; figure2.3).

Figure 2.2: ISO file system (dentsplay).

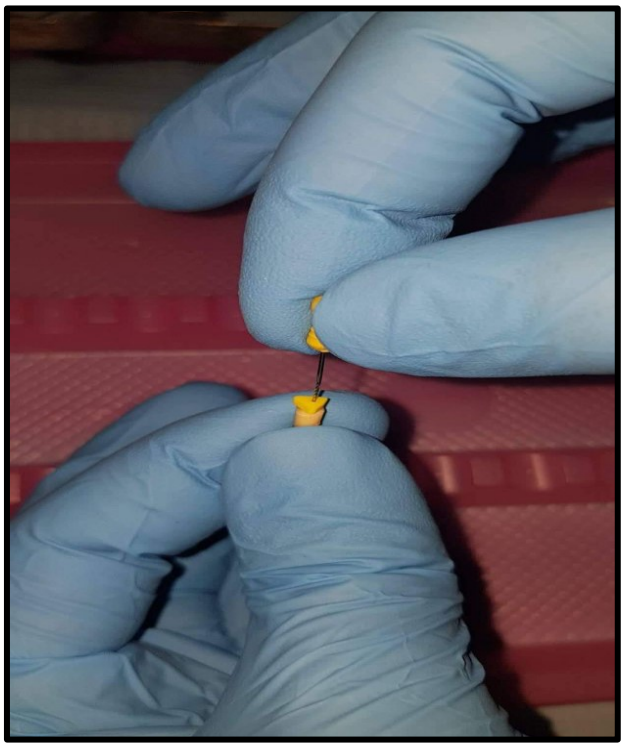

Figure 2.3: instrumentation of the root

2.3 Sterilization of the teeth

Sterilization of the teeth was accomplished by transferring them to a flask with normal saline in autoclave for 30 minute at 121 C° with 15 IB (Nagayoshi *et al.,* 2011). Specimens were randomly divided in to three groups.

2.4 Equipments and instruments

1- Adjustable micropipette with disposable tips (BIONEER, Korea).
2- Anaerobic jar (HP025-China).
3- Autoclave (steam sterilizer) type Rayapa-35064 (made in Spain).
4- Barbed broach (Produits Dentaires S.A., Vevery, Suisse).
5- Benzene burner (China).
6- Dental chair unites.
7- Disposable gloves (Comfit, Malaysia).
8- Disposable syringe 5cc (Kamadia, Iraq).
9- Disposable tube of 5ml (China)
10- Disposable tweezers
11- Glass marking pencil
12- High-speed hand piece (W&H Austria).
13- K file set (Densplay).
14- Mask (Mexpo International Company, USA).
15- Paper point (Korea)
16- Plastic disposable cups (China)
17- Plastic petri dishes (Japan)
18- Rubber polishing cup.
19- Test tube (China)
20- Vitek 2 machine for identification the bacteria.
21- Wire loop for bacterial inoculation.
22- X-ray film size -2 (Coda-ultra speed DF -58)

2.5 Solutions and chemicals

*Crystal violet (0.5%)

*Lugol iodine (potassium iodine 2.0%), (resublimed iodine 1.0%) and (safranin 1.0%).

6. 10% citric acid (Panreas Spain)
7. 17% EDTA (cercamed, polonia)
1. Alcohol spray.
2. Bile esculin Agar (Himedia India)
3. Blood agar base (Himedia)
4. Brain heart infusion broth (Mast USA).
8. Distal water (Iraq).
9. Doxycycline (Direvo Industrial Biotechnology Germany)
10. Normal saline 0.9 (I.V. Production plant, Iraq).
11. Pumice (Ainsworth Prophylaxis Pumice 400G)
5. Reagent composition
12. Sodium hypochlorite (Tehno Dent Chlora XD 3%)
13. 96% Dettol.

2.6 Sterilization

The test tubes, screw capped bottles and all steel instruments were sterilized by hot oven at 160 C° for one hour. Broth, culture media were sterilized by autoclave at 121c at 15 pounds per square inch for 15 minutes. 96%Dettol was used for sterilization the benches and floor of the lab.

2.7 Culture media

The culture media used in this study were:

1-Blood agar

2-Bile esculin agar

3-Brain heart infusion broth

2.7.1 Preparation of Blood agar

Composition of ingredient

1. Pancreatic digest of casein
2. Papatic digest of soy meal
3. NaCl
4. Agar
5. Distilled water

Procedure of preperation

Prepare the blood agar according to manufactures' instruction and sterilized by autoclave. Transfer the blood agar base to 50 C° water bath and left it to cool. Add sterilized blood agar and mix gently (avoid bubble formation). Disperse 15 ml amount of it in sterile petri plate. Label the media with date and store it in 2-8 C.

2.7.2 Preparation of Brain heart infusion broth

Brain heart infusion broth is highly nutritious media used for cultivation of fastidious organism and other organism.

Formula:	gm/ liter
Brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	5.0

PH 7.4 +- 0.2 at 25 C

Dissolve 37 g in litter of distilled water. Mix it well and then distribute in to final containers. (Storage at 10-30 C°), Figure (2.4).

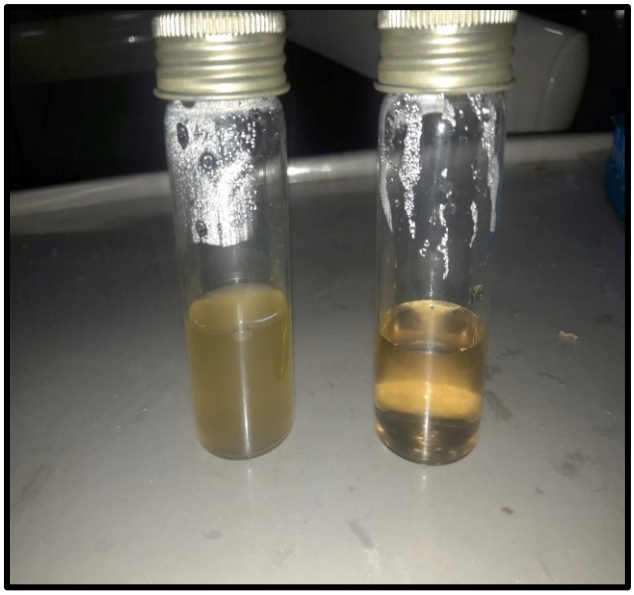

Figure 2.4: Brain heart infusion broth sterile clear (right)and with *E. faeclais turbid* (left)

2.7.3 Preparation of bile esculin agar

Esculin is glycosidic coumarin derivative (6- beta –glucoside -7-hydroxy coumarin). These two moieties of molecules which are (glucose and 7 hydroxy-coumarin) are bonded together by an ester bond through oxygen molecule. In this test, media that containing 4% of bile salt media was used for esculin incorporation. Bile esculin –positive bacteria have ability to grow with the presence of bile salt. Also able to hydrolyse esculin and result in formation glucose and esculetin Isolation of *E. faecalis*.

