

# Efficacy of antimicrobial property of two commercially available chemomechanical caries removal agents (Carisolv and Papacarie): An *ex vivo* study

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## Abstract

**Purpose:** The purpose of the study is to evaluate the antimicrobial efficacy of Carisolv and Papacarie. There are only a few studies comparing the primary teeth. The objective of this study is to assess the effects of Carisolv and Papacarie on cariogenic flora and to compare them. **Materials and Methods:** Fifteen children aged 4–8 years who had at least two primary molars with broad occlusal cavitated lesions showing brown and softened dentin samples were selected. The selected 30 teeth were randomly divided into two groups of 15 teeth each for Carisolv and Papacarie. Dentin samples of both groups were taken prior to and following caries removal. The total viable count and lactobacilli count were determined and expressed as colony forming units per milliliter. The two methods of caries removal were then compared and the data were statistically analyzed. **Results:** The data of both agents (groups) were analyzed by Wilcoxon signed-rank test before and after application and showed statistical significance. Inter-comparison of data of both groups was analyzed by Mann–Whitney U test for total viable count and total *Lactobacillus* count which showed no statistical significance. **Conclusion:** Carisolv and Papacarie have similar antibacterial efficacy against cariogenic flora as chemomechanical caries removal agents.

**Key words:** *Carisolv, chemomechanical caries removal agents, Papacarie*

## INTRODUCTION

Even though there has been a substantial reduction of prevalence of caries in developed countries, this disease continuous to be widespread throughout the world. Once it has formed, it is of fundamental importance to use

conservative procedure that simultaneously prevents lesion progression, minimizing loss of healthy tooth structure.

Traditionally, caries was removed mechanically with hand excavators and rotary instruments leading to overextended cavities, healthy tissue removal, pressure and heat on pulp, vibration, noise, pain stimulus, and need for local anesthesia.<sup>[1-3]</sup> Newer methods of caries removal have been developed as an alternative to traditional methods to overcome few of the above-mentioned drawbacks. These include laser, air abrasion, ultrasound, and use of chemomechanical caries removal (CMCR) agents.<sup>[4-6]</sup>

The usage of CMCR agents has gained high acceptance among children and patients with dental anxiety<sup>[6,7]</sup>

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.159955

because of its selective action on infected dentin, and being a simple, non-invasive, painless technique with proven clinical safety. With better understanding of the caries process and the tooth's potential for remineralization, development of new dental restorative materials, and advances in adhesive dentistry, the management of dental caries has drastically changed from G. V. Black's "extension for prevention" to "construction with conservation" (Tandon, 2008).

The objective of CMCR agents is to remove the most external portion (infected layer) which contains degraded collagen and bacteria that cannot be remineralized, leaving the affected demineralized dentin that is capable of being remineralized and repaired. This process of CMCR involves the selective removal of soft carious dentine without the painful removal of sound dentine.<sup>[8-11]</sup>

In the year 1997, Medi Team Dental AB, Sweden introduced Carisolv™ to the European market, which promised to be effective and easy to manipulate. Carisolv contains 0.5% sodium hypochlorite (NaOCl) and 0.1 M aminoacids (lysine, leucine, and glutamic acid), gel substance, sodium chloride, sodium hydroxide, and a color indicator (Erythrocin). When these components are mixed together, the aminoacids bind with chlorine to form high pH chloramines which are a potent disinfectant with tissue solvent activity. Despite its effectiveness, Carisolv was not a blockbuster, mainly because it requires extensive training and registration of professionals and customized instruments which increase the cost of the material.<sup>[12,13]</sup>

In 2003, a Papacarie gel was launched in Brazil which contained papain, chloramines, and toluidine blue. Papain interacts with collagen exposed by dissolution of dentin minerals by the microbial activity and also makes infected dentin friable, enabling its removal by blunt hand instruments.<sup>[14]</sup> Papain is an enzyme similar to human pepsin and acts as an anti-inflammatory and debriding agent.<sup>[13-16]</sup>

Several studies have evaluated CMCR agents with regard to patient comfort, clinical time for caries removal, and its effect on healthy tissue and pulp.<sup>[17,18]</sup> However, little research was done on evaluating the efficacy of these agents in reducing the total viable count and *Lactobacillus* count, which plays an important role in the progression of dentinal caries. Hence, the present study was aimed to evaluate and compare the antimicrobial efficacy of two commercially available CMCR agents, Carisolv and Papacarie.

