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Comparative Evaluation of Antimicrobial Activities of Different Types of Luting Cements

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ABSTRACT

Background and objectives: To minimize secondary caries, luting cements with antimicrobial properties are frequently used for the cementation of indirect restorations. The aim of this study was to investigate the antimicrobial effect of four dental luting cements

Materials and methods: Four luting cements (GC FUJI PLUS, GC FUJI I glass ionomer, GC Gold Label glass ionomer, and G-CEM ONE resin cement) were tested for antimicrobial activity against Streptococcus mutans and Candida albicans using agar diffusion test (ADT) (by preparation of seventy-two disc for each bacteria) and modified direct contact test (MDCT) at one hour, one day, and one-week intervals. In ADT, inhibition zones were measured in millimeters. In MDCT, bacterial suspensions were exposed to the cements, and colony-forming unit (CFU) counts were recorded at various time points to assess microbial survival.Top of Form

Results: In ADT, GC Gold Label glass ionomer exhibited the highest inhibition zones (17.75 mm) after one week, surpassing other intervals of the same cement and all other cements tested. At one hour, none showed antimicrobial activity against Candida albicans, except GC FUJI PLUS. In MDCT, none of the luting cements demonstrated growth against S. mutans, but all showed growth of Candida except for GC FUJI PLUS at one hour.

Conclusion: All evaluated luting cements exhibited antibacterial activity against S. Mutans in both tests. However, only RMGI at one hour showed antimicrobial activity against Candida Albicans. The conventional glass Ionomer cement (powder and liquid) showed superior antimicrobial activity, suggesting potential benefits for patients at high risk of caries.

Keywords: Antimicrobial effect, Glass ionomer cements, Growth inhibition.

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INTRODUCTION

In modern dentistry, the limitations of direct restorations—such as increased wear, polymerization shrinkage, and extended chair-side time—have made indirect restorations an attractive alternative. However, a challenge with indirect restorations is the potential for fluid leakage and micro-leakage after cementation, a frequent clinical issue. This leakage involves the penetration of oral fluids, ions, molecules, and bacteria between the tooth and the restoration interface, creating a favorable environment for cariogenic bacteria like Streptococcus mutans (S. mutans). This can lead to recurrent caries. To mitigate this issue, careful selection of an appropriate cement is essential.[1,2]

Cements for indirect restorations are primarily categorized by their setting mechanism. Some, such as Glass Ionomer, Resin-modified Glass Ionomer, Zinc Oxide Eugenol, Zinc Polycarboxylate, and Zinc Phosphate, set through acid-base reactions, while resin-based cements set through polymerization .[3] Glass Ionomer cements are noted for reducing demineralization around restoration margins. While they may have lower bending strength and offer less aesthetic polishability, they are valued for their sustained fluoride release, which can hinder demineralization and support remineralization of tooth tissue. Additionally, they can be recharged with fluoride ions in laboratory conditions, adding to their popularity as luting agents.[4]

Adhesive resin cements are now widely used for indirect tooth-colored restorations due to their strong bonding to both tooth and restorative material. Compared to traditional cements, these often provide a more durable and robust result.[5] Despite their advantages, the cementation layer and bond interface between the tooth and restoration remain susceptible over time. Margin integrity can be particularly compromised by cariogenic bacteria, which produce by-products and activate virulence factors that encourage bacterial growth.[6] This phenomenon is a key factor in the development of marginal gaps, secondary caries, and eventual failure of the restoration .[7]

Recurrent caries often arise at the margins of dental restorations, where biofilm containing bacteria like S. mutans produces acids that demineralize the tooth. Additionally, Candida albicans can contribute to certain oral diseases. Preventing bacterial colonization at restoration margins is essential to reduce caries and periodontal disease risk at the tooth-restoration interface. Despite preventive efforts, recurrent decay around restorations remains a common issue, affecting approximately 50–60% of restorations placed.[8] Routine disinfection of the tooth surface before cementation helps but may not completely prevent bacteria from infiltrating through marginal leaks if the adhesive or edge of the prosthesis deteriorates. Using luting cement with antibacterial properties could provide added protection by limiting biofilm formation along the exposed margin, thereby reducing recurrent caries risk.[5,8,9]

Given the need for effective antimicrobial protection, this study aims to evaluate and compare the antimicrobial properties of commonly used luting agents, including two types of conventional Glass Ionomer cement, Resin-modified Glass Ionomer cement, and resin cement against S. mutans and Candida albicans at three different time intervals.