Formula	gram / liter
Peptone	14.0
Bile salt	15.0
Ferric citrate	0.5
Aesculin	1.0
Agar	14

PH 7.1+- 0.2 at 25C

Direction according to the manfucture's instrument

Suspend 44.5 g in 1liter of distilled water and then bring gently to the broth to complete dissolving. (storage at dehydrated media at 10-30 C. (figure:2.5)

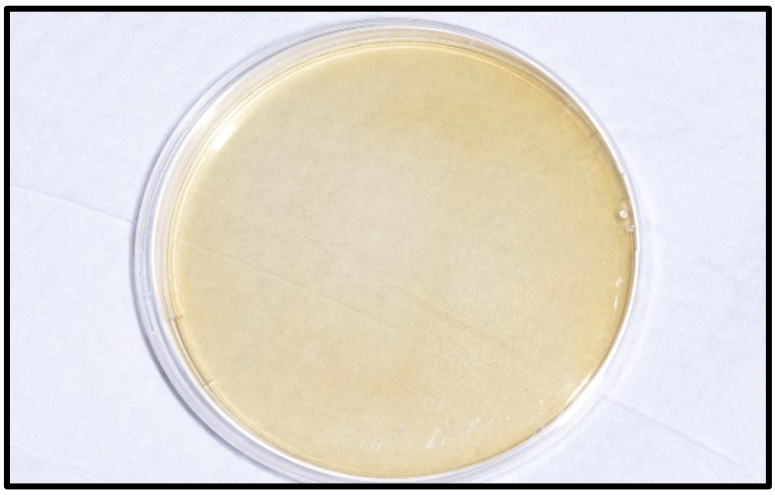

Figure 2.5: bile esculin agar plate

2.8 root canals specimen's collection for bacterial isolation

Several suspected teeth were chosen for testing the presence of *E.faecalis*. They were necrotic teeth, teeth with periapical lesion and teeth with failed endodontic treatment and requiring retreatment of endodontically treated teeth with the presence of apical periodontitis. Pulp vitality test, radiography and clinical examination are diagnostic methods used to confirm necrotic pulp or periapical lesion of the tooth (Mahmoudpour *et al.,* 2007). Samples were taken from healthy person. Any patients with systemic diseases, immunological diseases or under medications treatment were excluded from study. Consent was obtained from all patients. Access opening were made using sterile burs without any water spray. Iso type file (dentsply) was introduced to approximately 1.0 mm below the radiographic apex, and instrumentation of canal to #30 K file. without any irrigation. If the root canal appeared dry, sterile saline was introduced into it. Two paper points (Diadent, Korea) were inserted into the canal for about 1 min to soak up the fluid then papers point was used to inoculate bacteria from samples

to blood agar and cultivation for 24 hours, 37 C° in anaerobic atmosphere. Firstly, the collected samples were put in transport media (BHI), cultured in the blood agar to differentiate it from other microorganisms (In solid media enterococcus species appeared as smooth, white or creamy colonies, *E. faecalis* are non-hemolytic in blood agar).

2.9 Identification of bacteria:

Identification of *E.feacalis* was completed by using gram stain, bile – esculin, which is a selective media for *E.faecalis* (Wang *et al.,* 2012) and vitek 2 machine.

2.9.1 Gram staining procedure:

Reagent:

1. Crystal violet (primary stain)
2. Iodin solution/ grams iodin.
3. Decolorizer (e.g. ethanol)
4. Safranin (secondary stain)
5. Water.

Procedure of Gram stain:

Colonies of *E.faecalis* picked from the blood agar plate under the microscope and supjected to the Gram's stain procedure

2.9.2 Bile esculine test:

Using inoculation loop two or more suspicious colonies that had morphological similarity were touched and spread on bile esculin plate by streak or motion. Then, the inoculated samples were incubated for 24 hours at 35-37 C° (according to manufacturer instruction).

The principle of the bile esculin test depends on the ability of *E.faecalis* to hydrolyze esculin in the presence of bile (4%bile salt or 40% bile). In fact, many bacteria are able to hydrolyze esculin, but in the presence of bile few of them can do so. Reaction between esculin and ferric ions result in formation a black diffusible complex (Mohammad pour et al., 2007), As in figure (2.6).

2.9.3 Vitek 2machine:

1. Incubation of the isolated bacteria for 48 hours (that had been grown in selective media).
2. Testing the bacterial turbidity through dense check by suspension a number of isolated bacteria in 3 ml of normal saline. The result showed to be range from 0.5 -0.6 ml (range of McFarland standard).
3. Insert the bacterial suspension in the vitek 2 machine. 4-6 hours needed by vitek for identification the specific bacterial type and 8-12 hours for bacterial sensitivity (according to manufacturer instruction), Figure: 2.6.

Figure 2.6: vitek 2 machine

2.10 Inoculation of bacteria inside teeth:

Firstly, *E.faecalis* bacteria was cultured in brain heart infusion broth at 37C° for 48 hours, then 3ml of broth suspension was insulated inside sterile tooth specimen canal. Inculcated root canals specimens kept in brain heart infusion broth at 37C for further growth, 2 weeks period chosen for inoculation, as such a period could allow for the recognizable and appreciable colony of bacteria to produce and shown (Sohrabi *et al.,* 2016). Figure (2.7).

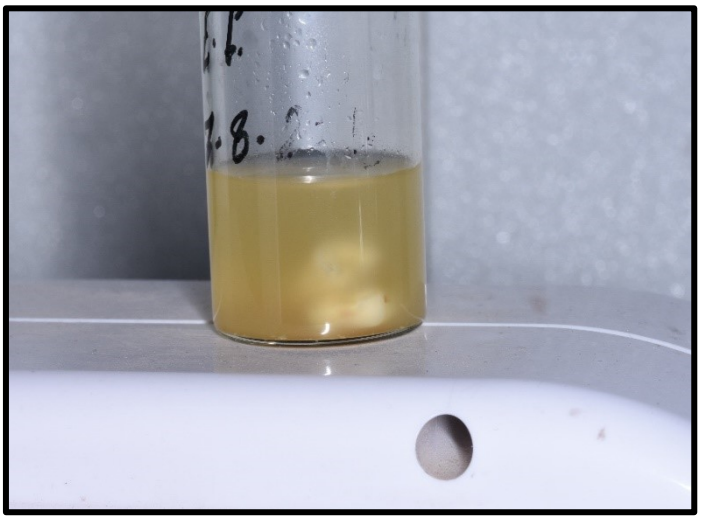

Figure 2.7: storage of the teeth in brain heart infusion broth

2.11 Counting the bacterial colony before experimental procedure:

In sterile environment, root canal firstly irrigated with 2 ml sterile normal saline solution to which a sterile paper point is inserted inside the canal for at least 60 second to soak up in the canal content, (figure 2.8). The sterile normal saline solution here acts as a carrying medium of bacterial colony from root canal to the blood agar plate. The paper point sample kept in Eppendorf tube filled with 1ml of normal saline, Figure (2.9).

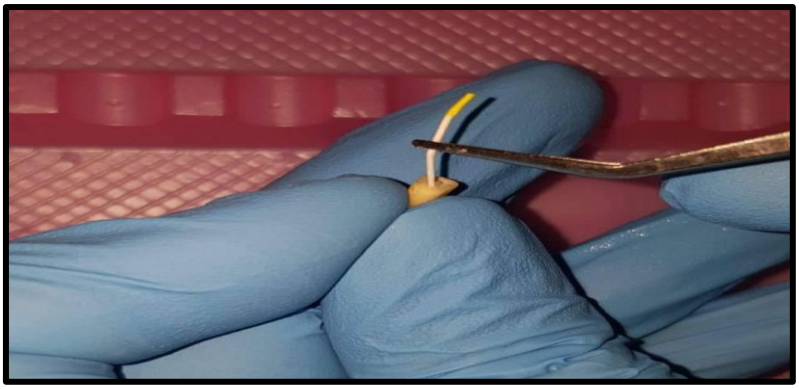

Figure 2.8: pre treatment samples by sterile paper point.

