

Evaluation of the irrigation efficacy of chlorhexidine gel in disinfection necrotic pulp root canals of anterior primary teeth (Clinical comparative bacteriological study)

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Abstract

Objectives: The study aimed to compare Qmix²ⁱⁿ¹ and sodium hypochlorite solution in eliminating Enterococcus faecalis bacteria within root canals of primary teeth.

Materials and Methods: The study sample consisted of 30 extracted primary molars, which were divided into two groups: Group 1 experimental (n=15) Qmix²ⁱⁿ¹, Group 2 control (n=15) 5.25% sodium hypochlorite. The root canals were prepared using Kedo-S file rotary then sterilized within autoclave and then contaminated with Enterococcus faecalis bacteria. The root canals were irrigated for 5 minutes in a quantity of 3 ml using a 31-gauge irrigation needle and then bacterial sample were taken then the bacterial colonies were counted and converted to logarithmic numbers..

Results: Both sodium hypochlorite and Qmix²ⁱⁿ¹ reduced the bacterial count of Enterococcus within root canals of primary molars. When comparing the two irrigant, significant statistically differences were observed (P-value = 0.001) in favor of Qmix²ⁱⁿ¹ group.

Conclusion: : Qmix²ⁱⁿ¹ can be considered as a good alternative to sodium hypochlorite in irrigation root canals of primary teeth.

Keywords: : Primary Teeth, Irrigation, Enterococcus Faecalis, Qmix²ⁱⁿ¹, Sodium Hypochlorite.

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تقييم الفعالية المضادة للجراثيم لمحلول Qmix²ⁱⁿ¹ مقارنة بمحلول هيبوكلوريت الصوديوم 5.25% في الأسنان المؤقتة (دراسة مخبرية)

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المخلص

الهدف: كان الهدف من هذه الدراسة المخبرية مقارنة محلول Qmix²ⁱⁿ¹ ومحلول هيبوكلوريت الصوديوم في القضاء على جراثيم المكورات المعوية البرازية Enterococcus faecalis داخل الأقنية الجذرية للأسنان المؤقتة.

المواد والطرائق: تألفت عينة البحث من 30 رحي مؤقتة مقلوعة تم تقسيمها إلى مجموعتين متساويتين عشوائياً وفقاً لسائل الإرواء المستخدم:

المجموعة الأولى (تجريبية): 15 رحي مؤقتة تم إروؤها باستخدام محلول QMix²ⁱⁿ¹
المجموعة الثانية (شاهدة): 15 رحي مؤقتة تم إروؤها باستخدام هيبوكلوريت الصوديوم 5.25%. جرى التحضير الآلي للأقنية الجذرية للأرحاء المؤقتة المقلوعة باستخدام مبادر Kedo-S

ثم تم تعقيم الأقنية الجذرية داخل جهاز تعقيم بالحرارة الرطبة. بعدها لُوثت العينات بجراثيم E. faecalis، بعد ذلك تم حضنها في مرق نقيع القلب والدماغ (BHIB) عند 37 درجة مئوية، وتركزت 48 ساعة للسماح للجراثيم باختراق عاج الأقنية الجذرية ثم أُخذت مسحة جرثومية أولية، بعدها تم إرواء الأقنية الجذرية لمدة 5 دقائق بكمية 3 مل بإبرة إرواء قياس 30gauge.

ثم تم أخذ مسحات جرثومية باستخدام أقماع ورقية وزرعها على أطباق بتري ومن ثم عد المستعمرات الجرثومية وتحولها إلى أرقام لوغاريتمية.

النتائج: قلل كل من محلول هيبوكلوريت الصوديوم و محلول Qmix²ⁱⁿ¹ من التعداد الجرثومي للمكورات المعوية داخل الأقنية الجذرية للأرحاء المؤقتة. عند مقارنة محلولي الإرواء بينت النتائج أن محلول QMix²ⁱⁿ¹ أدى إلى تقليل التعداد الجرثومي ضمن الأقنية الجذرية متموتة اللب أكثر من محلول هيبوكلوريت الصوديوم بفارق إحصائي دال (P-value= 0.001)

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الاستنتاج: : يمكن اعتبار Qmix²ⁱⁿ¹ بديل جيد لهيبوكلوريت الصوديوم في إرواء الأظنية
الجزرية للأرجاء المؤقتة.
الكلمات المفتاحية: الأسنان المؤقتة، إرواء الأظنية الجزرية، المكورات المعوية البرازية ،
QMix²ⁱⁿ¹ ، هيبوكلوريت الصوديوم.