MATERIALS AND METHODS

This study involved four types of luting cements, divided into four experimental groups. The manufacturer details for each cement type are provided in Table 1. The groups are as follows:

Group 1: Resin-Modified Glass Ionomer (RMGI)
Group 2: Conventional Glass Ionomer (capsule) (CCGI)
Group 3: Conventional Glass Ionomer (powder and liquid) (PCGI)
Group 4: Resin Cement (RC)

Each group underwent two types of antimicrobial activity tests—an agar diffusion test and a modified direct contact test—against Streptococcus mutans and Candida albicans. These tests were conducted at three time intervals: one hour, one day, and one week, to monitor changes in antimicrobial effectiveness over time.

The antimicrobial tests were performed under strict aseptic conditions in the Microbiology Department at Rizgary Hospital in Erbil, Iraq. The antimicrobial effectiveness of the luting cements was assessed using Streptococcus mutans (ATCC-25175) and Candida albicans (ATCC-10231) strains.

Agar diffusion test.

Microorganism strains were cultured in Brain Heart Infusion Broth (BHIB) for 24 hours. Bacterial suspensions were then prepared from these cultures and adjusted to a 0.5 McFarland standard for turbidity. Streptococcus mutans was cultured

on blood agar, while Candida albicans was cultured on Mueller-Hinton agar. For the tests, six discs of each material, each measuring 4 mm in diameter and 6 mm in depth, were prepared and placed on separate plates for each microorganism, ensuring adequate spacing between the discs and from the plate edges. The plates were incubated at 37°C, and inhibition zones were measured using a transparent ruler at one hour, one day, and one week intervals.[10]

Preparation of specimen's discs

A split cylindrical metal mold with double open ends, measuring 4 mm in internal diameter and 6 mm in height (Figure 1), was used to prepare each disc according to the manufacturer's instructions for each material.[8] A total of 72 discs were prepared (18) per group of luting cement), with each group further divided into three subgroups based on time intervals (6 discs per subgroup). The process began by positioning the metal mold on a glass slab covered with a Mylar strip.

For encapsulated cements (Groups 1 and 2), the cement capsules were activated and triturated in an amalgamator according to manufacturer guidelines, loaded into a capsule applicator, and extruded directly into the mold through the capsule nozzle.

For the powder and liquid cement (Group 3, PCGI), a ratio of 1.8 g powder to 1.0 g liquid (1 scoop of powder to 2 drops of liquid) was used. The components were dispensed onto a pad and mixed with a plastic spatula for 20 seconds, then applied to the mold per manufacturer instructions

For resin cement (Group 4), packaged in dual-barrel syringes with single-use automix tips, the components were mixed through a spiral mixer within the syringe and applied directly to the mold. The cement was layered in three 2-mm thick layers, each cured with an LED unit emitting 1500 mW/cm² of light at 440–480 nm, per manufacturer instructions.

After filling each mold, an additional Mylar strip and glass plate were placed on the slightly overfilled open end to eliminate excess material. The specimens were then removed from the molds and soaked in distilled water at 37°C for 24 hours. Each disc's dimensions were measured precisely using a micrometer with 0.001 mm accuracy.

Table 1: The commercial name, classification, manufacture and properties of the luting materials used in the study.

Materials used in this study	Code	Materials classification	Composition	Materials Manufactures	Powder/ Liquid ration (g/ g)	Curing mode	Delivery system
GC FUJI PLUS	RMGI	Resin modified glass ionomer	Powder: Fluoro-alumino-silicate glass, initiator, pigment Liquid: Methacrylate, distilled water, polyacrylic acid, dimethacrylate, carboxylic acid, stabilizer	GC Corporation, Japan	0.36/0.18	Chemically cured	Capsule mixing/ delivery
GC FUJI I glass ionomer	CCGI	Conventional glass ionomer	Powder: strontium-alumino-flouro-sillicate glass Liquid: polybasic carboxylic acid	GC Corporation, Japan	0.33/0.18	Chemically cured	Capsule mixing/ delivery
GC Gold Label glass ionomer	PCGI	Conventional glass ionomer	Powder : Fluoro Alumino-silicate glass (amorphous), strontium glass, polyacrylic acid. Liquid: Distilled water (50-55%) polyacrylic acid (30-40%)	GC Corporation, Japan	1.8/1.0	Chemically cured	Powder and Liquid
G-CEM ONE resin cement	RC	Self-adhesive resin cement	water and functional monomers (4-MET and phosphoric acid ester	GC Corporation, Japan		Light cured	Twin tube with automix tip