Figure 2.9: pre and post sample

2.12 Irrigation procedure:

Group 1:

Standard irrigation with 0.9 normal saline then 1ml of 3% NaOCL used for irrigation in this group. Followed by irrigation with 5 ml of 17% EDTA and final irrigation with 0.9 normal saline. A ProRinse needle 30-gauge needle was used total irrigation time is 2 minute (Kho and Baumgartner, 2006), (Figure 2.10; 2.11)

Figure 2.10 :3% NaOCL

Figure 2.11: 17% EDTA

Group 2:

Standard irrigation with 0.9 normal saline was used followed by thorough irrigation with 2 ml of MTAD solution and then left it for 5 minute. A 30-gauge ProRinse needle was used to deliver the irrigation material. Final irrigation was with 0.9 normal saline (Nara *et al.,* 2010).

Group 3:

Standard irrigation with 0.9 normal saline then followed by 1 ml of 3% NaOCL, Followed by irrigation with 5 ml 10% citric acid for 1 min, and finally irrigation with 0.9 normal saline again. A 30 gouge ProRinse needle was used, the total irrigation time was 2 minute (Reis *et al.,* 2008; Arslan *et al.,* 2014) (figure 2.12).

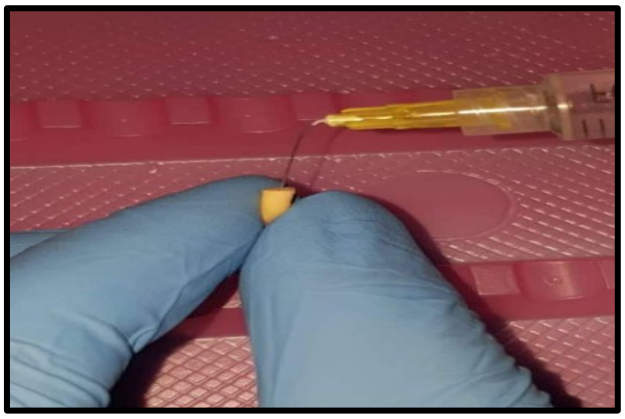

Figure 2.12: irrigation of the root canal.

After completion of the irrigation procedure, a sterile paper point was inserted inside the full working length of the canal and left for 60 second to allow enough time for the bacterial inoculation from the canal surface. The post irrigation sample kept in 1ml of normal saline in Eppendorf tube for complete bacterial colony counting, Figure (2.13).

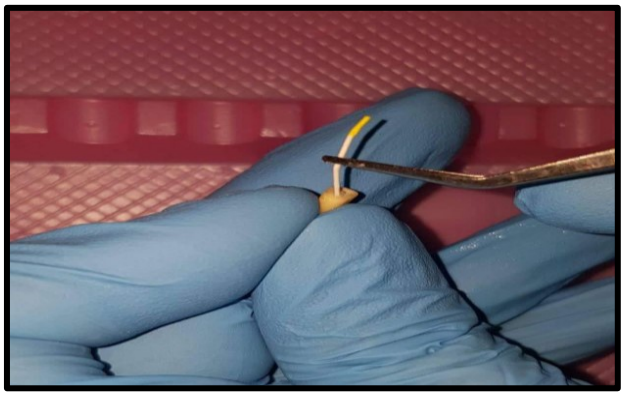

Figure 2.13: post operative samples by sterile paper point

2.13 Counting bacterial colony:

Both pre and post samples transferred to the laboratory for colony counting, figure (2.14). Paper point used to viably count the bacterial colony in the blood agar plate. The Eppendorf tubes containing the socked paper points were shaken using a vibrator and mixed well by the means of a pipette to make

sure that bacteria are evenly distributed through the solution. After that, each sample was serially diluted for four dilutions. The dilution factor was 10^{-1}, 10^{-2} 10^{-3} and 10^{-4} consequently figure (2.15). Following an adequate mixing, a 10 µl of each dilution was spread on a blood agar. Then blood agar incubated for a period of 24 hours at 37C figure (2.16). The number of colony was obtained by cfu/ml (colony forming unit per ml) to both pre and post samples to compare the effectiveness of each irrigant. The viable counting cfu/ml were performed using the following equation, (Nagayoshi *et al.,* 2011).

$$\left(\frac{cfu}{ml} = \frac{\text{no. of colonies}}{\text{diluton factor} * \text{amount of plate solution(ml)}}\right)$$