Introduction

Primary teeth act as natural space maintainers, guiding the eruption of permanent teeth into their optimal position in the dental arch. Therefore, preserving primary teeth in the dental arch in their natural, disease-free state is of utmost importance. Literature indicates that maintaining the integrity of primary teeth aids in mastication, preserves aesthetics, ensures the normal eruption of posterior teeth, prevents abnormal tongue movements and speech development issues, and avoids the psychological effects associated with tooth loss (Panchal et al., 2019).

When primary teeth are affected by dental caries, treatment often involves root canal therapy. The success of endodontic treatment largely depends on the thorough disinfection of the root canal system to completely eliminate or significantly reduce microorganisms and their toxic byproducts before obturation (Agnihotri et al., 2020). The anatomical structure of root canals in primary teeth exhibits a wide range of variations and unexpected configurations. As a result, abnormal canals pose a challenge for dentists (Reddy et al., 2018).

Pulp therapy in primary teeth presents a significant challenge due to anatomical complexities such as accessory and abnormal canals. Additionally, physiological resorption can lead to structural changes and increased root surface permeability to various bacterial toxins. Furthermore, internal resorption can alter the root canal system (Ahmed et al., 2020).

Necrotic pulp and periapical lesions harbor a vast number of polymorphic bacteria, with *Enterococcus faecalis* being the most common microorganism found within root canals and associated with untreated chronic periapical inflammation (Lim et al., 2020).

Due to the advantages *Enterococcus faecalis* possesses, being the most prevalent bacterium in post-endodontic infections, highly resistant to harsh environmental conditions, and resistant to many types of medicaments, it has been increasingly cited as a serious challenge in endodontic treatment (Jhajharia et al., 2015).

Enterococcus faecalis thrives in extreme environments, tolerating a wide pH range (4–11), salt concentrations of up to 6%, temperatures between 10°C and 45°C, and can survive at 60°C for 30 minutes. It can also persist in nutrient-poor conditions (Jhajharia et al., 2015). *Enterococcus faecalis* can survive in sodium hypochlorite

concentrations as high as 6.5% (Del Fabbro et al., 2014).

Various irrigants have been used for root canal disinfection, the most common being sodium hypochlorite, chlorhexidine, and hydrogen peroxide. Although most studies have proven the effectiveness of sodium hypochlorite at different concentrations in accomplishing this task, some have shown its inability to eliminate *Enterococcus faecalis* within the canal due to the bacterium's high resistance, leading to long-term endodontic treatment failure (Gomes et al., 2001).

QMIX 2in1 solution exhibits excellent antimicrobial properties, eliminating more than 99.99% of planktonic microorganisms, including resistant strains of *Enterococcus faecalis* and *Candida albicans* (Kishore and Saurav, 2021). Therefore, this study was conducted to evaluate QMix 2in1 as an irrigating solution in eliminating bacteria within the root canal system.

Research Objective

To evaluate the antibacterial efficacy of QMix 2in1 as an irrigating solution against *Enterococcus faecalis* in the root canals of primary teeth with necrotic pulp, compared to 5.25% sodium hypochlorite (NaOCl).

Materials and Methods

Study Design

A comparative in vitro study assessing the antibacterial activity of 5.25% NaOCl and QMix2in1 against *Enterococcus faecalis* (*E. faecalis*) in the root canals of primary molars with necrotic pulp. The Scientific Research and Postgraduate Council approved the study protocol at the University of Damascus. The Ethics Committee at the University of Damascus, Syria (Approval No.: UDDS-453-23082019 / SRC-1450).

Sample Description:

Using the PS Power and Sample Size Calculation Program (Version 3.0.43) and based on a previous similar study (Wang et al., 2007), with a 5% significance level and 90% confidence interval, the study sample consisted of 30 extracted primary molars.

The sample was randomly divided into two groups (n=15 each) using <http://www.randomization.com>, based on the irrigant used:

- Experimental Group (n=15): QMix 2in1 (Dentsply Sirona, America).
- Control Group (n=15): 5.25% Sodium Hypochlorite (NaOCl).

Inclusion Criteria

1. Root resorption is less than one-third of the root length (Dentistry, 2020; Coll et al., 2020).
2. No cracks, caries, or fractures in the root.
3. No previous endodontic treatment.