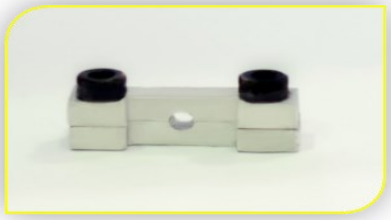

Figure 1: Metallic mold used for disc preparation

Modified Direct Contact Test

Samples grouping.

Each experimental group consisted of 58 microtubes. Additionally, 58 microtubes containing a bacterial solution without cement were designated as the positive control group. For negative controls, five microtubes were filled with test cements without bacteria. Another five microtubes contained only culture media without cements or bacteria to ensure sterility of the microplates.[2]

In each experimental group, a 1 mm layer height of the selected cement was applied to the microplates (Figure 2). For the resin cement group, polymerization was carried out according to the manufacturer's instructions. The microplates were then aged by immersing them in phosphate-buffered saline at 37°C with 95% humidity for intervals of 1 hour, 1 day, and 1 week. During this week-long aging, the saline solution was replaced every 24 hours. At each interval, the contents of the microplates were removed, and 10 µL of a microorganism suspension containing approximately 10^6 bacteria were added to each microplate.

The microplates were incubated at 37°C in a humid environment for 60 minutes, allowing microorganisms to come into direct contact with the exposed cement surfaces. After this period, 240 µL of Brain Heart Infusion (BHI) culture medium was added to each microplate and mixed for 2 minutes. Serial dilutions were then prepared from the microtube contents in BHI culture medium, and 20 µL of each dilution was spread onto BHI agar plates using the spreading technique. The bacterial counts were measured as colony-forming units per milliliter (CFU/mL).

Statistical analysis:

Data collection and analysis were performed using SPSS for Windows, version 26 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used, and mean differences among groups were analyzed using One-Way ANOVA. To assess potential differences between group means over time, Repeated Measures ANOVA was applied. Duncan's test was conducted to determine any significant differences among the time intervals (1 hour, 1 day, and 7 days) for each group. The F value was calculated, and significance levels (P values) were interpreted as follows: if $P \geq 0.05$, the difference was considered not significant (N.S); if $P < 0.05$, the difference was considered significant (S).

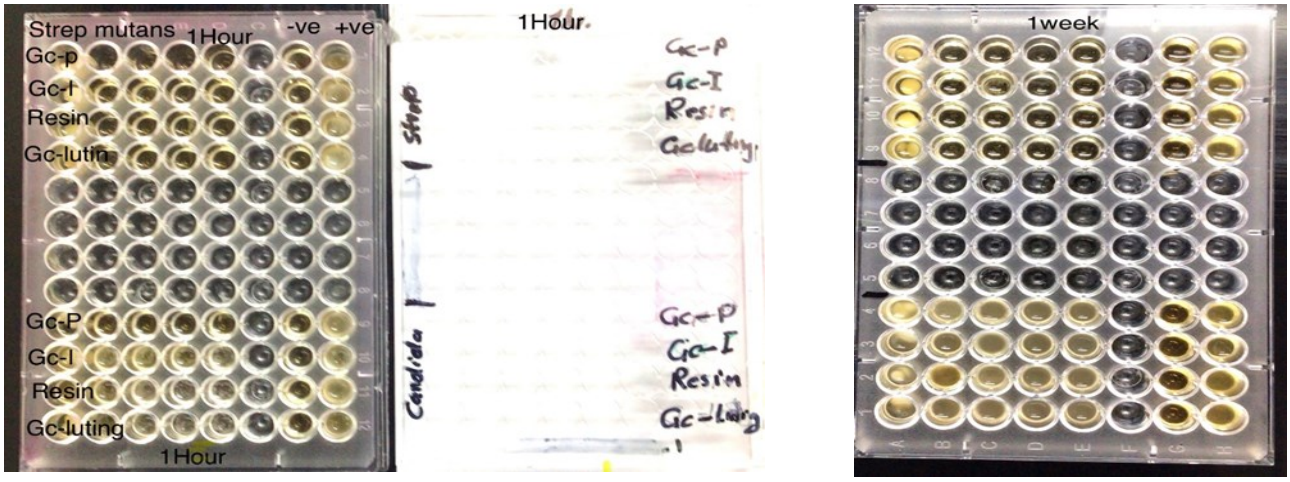

Figure 2: Microplate for MDCT, each with 1mm of the luting cement at one hour and one week.