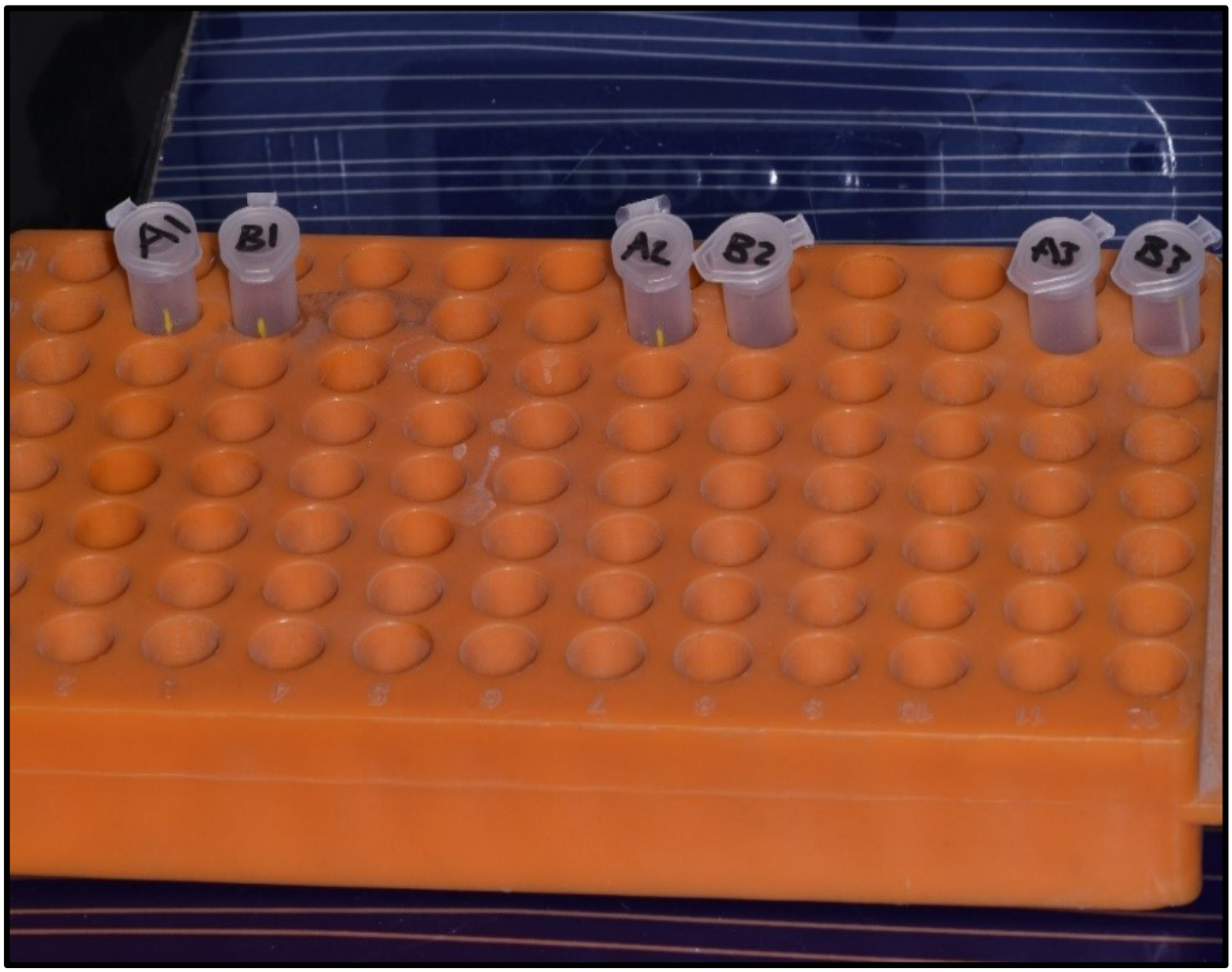

Figure 2.14: pre and post samples in 1ml of normal saline send to the lab

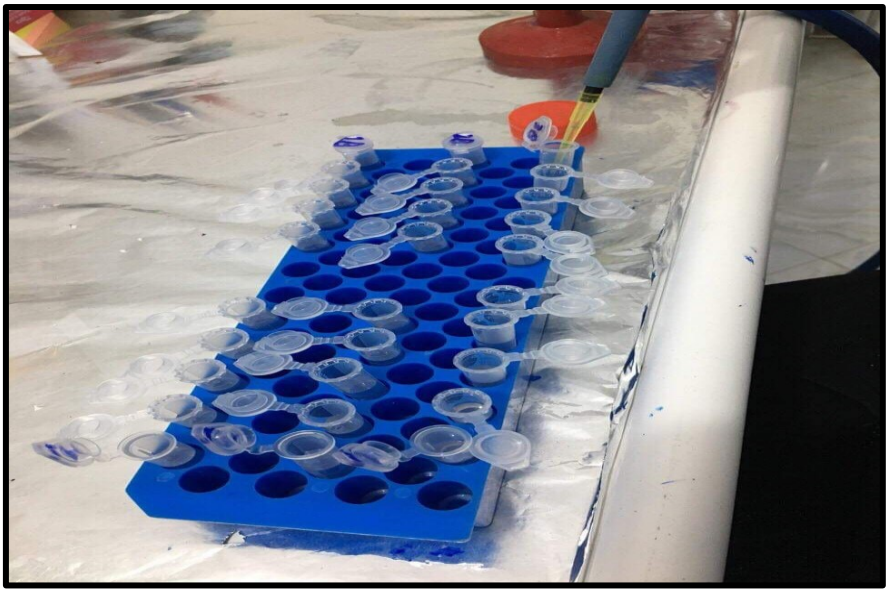

Figure 2.15: serial dilution

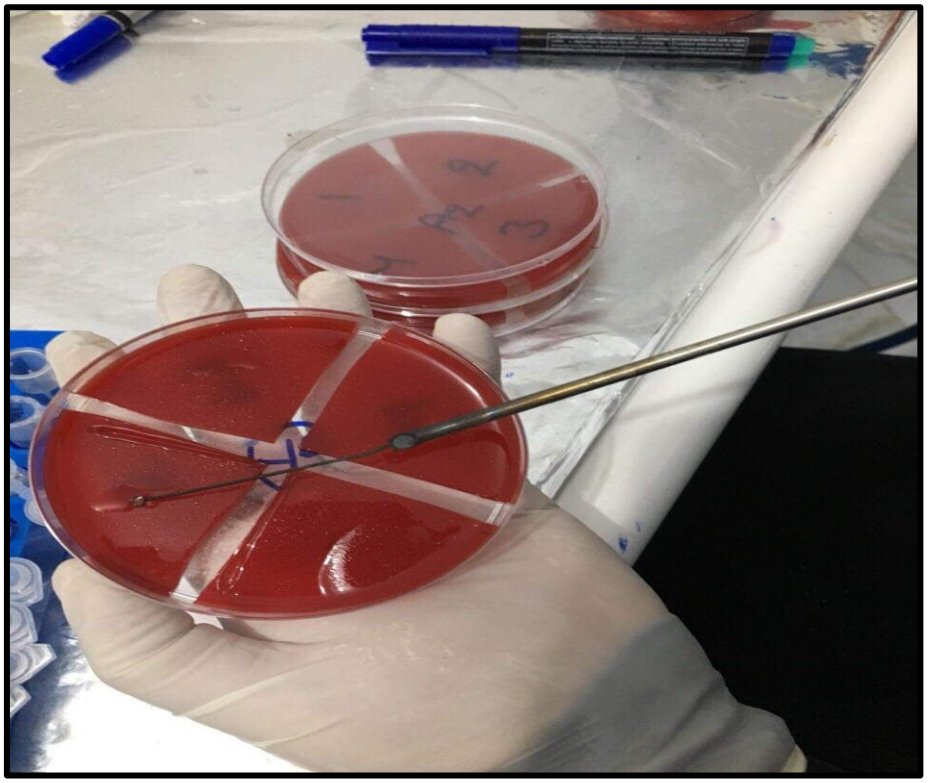

Figure2.16: inoculation of the bacteria in the blood agar

Statiscal analysis of data

A one-way ANOVA with post-hoc Dunnett t3 test analysis were performed with the percentage of of the microorganisms as the dependant variable and type of treatment mode as factors. The level of significance was set at $p<0.05$. Statistical analysis of data was performed using SPSS 20 (IBM, USA).

Results

3.1 result of biological test

Microscopic examination

The morphology of *E.faecalis* colonies under the microscope was Gram positive arranged in short chain. *E.faecalis* are Gram's positive, circular, convex colonies with entire margin arranged in short chain, Figure 3.1.