Exclusion Criteria

1. Roots with prior endodontic treatment.
2. Roots with calcified canals.
3. Curved or constricted roots.

Randomization and Blinding

Randomization was performed electronically using <http://www.randomization.com>. Double-

blinding was applied, where neither the evaluator nor the statistician knew which irrigant was used.

Methodology

After extraction, the root surfaces were cleaned using ultrasonic scaling to remove periodontal ligament remnants and tissue debris. Samples were immersed in sterile saline solution. Crowns were sectioned using diamond discs. Root canal preparation was performed using K-files with sterile saline irrigation until the file tip was visible at the apical foramen (Figure 1).



Figure (1): Prepared primary molar samples showing root canals after coronal access and mechanical instrumentation

Mechanical preparation was done using Kedo-S rotary files (Reeganz Dental Care Pvt. Ltd). For standardization, the palatal canals of maxillary second molars and distal canals of mandibular second molars were prepared (Walia et al., 2019). D1 Kedo-S rotary file was used for canal shaping. After drying the roots, 37% phosphoric acid was applied, followed by rinsing and drying. A bonding agent was applied and light-cured for 20 seconds before sealing the root apex with composite resin. To prevent bacterial leakage, the roots were coated with two layers of red nail polish, and a cotton pellet was placed in each root canal orifice before sealing with a temporary filling and embedding the roots in

cold-cure acrylic resin molds using pre-prepared rubber molds (Figure 2). Following complete acrylic polymerization, the temporary fillings and cotton pellets were removed, and the orifices of unprepared canals were sealed with Teflon tape before individually placing the samples in autoclave sterilization bags for moist heat sterilization at 121°C for 15 minutes. To confirm sterility, three randomly selected samples underwent microbial swab testing, which yielded negative cultures, verifying the absence of contamination before experimental procedures



Figure (2): Representative samples fixed in cold-cure acrylic resin molds for standardized testing.

The *Enterococcus faecalis* strain was isolated from infected root canals of necrotic primary teeth with abscesses obtained from the Pediatric Dentistry Department at Damascus University, then cultured in Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 hours at the Medical Technical Institute, Faculty of Medicine. Bacterial identification was performed through culture diagnosis on selective Azide Agar Esculin Bile medium, where characteristic glossy colonies turned the medium black due to esculin hydrolysis, and further confirmed using the PHOENIX™ automated

microbial identification system. For experimental contamination, a bacterial suspension was injected into the prepared root canals using a 31-gauge needle, after which all samples were immersed in BHI broth and incubated at 37°C for 48 hours to ensure adequate bacterial penetration into the root canal dentinal tubules, following established protocols (Atiyah & Al-Khafaji, 2020; Abdulrazzaq & Al-Nasrawi, 2020; Walia et al., 2019), as illustrated in Figure 3



Figure (3): Immersion of all root samples in BHIB broth containing *E. faecalis* colonies for standardized bacterial contamination.

Initial Bacterial Colony-Forming Unit (CFU) Count Procedure: Following the incubation period, primary microbial sampling was conducted for each root canal by first irrigating with sterile saline and performing circumferential filing using a heat-sterilized H-file, after which three sterile paper points matching the canal preparation size were sequentially inserted to full working length for 60 seconds each to collect bacterial samples; these paper points were then transferred to a sterile Eppendorf tube containing 1 mL of sterile saline, with the process repeated three times per canal to ensure representative sampling. The collected paper

points were vortexed for 1 minute using a Biovortex mixer to create a homogeneous bacterial suspension, from which a 50 µL aliquot was extracted with a micropipette and plated onto pre-prepared agar plates for subsequent incubation and CFU enumeration, ensuring accurate quantification of the initial bacterial load before experimental interventions. Following the initial sampling, serial decimal dilutions (10-fold dilutions) were prepared from the homogenized bacterial suspension according to standardized protocols (Sabry et al., 2019) (Figure 4).

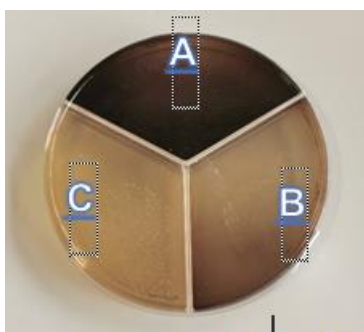


Figure (4): Petri dish containing Bile Esculin Azide Agar after bacterial plating and serial dilution of a sample root during initial CFU counting prior to root canal irrigation: (A) Undiluted (10^0) suspension, (B) First dilution (10^{-1}), (C) Second dilution (10^{-2}).