RESULTS

Agar diffusion test:

Table 2 displays the results of the microbiological analysis conducted via agar diffusion tests for all materials tested against Streptococcus mutans. The findings indicate that conventional glass ionomer cement (Group 3) consistently showed the largest inhibition zone at the one-week interval

Table 2: The mean values of the inhibition zones of the tested materials in mm on S. mutans.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
RMGI	one hour	6	6.583 a	.4916	.2007	6.067	7.099	6.0	7.0
	one day	6	11.417 b	.3764	.1537	11.022	11.812	11.0	12.0
	one week	6	16.583 c	.3764	.1537	16.188	16.978	16.0	17.0
CCGI	one hour	6	13.583 c	.3764	.1537	13.188	13.978	13.0	14.0
	one day	6	12.583 b	.3764	.1537	12.188	12.978	12.0	13.0
	one week	6	10.583 a	.3764	.1537	10.188	10.978	10.0	11.0
PCGI	one hour	6	16.583 a	.3764	.1537	16.188	16.978	16.0	17.0
	one day	6	17.000 a	.5477	.2236	16.425	17.575	16.0	17.5
	one week	6	17.750 b	.2739	.1118	17.463	18.037	17.5	18.0
RC	one hour	6	6.667 ab	.3764	.1537	6.188	6.978	6.0	7.0
	one day	6	7.083 b	.4082	.1667	6.238	7.095	6.0	7.0
	one week	6	6.583 a	.3764	.1537	6.688	7.478	6.5	7.5

*Different letters indicate significant difference (P < 0,05). Lower-case letters indicate differences in vertical directions

Table 1: ANOVA test between and within three groups of PFS due to compressive test.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
RMGI	Between Groups	300.111	2	150.056	857.460	.000
	Within Groups	2.625	15	.175		
	Total	302.736	17			
CCGI	Between Groups	28.000	2	14.000	98.824	.000
	Within Groups	2.125	15	.142		
	Total	30.125	17			
PCGI	Between Groups	4.194	2	2.097	12.177	.001
	Within Groups	2.583	15	.172		
	Total	6.778	17			
RC	Between Groups	.861	2	.431	2.870	.088
	Within Groups	2.250	15	.150		
	Total	3.111	17			

and across all three intervals, outperforming the other groups. Notably, Groups 1 and 2 demonstrated a gradual increase in inhibition zone size over time, while Group 2 showed a decrease. In comparison, resin cement (Group 4) had the smallest inhibition zone across all intervals relative to the other luting cement groups.

The results of the ANOVA test for antibacterial activity test are presented in Table 3 which show that there is a significant difference among all different categories of materials (P <0.05) except for the resin cement group (P (0.08). The agar diffusion test results assessing the antimicrobial activity of the selected luting cements against Candida albicans showed that RMGIC (Group 1) exhibited significant antimicrobial effects only during the first hour. However, no detectable antimicrobial activity was observed at later time intervals, as presented in Table 4 and illustrated in Figure 3.

Table 4: The inhibition zones (measured in millimeters) of Resin Modified Glass Ionomer Cement against C. albicans at various time intervals.

Time	N	Mean (mm)	Std. Error
One hour	6	8.00 b	0.1291
One day	6	0 a	
One week	6	0 a	

*Different letters indicate significant difference (P < .05).
Lower-case letters indicate differences in vertical directions

Modified direct contact test (MDCT):

The results from the MDCT analysis against Streptococcus mutans, shown in Table 5, indicate that no bacterial growth (N.G) was detected for any of the tested luting cements at any of the three intervals. This finding suggests that these luting cements have antibacterial properties against S. mutans, supporting the results obtained from the ADT.