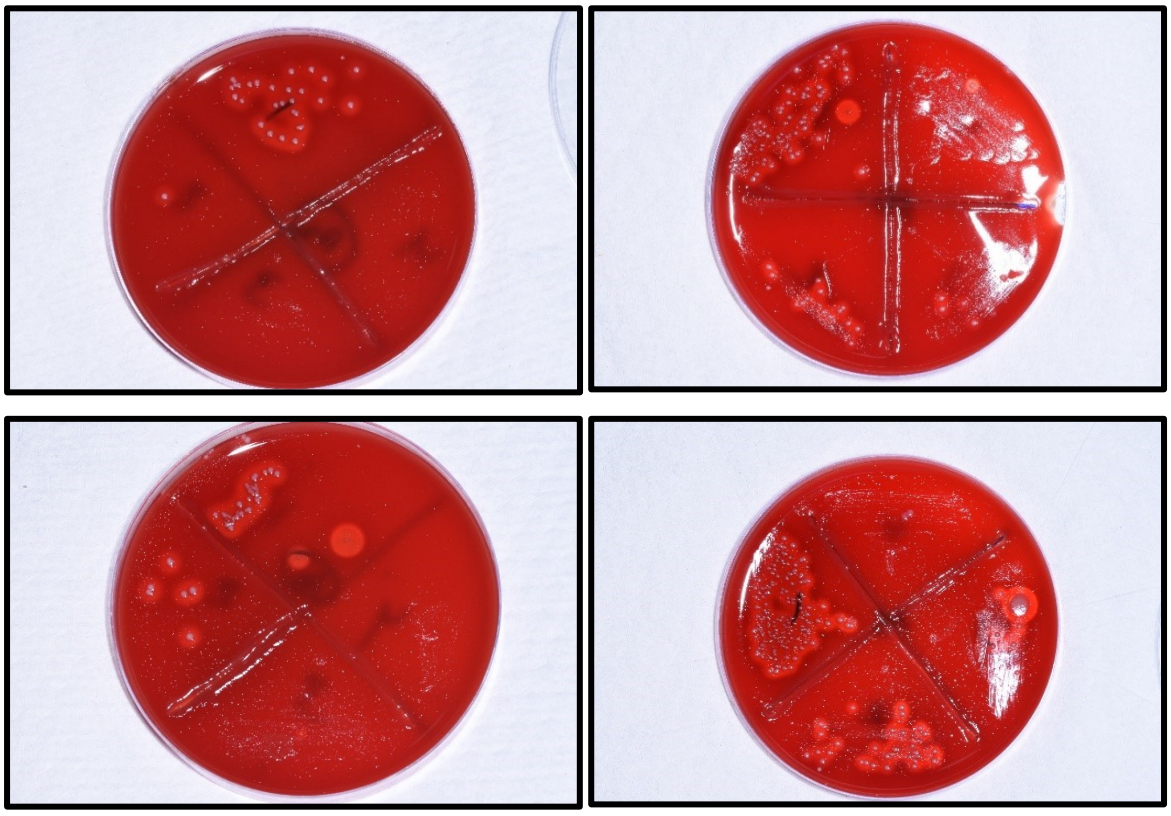

Figure 3.1: E.faecalis in the blood agar

Result of bile esculin test

Black haloes that observed around colonies and any black area consider positive. As the bile esculin is a selective test for enterococcus and non-enterococcus group D streptococcus, so further detection by vitek 2 system was needed as it is very accurate in detection of the *E.faecalis* (figure 3.2).

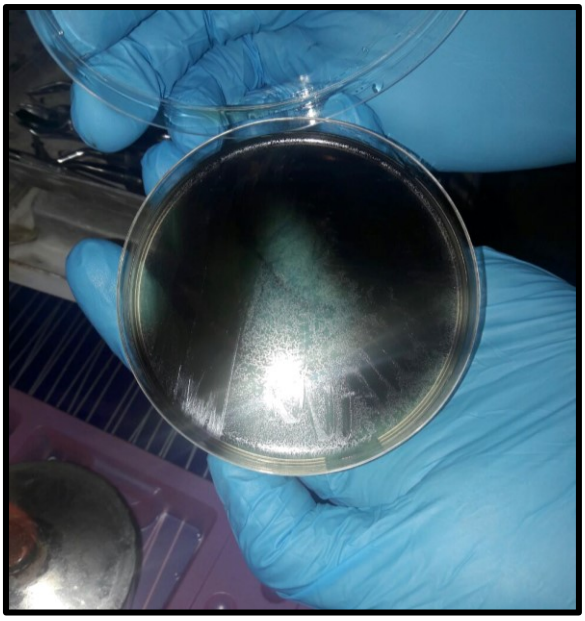

Figure 3.2: black deposit of E.faecalis in bile esculin agar plate

Result of vitek:

The vitek 2 system showed the presence of *E. faecalis* with 97% probability and excellent identification.

3.2 result of bacterial count (cfu/ml) for pre and post samples (table 3.1):

Groups	Pre*10^4	Post*10^4	diff post- pre*10^4
mtad	3.7	0.1	3.6
mtad	2.2	0.1	2.1
mtad	1.9	0.038	1.86
mtad	4.5	0.1	4.4
mtad	5.56	0	5.56
mtad	2.7	0.081	2.62
mtad	4.3	0.08	4.22
mtad	5.7	0.075	5.63
mtad	4.5	0.09	4.41
mtad	5.1	0.1	5
mtad	2.9	0.07	2.83
mtad	3.1	0.06	3.04
mtad	2.32	0.03	2.29

mtad	2.55	0.025	2.53
mtad	4.1	0.05	4.05
edta	2.25	0	2.25
edta	1.95	0	1.95
edta	1.95	0.117	1.83
edta	2.6	0	2.6
edta	2.15	0.02	2.13
edta	2.9	0	2.9
edta	1.44	0	1.44
edta	1.23	0	1.23
edta	3.05	0	3.05
edta	1.2	0	1.2
edta	2.95	0	2.95
edta	1.65	0	1.65
edta	2.23	0	2.23
edta	1.73	0	1.73
edta	1.33	0.07	1.26
cITRIC ACID	1.9	0.3	1.6
cITRIC ACID	4.4	0.925	3.48
Citric acid	4.7	0.94	3.76
Citric acid	1.58	0.45	1.13
Citric acid	4.65	1.25	3.4
Citric acid	1.75	0.56	1.19
Citric acid	1.78	0.5	1.28
Citric acid	2.7	0.65	2.05
Citric acid	4.2	0.882	3.32
Citric acid	3.15	0.97	2.18
Citric acid	3.7	1.35	2.35
Citric acid	1.9	0.58	1.32
Citric acid	3.2	0.8	2.4
Citric acid	2.65	0.6	2.05
Citric acid	3.4	0.68	2.72

3.3 Measuring the effectiveness of irrigation material:

The effectiveness of each sample was calculated by subtraction the result of dividing the viable counting of the post-irrigant on that of pre-irrigant from 1 as follows (Dunavant *et al.,* 2006) (figure2.18).

$$The\ effectiveness\ of\ a\ sample = 1 - \frac{\frac{cfu}{ml}(post\ irrigation)}{\frac{cfu}{ml}(pre\ irrigation)} \text{x}100\%$$

3.4 Descriptive statistics:

Table (3.2) provides several useful descriptive statistical values, including the mean(cfu/ml), slandered deviation, minimum and maximum statistic values of the antibacterial effect of three irrigant methods.

Figure (3.3) illustrates the mean percentage distribution of antibacterial effect of three groups and show that the largest percentile mean for (NAOCL - EDTA) group is 99.19 followed by MTAD 98.04, while the least one is in (NaOCL-citric acid) group 74.48.

Table (3.2) description statistical values of antibacterial effect of the three irrigant methods used in the study.

Groups	Mean	Std. Deviation	Minimum	Maximum
NaOCL & EDTA	99.187	1.975	94.00	100.00
MTAD	98.037	1.029	95.45	100.00
NaOCL & CITRIC ACID	74.481	5.602	63.51	84.21

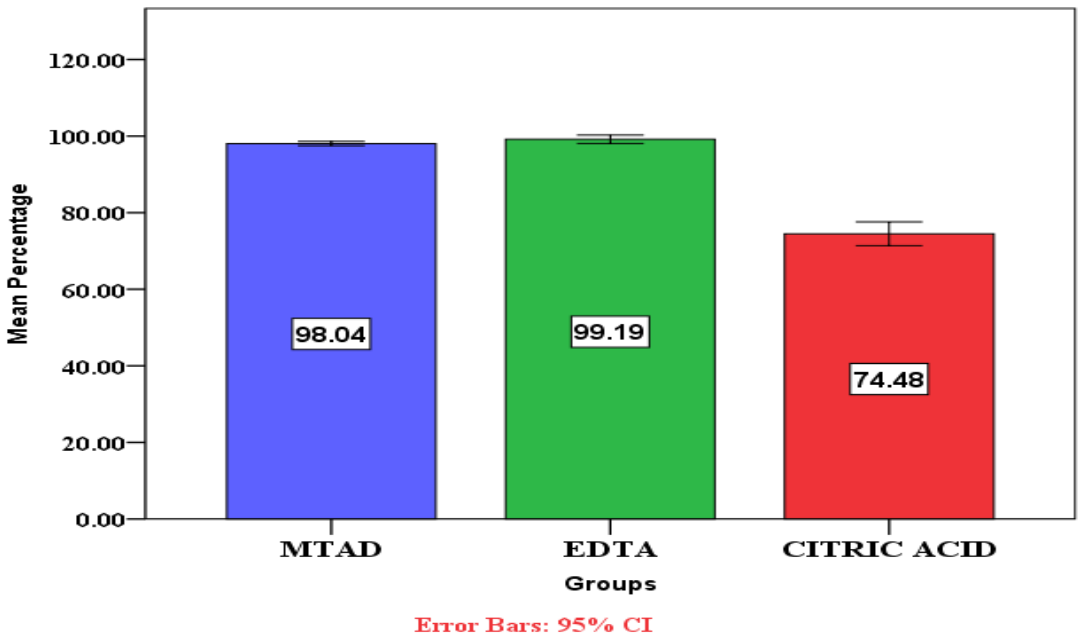

Figure (3.3) distribution the mean percentile of antibacterial effect for each irrigation methods.