Irrigation Protocol and Microbiological Analysis: The root canals in Group 1 (QMix 2in1) were irrigated with 3 mL of solution for 5 minutes using a 30-gauge NaviTip needle, followed by 2 mL saline flush, while Group 2 (5.25% NaOCl) received identical irrigation parameters with a 31-gauge needle (Goztas et al., 2014) (Figure 5). Post-irrigation, canals were filled with saline and circumferentially filed with an H-file before collecting microbial samples using three sterile

paper points (60 seconds each), which were then vortexed in 1 mL saline for 1 minute; 50 μ L aliquots of the homogenized suspension were plated on Bile Esculin Agar and incubated aerobically at 37°C for 24 hours (Figures 6-7). Bacterial colonies were counted visually, with CFU counts per 1 mL converted to logarithmic values for statistical analysis, ensuring standardized comparison of antibacterial efficacy between irrigants.



Figure (5): Root canal irrigation.

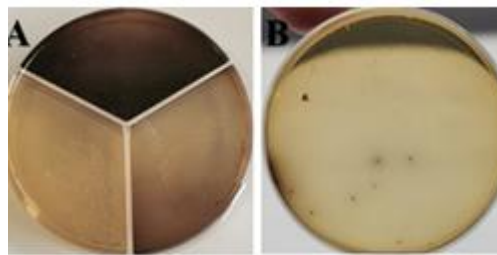


Figure (6): Representative samples from the sodium hypochlorite group showing bacterial growth before irrigation (A) and significant reduction after irrigation (B) on Bile Esculin Azide agar plates.



Figure (7): Representative samples from the QMix group demonstrating (A) initial bacterial colonization and (B) post-irrigation microbial reduction on Bile Esculin Azide agar

Results

The study sample consisted of 30 extracted primary molars equally divided into two main groups based on the irrigation solution used (QMix2in1 solution and 5.25% sodium hypochlorite). Aerobic bacterial colonies were counted, and the decimal logarithm of colony counts was calculated at two different stages (pre-irrigation and post-irrigation) for each tooth in the study sample. Descriptive statistics, including mean, standard deviation, and standard error, were calculated for the logarithmic values of bacterial colony counts according to the irrigation solution and study phase, as shown in Table 1 and Chart 1. The Wilcoxon signed-rank test was performed to examine significant differences in mean logarithmic bacterial colony counts between the two study phases (pre- and post-irrigation) for each irrigation group, as presented in Table 2. To compare the

mean logarithmic bacterial colony counts between the two irrigation groups (QMix2in1 vs 5.25% NaOCl) according to study phase and bacterial characteristics, a Student's t-test was conducted. Table 1 shows the t-test results, indicating that the significance level was greater than 0.05 before irrigation (no statistically significant difference at 95% confidence level), while a statistically significant difference in mean logarithmic bacterial colony counts was found after irrigation. Descriptive statistics for the reduction percentage in logarithmic bacterial counts were calculated, including mean, standard deviation, median, and minimum/maximum values for each group before and after irrigation, as shown in Table 3. These results demonstrate the comparative efficacy of the two irrigation solutions in reducing bacterial load within root canals of primary teeth.

Table (1): Shows the baseline values for the two study groups and the results of the t-test.

Study stage	Mean	SD	Min	Max	t-value	p-value
Pre-irrigation	7.95	0.184	7.639	8.179	0.182	0.857
Post-irrigation	7.963	0.179	7.653	8.166		
Pre-irrigation	0.872	0.856	0.000	2.000	3.947	0.001
Post-irrigation	0.000	0.000	0.000	0.000		

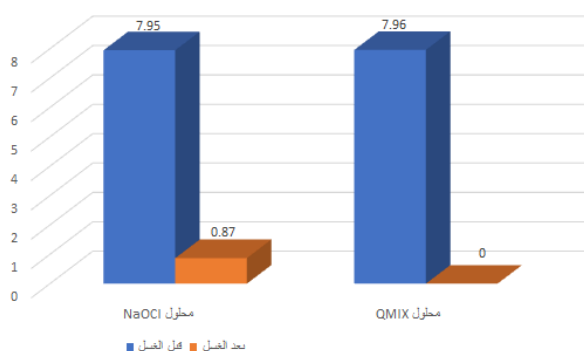


Chart (1): Mean logarithmic values of bacterial counts in the study sample according to treatment phase and irrigation solution used.

differences in mean log₁₀ bacterial colony counts between pre- and post-irrigation phases for each irrigation solution group.