Table 5: MDCT of luting cements against Str. mutans

MDCT	N	RMGI	CCGI	PCGI	RC
one hour	6	N.G	N.G	N.G	N.G
one day	6	N.G	N.G	N.G	N.G
one week	6	N.G	N.G	N.G	N.G

Table 6: MDCT of luting cements against Candida albicans

MDCT	N	RMGI	CCGI	PCGI	RC
one hour	6	N.G a	10^5 a	10^5 a	10^4 a
one day	6	10^5 b	10^5 a	10^5 a	10^5 b
one week	6	10^5 b	10^5 a	10^5 a	10^5 b

10^4 cfu/ml: mean 10 – 100 colonies

10^5 cfu/ml: mean more than 100 colonies

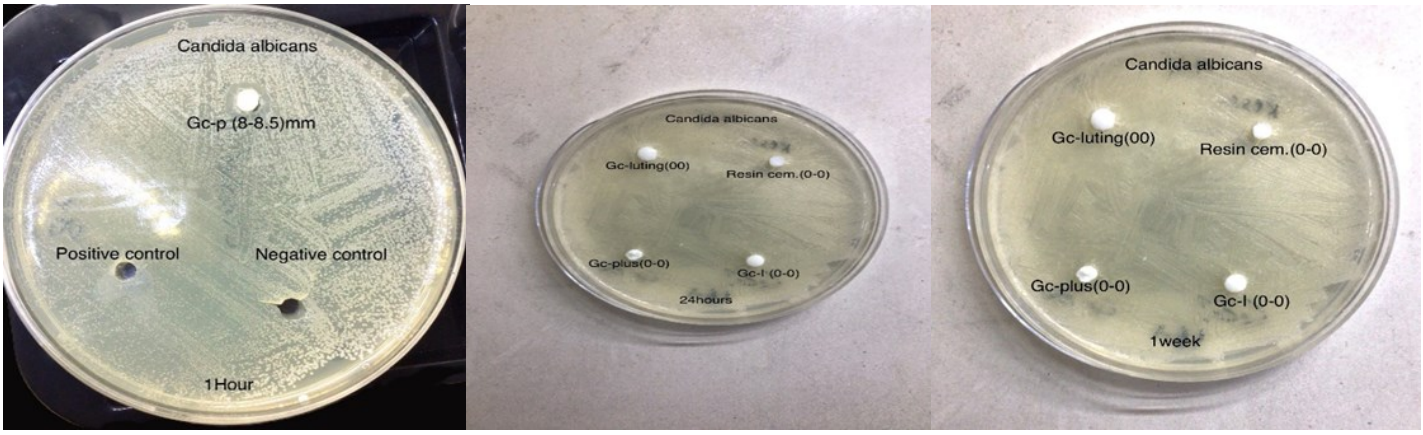

Figure 3: Agar diffusion test of tested luting Cement against C. albicans at various time intervals.

The results from the MDCT analysis against Candida albicans, shown in Table 6, revealed that Candida growth occurred for all tested luting cements at all three intervals, with the exception of the RMGI group at the one-hour mark. This suggests that most of the tested luting cements lack antimicrobial activity against Candida albicans.

DISCUSSION

Tooth preparation for crown and bridge exposes the dentinal tubules. These tubules provide a pathway for the ingress of microorganisms that may infect the pulp and periradicular tissues. Luting cements with antimicrobial potential may protect teeth from ingress of microorganisms, secondary caries and pulp involvement. Modern luting cements are suggested to obtain antimicrobial efficacy. Additionally, they release fluoride ions, which help to remineralize initial carious lesions and hamper the progression of dental caries.[4]

The microbial strains examined in this research encompass some of the prevalent microorganisms responsible for oral health issues. S. mutans stands out as the primary culprit behind dental caries, a pervasive ailment affecting a significant portion of the population. C. albicans, on the other hand, represents one of the most common fungal species found in the oral cavity. It is associated with various opportunistic infections like geographic tongue and angular cheilitis, as well as contaminating denture bases by infiltrating the microporosities of polymethyl methacrylate materials.[8,11] The current research examined and contrasted the antibacterial efficacy of four luting cements against Streptococcus mutans and Candida albicans at three time points (one hour, 24 hours, and one week). The tested cements included resin-modified glass ionomer cement, two variations of conventional glass ionomer cements, and resin cement. Antimicrobial activity of the specimens was evaluated using agar diffusion test and modified direct contact test.