3.5 Normality test

Table (3.3) shows the normality test of percentage among groups using Shapiro- wilk to assume the normality of the sample, p value for MTAD was 0.345, (NaOCL-EDTA) was 0.065 and (NaOCL-citric acid) was 0.947. Which are > 0.05 that means the independent variable were normally distribute among the groups.

Table (3.3) normality test of shapiro-wilk.

Groups	Shapiro- Wilk		
	Statistic	df	Sig.
NaOCL & EDTA	0.889	15	0.065
MTAD	.937	15	.345
NaOCL & CITRIC ACID	.977	15	.947

3.6 Comparison between the groups

The output of one way anova analysis showed that there is a highly significant difference in the antibacterial activity between the three irrigants groups. The p value is .000 < 0.01.

Table (3.4) descriptive statistical values of one-way ANOVA analysis for three Groups.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5832.821	2	2916.410	240.724	.000 [HS]
Within Groups	508.837	42	12.115		
Total	6341.658	44			

Levene statistics=17.993, df=2, p value=0.000[HS].

3.7 Normality test of pre and post samples

Table (3.5) illustrates that pre and post sample are normally distributed among the groups as the p value for all three groups > 0.05.

Table (3.5) normality test of difference of shapiro-wilk between pre and pos Samples among groups.

Groups	Shapiro-Wilk		
	Statistic	df	Sig.
NaOCL & EDTA	.931	15	.280
MTAD	.937	15	.348
NaOCL & CITRIC ACID	.922	15	.205

3.8 Multiple comparison between methods

As shown in Table 3.6 it is cleared that there was, highly significance statically difference between the three groups as a whole. Multiple comparison between groups using dunnett t3 test shows that there was no statically significant difference between MTAD group and (NaOCL- EDTA) group as the p value was .161 (p> 0.05). on the other hand Highly significant difference between MTAD

and (NaOCL –citric) group was found with p value .000 (p<0.01) as well as a highly significant difference between (NaOCL- EDTA) and (NaOCL _citric acid) group as the test shows p value of .000 (p<0.01).

Table (3.6) multiple comparison between the antibacterial effect of the Three irrigants methods by Dunnett t3 post hoc test.

Multiple Comparisons between groups using Dunnett T3					
(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
MTAD	NaOCL &EDTA	-1.151	.161 [NS]	-2.636	.335
	NaOCL & citric acid	23.555	**.000[HS]**	19.628	27.482
NaOCL&EDTA	NaOCL & citric acid	24.706	**.000 [HS]**	20.677	28.735

3.9 Effectiveness of the irrigant material

Table 3.7 showns that there was a reduction in the mean of the post values for all three groups' samples in relation to the pre samples. However, the largest effect size was 3.200 in the (NaOCL- EDTA) group, followed by MTAD group with 2.892 effect size, while the least effect size was 2.554 in the (NaOCL- citric acid) group as shown in Figure 3.4.

Table (3.7) The paired samples statistics of effective size for pre and post Samples for all groups.

Groups	Paired Samples Statistics				T value	P value	Effect size
	PRE		Post				
	Mean	SD	Mean	SD			
NaOCL& EDTA	2.041	.626	.014	.034	12.394	**.000[HS]**	3.200
MTAD	3.675	1.251	.067	.032	11.202	**.000[HS]**	2.892
NaOCL &citric acid	3.044	1.115	.762	.293	9.891	**.000[HS]**	2.554

Df =14

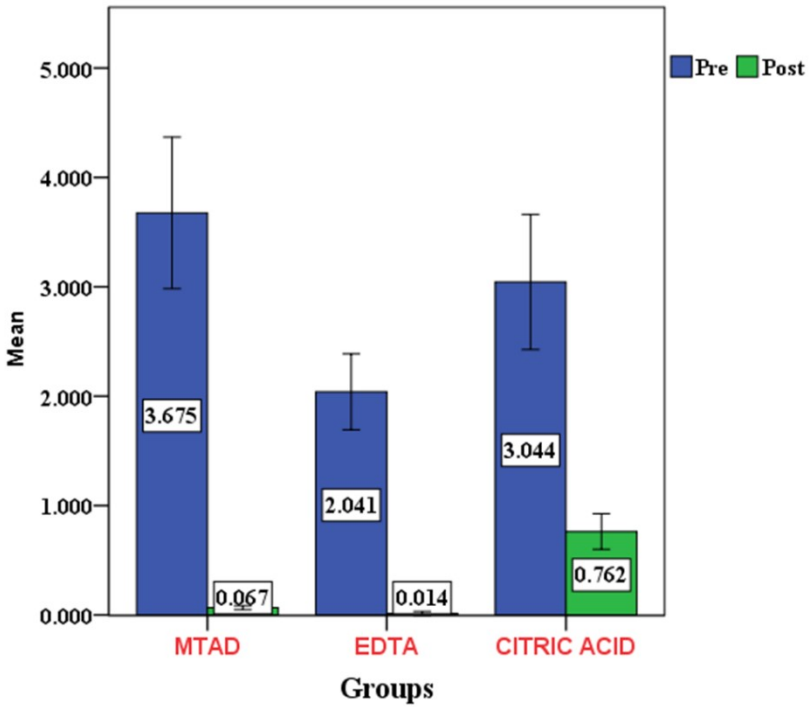

Figure (3.4) percentage of mean time distribution of pre and post samples for all groups.

Discussion

Disinfection plays an essential role in endodontic treatment of primary teeth (Olivi *et al.,* 2016). So one of the primary goals of root canal treatment is to remove the bacteria, their metabolic product and substrate from the root canal (Nogo-Zivanovic *et al*.,2018)

Using an irrigant agent in this process is fundamental to ensure the elimination of bacteria and eradication of remnant tissue (Shen *et al.,* 2012). Maximum antimicrobial effect and maximum ability to dissolve the necrotic tissue with least toxic consequences to periapical tissue, are essential features for ideal irgant solution. Complete disinfection of root canals is very difficult because of the complex anatomy of primary teeth roots. Therefore, there is a high chance of endodontic failure (Nara *et al.,* 2010).

Root canal treatment of primary teeth is commonly practiced in dentistry. Infection control is very important because there is a chance of dissemination the infection to the medullary bone space, and also may affect the permanent teeth due to close proximity of developing permanent teeth germs and the roots of the primary teeth (Da Silva *et al.,* 2008).

In the present study, a total number of 45 primary single rooted teeth fulfilling the inclusion criteria were selected to compare the antimicrobial efficacy of 3%NaOCL-17% EDTA, 3%NaOCL-10% citric acid and MTAD against *E.faecalis* that isolated from necrotic primary teeth. Bacterial counting by (cfu/ml) was used for the evaluation of the three irrigant methods and post hoc dunnett t3 test was used for statistical analysis of data to multiple comparisons between groups.