Table (2): Results of Wilcoxon signed-rank test analyzing significant

Irrigant	z-value	p-value
NaOCl	-3.408	0.001
QMix2in1	-3.408	0.001

Table (3); Descriptive statistics of the reduction percentage in log₁₀ bacterial counts before and after irrigation, including mean, standard deviation, median, and minimum/maximum values.

Study stage	Mean	SD	Median	Min	Max
Pre-irrigation	-89.21	10.65	-83.38	-100	-75.54
Post-irrigation	-100	0.000	-100	-100	-100

Discussion

Primary teeth hold equal importance to permanent teeth, serving as natural space maintainers while fulfilling crucial masticatory, speech, and aesthetic functions. When affected by dental caries, their treatment often necessitates root canal therapy. However, the complex anatomy of primary molars—featuring delta regions and lateral canals (present in 10-20% of cases)—poses significant challenges for debridement, underscoring the urgent need for optimal irrigation solutions (Farooq et al., 2021). While sodium hypochlorite (NaOCl) remains the gold standard endodontic irrigant due to its potent antibacterial action via hypochlorous acid (HOCl) release—which disrupts microbial sulfhydryl groups and metabolic processes—it carries notable drawbacks, including periradicular tissue toxicity, potential harm to permanent tooth buds, and unpleasant taste/smell (Afkhami et al., 2017).

To address these limitations, QMix 2in1—a ready-to-use irrigant co-developed with endodontic specialist Dr. Markus Haapasalo—was investigated. This innovative solution combines 2% chlorhexidine (CHX), 17% EDTA (a calcium-chelating agent), saline, and surfactants, demonstrating efficacy in simultaneous smear layer removal and eradication of resistant pathogens like *Enterococcus faecalis* (Gündoğar et al., 2018; Siqueira and Rôças, 2019). Its unique chemical design prevents the white precipitates typical of CHX-EDTA mixtures (Bindui et al., 2019) while offering prolonged residual antimicrobial activity (up to 120 days) and deep dentinal tubule penetration (500 µm) without causing erosion (Zhang et al., 2015; Baldasso et al., 2017).

E. faecalis, a gram-positive facultative anaerobe implicated in persistent endodontic infections (Singh, 2016; Mergoni et al., 2021), was selected as the test organism due to its prevalence in treatment

failures. To standardize anatomical variables, palatal roots of maxillary second molars and distal roots of mandibular second molars were used (Walia et al., 2019). Samples were stored in sterile saline to prevent preservative artifacts (Abdulrazzaq and Al-Nasrawi, 2020) and prepared using the pediatric Kedo-S rotary system, which enhances efficiency and reduces procedural stress (Lakshmanan et al., 2020; Jeevanandan and Govindaraju, 2018). Apical sealing with composite created a closed-system model mimicking clinical conditions (Jamleh et al., 2018). A standardized irrigation protocol (3 mL/5 min) was employed, with solutions delivered via side-vented 31-gauge needles to optimize fluid dynamics (Darcey et al., 2016). Microbial assessment used paper-point sampling augmented by H-file filing to capture intratubular bacteria (Sandini et al., 2021), with colony counts quantified via plate counting (Purushotham, 2016). Both irrigants significantly reduced bacterial loads ($p < 0.05$), with QMix2in1 demonstrating superior efficacy to NaOCl ($p < 0.01$), attributable to its synergistic components—surfactants enhancing wettability and penetration, EDTA disrupting gram-negative cell walls through Mg^{2+}/Ca^{2+} chelation (Elakanti et al., 2015), and CHX providing sustained antimicrobial activity. These results align with Kishore & Saurav (2021) and Elakanti et al., but contrast with Ordinola-Zapata et al. (2013), potentially due to extended exposure times (5 min vs. 60–90 sec).

Conclusions

QMix2in1 emerges as a superior alternative to NaOCl for primary teeth endodontics, combining enhanced antibacterial action with clinical safety, while NaOCl remains a cost-effective option; future research should evaluate long-term clinical outcomes and cost-benefit analyses to validate QMix adoption.

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