The current investigation demonstrates that all luting cement varieties exhibited uniform antimicrobial efficacy across both agar diffusion and direct contact tests. This outcome is attributed to the synergistic impact of fluoride release and low pH levels. These findings align with those reported by Vermeersch et al. in their 2005 study[12]

The findings of this study illustrate that each of the examined glass ionomer materials exhibited antimicrobial properties against S. mutans. This observation aligns with prior research indicating that glass ionomer cements possess the capability to diminish the presence of S. mutans both in vitro and in vivo.[13, 14 1nd 15]

The observed antimicrobial effects of luting cement materials are likely attributed to several factors, including the release of fluoride, the low pH during initial setting, and other antimicrobial components present in the cement powder. Studies have indicated that fluoride ions primarily exhibit a bacteriostatic effect. However, under specific conditions, such as high concentrations, fluoride ions can also exhibit a bactericidal effect and have the potential to prevent caries.[4]

In vitro fluoride release from materials is influenced by various factors, including the fluoride concentration within the set materials, the size and composition of inorganic fillers, the powder-liquid ratio, the mixing procedure, and the porosity within the material. Fluoroaluminosilicate glass, a primary filler in GICs, is soluble and capable of releasing fluoride. Additionally, particle size plays a crucial role in determining fluoride release.[16, 17]

In the agar diffusion test, the conventional GIC (P & L) produced the maximum zone of inhibition with a value of 17.75 mm at one week among all groups, and it is significantly higher than other two measured interval (one hour and 24 hours) with no significant difference between the two intervals.

The greater antibacterial activity of conventional glass ionomer cement (powder and liquid type) is related to the greater fluoride release from this luting cement which could attributed to introduction of porosity during hand-mixing of glass ionomer cements. It was reported that significant differences in the porosity of glass-ionomer cements were found between the hand-mixed and capsule-mixed equivalents tested.[18]

The method of hand mixing used to handle glass ionomer luting cement can result in un even dispersion of unreacted glass filler particles within the plastic mass, which may lead to porosity formation. This mixing technique significantly impacts the fluoride ion release process. Initially, the acid acts on the glass filler particles, triggering the release of calcium and aluminum ions. These ions subsequently interact with the polyacrylate chains present in the cement, forming a salt matrix. [19]

During the acid attack on the glass filler, fluoride ions are released and become embedded within the

forming matrix, despite not participating further in the setting reaction. Moreover, research indicates that higher porosity permits deeper diffusion of recharge agents into the sample, leading to enhanced fluoride storage and release. Furthermore, porosity in glass ionomer materials can amplify fluoride release for several reasons.

Firstly, porous structures offer a greater surface area compared to non-porous ones. This increased surface area fosters increased interaction between the glass ionomer and the surrounding environment, thereby promoting the leaching of fluoride ions.[20, 21] Capsulated conventional glass ionomer cements (GICs) exhibit an ion release pattern characterized by a significant concentration of fluoride in the first hour, often termed the "burst effect." Following this initial surge, fluoride levels decline rapidly after 24 hours, with a tendency to stabilize within the first week. The goal is to achieve a high release of fluoride ions without compromising the integrity of the filling material. The initial fluoride burst effect is advantageous as it aids in reducing viable bacteria remaining in the dentin and promotes remineralization of enamel and dentin. The subsequent decline in fluoride release is primarily attributed to the dissolution of fluoride from the glass particles during the setting reaction with polyalkenoate acid. Additionally, the initial superficial rinsing may contribute to the high fluoride release on the first day, while continuous fluoride release in the following days occurs due to fluoride's ability to diffuse through cement pores and fractures.[21,22]

Another interpretation for this phenomenon is the "cleaning effect" induced by water on the material's surface, followed by the release of fluoride. This process is regulated by the diffusion of water through the micropores and the cement mass. [21]

The present study showed that None of the luting cement showed inhibition zones formed around Candida. Except the RMGI at one hour interval. This result is in accordance with the study of Bora et al, 2018, in which only RMGI (among conventional glass ionomer and resin cement) showed antimicrobial activities against Candida albicans. [10] Therefore, the results suggested that Candida is resistant to selected luting cement as there is no significant zone of inhibition formed around it and this result is in accordance with a study done by Naguib et al., 2022 .[8]

CONCLUSION

Within the limitation of this study, it can be concluded that all the evaluated luting cement demonstrated antibacterial activity against S. mutans using both tests. While none of them (except RMGI at one hour) showed antimicrobial activity against Candida albicans. The superior antimicrobial activity was demonstrated by conventional (powder and liquid). Hence, it could be beneficial in patients with high caries risk.

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