Selection of *E.faecalis* in this study was based on their important role in failure of endodontic treatment. *E.faecalis* detected in 55% of necrotic primary maxillary molars according to Tulsani *et al.* (2014). Furthermore, *E.faecalis*

played an important role in the etiology and in persistence the periapical lesion after complete endodontic treatment (Rocas *et al.,* 2004).

Several studies evaluate the presence of E.faecalis in primary teeth, From 48 necrotic primary teeth selected for the study *E.faecalis* detected in 37 teeth representing about 75% of samples (Dutta *et al.,* 2017).

The results of the present study showed high antibacterial efficacy of 3%NaOCL-17% EDTA against *E.faecalis* with mean percentage of bacterial eradication is about 99.187 that means there was highly significant reduction in the numbers of bacteria following irrigation to reach zero in most of the tested post samples. This was agreement with Dunavant *et al* (2006).

It is known that Sodium hypochlorite is the most common root canal irrigant that employed in practice dentistry (Nara *et al.,* 2010; Haapasalo *et al.,* 2014; Paul, 2014), the antimicrobial activity of sodium hypochlorite is result from releasing the hypochlorous acid (Hocl) and by strong oxidation action of sulfhydryl groups of bacterial enzyme result in disruption to the metabolic process of the microorganism. The decision to choose 3% NaOCL is supported by the fact that there is no significant difference in the antimicrobial activity in comparison with higher concentration. as well as, copious irrigation with sodium hypochlorite keeps the chlorin reserve that is enough to eliminate the bacterial cell and compensate the concentration effect (Siqueira *et al.,* 2000)

MTAD composed of 3% doxycycline, 4.25% citric acid and 0.5% detergent polysorbate (tween 80). In aqueous solution, MTAD has been showed to be a good biocompatible material and effective root canal irrigant (Park *et al.,* 2004).

Statistical analysis of this study showed that there was no significant difference between MTAD and 3% NaOCL- 17%EDTA groups, p value= .161 which >0.05. Thus, both irrigation protocols showed similar significant effectiveness against *E.faecalis*. This was in accordance with study in primary teeth (Tulsani *et al.,* 2014) as well as several studies in permanent teeth (Siqueira

*et al.,*2000; Torabinejad *et al.,* 2003; Torabinejad and Shabahang , 2003; Dubey, 2016).

Ahangari *et al.* in 2008 showed that there was no significant difference between the antimicrobial effect of 2.5% NaOCL, MTAD and 2% chlorhexidine gluconate against *E.feacalis* in single rooted permanent teeth.

The results of this study agrees with Kho and Baumgartner, 2006 who reported that there was no significant difference in the antimicrobial effectiveness of irrigation with the 5.25%NaOCL - 15% EDTA versus irrigation with 1.3% NaOCL- MTAD in the roots canal of the permanent teeth infected with *E.faecalis.*

Several studies reported that combination of EDTA- NAOCL was the most effective irrigant for smear layer removal (O'Connell *et al.,* 2000; Calt *et al.,* 2002).

In the present study, MTAD showed good results in eradication of *E.faecalis* with the mean percentage 98.037. and these result was in accordance with (Tulsani *et al.,* 2014).

Several reports had been confirmed that MTAD possess high binding affinity to the dentin. This is because the presence of doxycycline which is an important agent for prolonged the antimicrobial activity of MTAD (Machnick *et al.,* 2003; Zhang *et al.,* 2003). Besides that, the concentration in MTAD is relatively high for effective bacterial killing. The main factors for effectiveness of doxycycline are its low pH (2.15) according to (Beltz *et al.,* 2003; Zhang *et al.,* 2003), anti-collagenous property, binding to dentinal tissue and able to gradual release over time (Rizvi et al., 2004).

Half-life of doxycycline in unobturated root canal is 3 weeks (Rasimick *et al.,* 2010). MTAD eliminate most of microorganism that resistant to common endodontic irrigant agents (Shabahang and Torabinejad, 2003) including *E.faecalis* (Torabinejad *et al.,* 2003). Portenier, et al in 2006 reported that doxycycline with minimum concentration 0.3% (3mg/ml) maintain its

bactericidal activity against *E.faecalis* in the presence of dentinal contact for 24h. Newberry *et al.* in 2007 illustrated that MTAD able to kill most strains of *E.faecalis* after upon 1:512 dilution following that doxycycline concentration would be 0.05mg. According to Bolhari *et al.* (2013) doxycycline in MTAD might able to eradicate most strain of *E.faecalis* for a period of 6 weeks. The mechanism of action of doxycycline involves restriction the protein synthesis process in the bacterial cells so inhibit the bacterial growth.

Torabinejad *et al.* in 2003 reported that *E.faecalis* was highly susceptible to MTAD even after 200x dilution while NaOCL missed its antimicrobial effect against the same bacteria beyond 32x dilution.

Second ingredient of MTAD is 4.25% citric acid that possess chelating property which effective against *E.faecalis* (Patil *et al.,* 2018). Polysorbate 80 (detergent) of MTAD is that derived from polyethoxyylated sorbitan and oleic acid and it is nonionic component of MTAD, it reduced the surface tension of solution result in better penetration in to the dentinal tubules (Torabinejad *et al.,* 2003). This deep penetration enhances the antibacterial activity of MTAD against *E.faealis*.

As well as, the synergistic action of doxycycline, citric acid, and detergent results in smear layer removal (Zehnder *et al.,* 2005).

The findings of the present study expressed a highly significant difference between (3% NaOCL- 10% citric acid) group with the other two groups (3%NaOCL- 17%EDTA and MTAD). Multiple comparison between groups showed that p value between 3%NaOCL-10% citric acid and MTAD is .000 and between NaOCL-citric acid and 3%NaOCL-17%EDTA is .000 and both of them were < 0.01(a highly significant difference).

NaOCL is confirmed as a very effective irrigant because it is excellent antibacterial activity and tissue dissolving capacity. However, it has only limited ability in smear layer removal (Zehnder *et al.,* 2002). The least efficacy obtained

by the third group (NaOCL- citric acid) can be related to the presence of smear layer which may be incompletely removed by citric acid.

The mean percentage of bacterial eradication by 3% NaOCL- 10%citric acid was 74.481 which was the least one among group incomplete removal of smear layer by citric acid might be attributed to the presence of bacterial colonies in the post samples.

Citric acid is weak organic acid that is used to eliminate the inorganic part of smear layer after first rinsing of root canal (Herrera *et al.,* 2013). The presence of H ion in citric acid will cause release of ions from the hydroxyl apatite result in formation of soluble chelate complex (Agianni *et al.,* 2007). Citric acid used in various concentration 10,25,50% (Paul *et al.,* 2013). 10% citric acid solution being the most commonly used at the end of instrumentation after NaOCL irrigation (Haapasalo *et al.,* 2010).

In comparison with EDTA, citric acid had a significant erosion effect on dentin (Qian *et al.,* 2011). The results of this study corporate with Darrag *et al.* in 2014 who showed MTAD and EDTA had similar effect on smear layer removal and better than citric acid in the middle and apical third canal. As well as, finding agrees with the those of Wu, et al in 2012 who revealed that MTAD had superior activity to citric acid and EDTA in smear layer removal. NaOCL and citric acid should not be present together in root canal treatment either sequentially or admixed as they loss their ability to dissolute the organic tissue (Wright *et al.,* 2017)

Findings of the present study disagreed with Mancini *et al.* (2009) who reported that citric acid had better effect on smear layer removal than 17% EDTA and MTAD. This disagreement can be explained by the large difference in the concentration of citric acid as he used 42% on his study.

MTAD showed more effectiveness than 3%NaOCL- 10%citric acid group against *E.faecalis* this finding was in accordance with Paul *et al.* (2003) and Irfan *et al.* (2013).

This conflicting of the results could be due to the difference in methodology, microbial sampling procedures and concentrations of irrigation solutions or regimes of irrigation that have been followed.

Conclusions and Suggestions

5.1 Conclusion:

1. Within the limitation of the present study it can be concluded that 3% NaOCL/ 17% EDTA and MTAD protocol were statistically equally effective,

2. the antimicrobial effect of 3% NaOCL - EDTA is significantly greater than 3% NaOCL -10% citric acid against *E.faecalis*.

3. MTAD showed a greater effect against *Enterococcus faecalis* than the effect of 3% NaOCL -10% citric acid.

4. MTAD is a substitute to NaOCL and can be used as a single active irrigant solution with promising favorable effects.

5.Although MTAD proved to be an effective irrigant solution for the primary teeth, several limitations such as its cost, low shelf life and difficult to be available make it less to be chosen than NaOCL in dental practice.

5.2 Suggested studies

1. Further in vivo studies on MTAD are needed to confirm its effectiveness in the clinical practicing.
2. More in vivo studies including larger sample size with the respect to different clinical scenarios are needed.
3. studies on primary teeth, with follow up till eruption of permanent teeth, to ensure that there is no unfavorable effect from irrigation with MTAD.
4. studies required to evaluate the antimicrobial activity of MTAD against more than one specious strain of *E. faecalis*.

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Appendix: Results of Vitek 2 system

مختبر الأمل التخصصي

bioMerieux Customer:
System #:

Laboratory Report

Printed Jun 6, 2018 17:24 ADT
Printed by: LabAdmin

Patient Name: teeth search
Isolate Group: 01-1

Patient ID: 01

Card Type: GP Testing Instrument: 000014EEE4E4 (9513)

Bionumber: 116002765773671
Organism Quantity:

Comments:	

Identification Information	Card:	GP	Lot Number:	2420322103	Expires:	Oct 14, 2018 12:00 AST	
	Completed:	Jun 6, 2018 01:01 ADT	Status:	Final	Analysis Time:	6.00 hours	
Selected Organism	97% Probability		Enterococcus faecalis				
	Bionumber:	116002765773671			Confidence:	Excellent identification	
SRF Organism							
Analysis Organisms and Tests to Separate:							
Analysis Messages:							
Contraindicating Typical Biopattern(s)							
Enterococcus faecalis	LeuA(79),AGLU(83),						

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Installed VITEK 2 Systems Version: 07.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بغداد
كلية طب الأسنان

تقييم تأثير المضاد الميكروبات بين هيبوكلوريت الصوديوم مع EDTA وحمض الستريك و MTAD ضد المكورات المعوية البرازية في القنوات الجذرية للأسنان الأولية (دراسةمختبرية)

رسالة

مقدمة إلى مجلس كلية طب الأسنان في جامعة بغداد في استيفاء متطلبات درجة ماجستير العلوم في طب أسنان الأطفال

من قبل:

سارة توفيق جابر

بكالوريوس طب وجراحة الفم والاسنان

باشراف

الاستاذ مساعد

د. عبير محمد حسن

بكالوريوس طب وجراحة الفم والاسنان

ماجستير طب اسنان الأطفال

2019 م

1440 هـ

الخلاصة

المقدمة: الكائنات الحية الدقيقة هي المسؤولة عن مسببات أمراض اللب السني وامراض ما حول الجذر. تعتمد المعالجة لحشوة الجذر الناجحة على تنظيف القناة وتعقيمها وتطهيرها وملئها جيدا. خطوة غسل السن بمادة معقمة مهمة جدا في معالجة قناة الجذر لأنها مسؤولة عن تطهير نظام القناة. التنظيف الكامل لقناة الجذر أمر صعب للغاية بسبب التشريح المعقد للقناة. كذلك، يمكن للكائنات الحية المجهرية الدقيقة البقاء على قيد الحياة حتى في البيئة ذات تغذية ضئيلة بوجود الحد الأدنى من التكافؤ مع البكتيريا الأخرى. واحدة من أكثر البكتيريا شيوعا التي تم الكشف عنها في حالة فشل القناة الجذرية هي المكورة المعوية، فهي قادرة على البقاء حتى في حالة وجود الظروف القاسية عن طريق تشكيل مستعمرات بكتيرية مقاومة للعديد من المحاليل والأدوية.

الهدف من الدراسة: تهدف الدراسة الحالية لمقارنة الفعالية المضادة للميكروبات بين 3% هيبوكلوريت الصوديوم - 17%EDTA و MTAD و 3% هيبوكلوريت الصوديوم - 10% حمض الستريك ضد المكورات المعوية البرازية في قناة الجذر في الأسنان اللبنية للأطفال.

المواد وطرق العمل: في هذه الدراسة، تم استخدام 45 سنًا لبني جمعت من أطفال اعمارهم بين 4-6 ويتم قطع تيجان العينات متبوعًا بأدوات تنظيف الجذر والتعقيم. تم جمع عينات اخرى من مرضى لهم اسنان متعفنة العصب لعزل المكورات المعوية البرازية. بعد جمع العينات، خضعت هذه العينات إلى ثلاثة اختبارات مكروبيولوجية مختلفة للكشف البكتيري. أولا زرعت في أوساط معينة لتشخيصها، وأخيرا من قبل نظام vitek2 تم تشخيص البكتيريا. بعد الحصول على خلايا بكتيرية من المكورات المعوية البرازية، تم تلقيح البكتيريا داخل قنوات الجذرفي العينات وتركها في محلول مغذي لها لمدة 2 أسابيع. قبل إجراء عملية غسل السن، تأخذ مسحة تستخدم لفحص عينات ما قبل عملية غسل الجذر لضمان وجود البكتيريا وتعداد عدد المستعمرات البكتيرية في قناة الجذر. تنقسم العينات عشوائياً إلى 3 مجموعات (15 لكل مجموعة). المجموعة الأولى تغسل بواسطة 3 %NaOCL- 17% EDTA والمجموعة الثانية تغسل مع MTAD والمجموعة الثالثة تغسل معcitric 10% -3%NaOCL. تم تنفيذ الغسل حسب تسلسل التعليمات والبروتوكول لكل مادة غسل. أخذت عينات بعد العملية من القنوات للمقارنة مع العينات السابقة. لتحديد العدد البكتيري.

النتائج: أظهر التحليل الإحصائي للبيانات عدم وجود فرق معنوي كبير في القضاء على البكتيريا بين 3%NaOCL- 17% EDTA ومجموعة (MTAD) حيث كان متوسط النسبة المئوية من cfu / ml لمجموعة 3% NaOCL-17%EDTA (99.187) و لمجموعة MTAD هو (98.037)

ولمجموعة 3%NaOCL 10% citric acid هو (74.481). اظهرت النتائج ان المجموعة الأخيرة لها أقل تأثير مضاد للميكروبات بين المجموعات الأخرى. كما كشف التحليل الإحصائي للبيانات عن اختلاف كبير بين3%NaOCL- 10%citric acid مع مجموعتين أخريين

الاستنتاج: كلا طرق الري 3% NaOCL- 17% EDTA وMTAD اظهرت نفس الفعالية ضد المكورات المعوية البرازية ويمكن استخدام MTAD كبديل لصوديم هايبوكلورايت في قناة جذر الأسنان اللبنية. اما المجموعة الثالثة 3%NaOCL-10% citric acid أظهرت فعالية اقل من بقية المجموعات ضد نفس البكتيريا المعزولة.