## MATERIALS AND METHODS

The present *ex vivo* study received consent from the research ethics committee, CKS Theja Institute of Dental Sciences, Tirupati. Research was conducted in association with the Department of Microbiology, SV Institute of Medical Sciences, Tirupati, Andhra Pradesh.

Healthy and cooperative children who attended as outpatients to the Department of Pedodontics and Preventive Dentistry, CKS Institute of Dental Sciences were selected for this study.

The study group consisted of 40 primary molars that were free of any developmental anomalies and were selected from 20 children aged between 4 and 8 years without sex predilection. Out of 40 samples, only 30 were selected; 10 samples (5 children) were eliminated from the study because of technical errors. The degree of destruction was clinically determined by using a dental probe. Only large occlusal cavities with brown and softened dentin with broadly comparable depth (moderate or deep-seated caries) were chosen for sample taking and the depth of caries progression was confirmed with an intraoral periapical radiograph such that radiolucency did not involve the pulp. For comparison, two primary molars on contralateral sides were selected in each patient to remove caries using two commercially available CMCR agents (Carisolv and Papacarie).

A brief case history was recorded and thorough examination of the selected teeth was done both clinically and radiologically, prior to the study. Consent for the children participating in the study was obtained from the children's guardians after explaining them about the study design. All the patients received provisional teeth cleaning and oral hygiene instructions 1 week prior to the experiment.

### Inclusion criteria

- Primary first/second molars with large occlusal carious lesion having moderate depth and clinically visible brownish colored softened dentin
- No clinical symptoms or evidence of pulp degeneration, such as pain on percussion and history of swelling or sinus tracts. Intraoral periapical radiographs with lesions having radiolucency extending into, but confined only to dentin.

### Exclusion criteria

- Uncooperative patients
- Completely broken down tooth where isolation with rubber dam is not possible
- Patients on any antibiotic regimen either on the day of treatment or for at least 2 weeks prior to the study
- Radiographic evidence of external or internal root resorption, furcal or periapical radiolucency.

A total of 40 teeth from 20 patients were randomly divided into two groups of 20 teeth each – group I (Carisolv) and group II (Papacarie). In both the groups, the teeth were isolated with rubber dam without local anesthesia administration. Carisolv and Papacarie were removed from the refrigerator approximately 1 h before treatment, as per manufacturer's instructions.

Before taking the sample, the outer surface of the carious lesion was washed with a flurry of water to avoid contamination of plaque bacteria. Two samples before and after caries excavation was taken by a single operator to avoid operator bias. First sample was taken using specialized hand instrument designed for Carisolv gel. After removal, the dentin samples were immediately transferred to a sterile vial with screw cap which contained sterile isotonic saline.<sup>[19]</sup>

In group I, the Carisolv gel components were extruded by pressing the plunger of the multi-mix pack which was dispensed directly into the occlusal carious lesion. After 30 s, the soft carious dentin was gently scraped using Carisolv hand instrument. More gel was applied and the procedure was repeated until the liquid no longer turned cloudy.<sup>[4,20]</sup> The cavity was carefully inspected for complete caries removal, and the procedure was repeated if any carious dentin remained. The cavity was tested according to visual (absence of any discoloration) and tactile (smooth passage of explorer and absence of a catch or a tug back sensation) clinical criteria using a blunt straight probe. Caries was considered to be totally removed when the explorer did not stick in dentin and gave no tug back sensation.

In group II, Papacarie gel was filled into the cavity and allowed to work for 60 s. On initial application, fresh gel was clear, which then turned opaque or turbid with debris from the lesion. The softened, decayed dentin was scraped away with an excavator which was used for Carisolv. The gel was reapplied whenever a dark color appeared, which indicated that the decomposition of the decayed tissue was still in process. The procedure was

repeated until the gel no longer turned turbid and the surface felt hard.<sup>[21]</sup>

In both the groups, after completing caries removal, the cavity was cleaned with sterile cotton pellets, washed, and dried. A second sample of dentin was taken from different sites of the cavity, including the walls and floor, using a Carisolv hand instrument. The contents of the second samples were directly placed in sterile vials containing 1 ml of isotonic saline and used for further evaluation.<sup>[19]</sup>

The dentin samples were processed in the microbiological lab within 1 h of collection.<sup>[22]</sup> Each sample was vortexed for about 30 s in order to dislodge the bacteria from the dentin. The samples were then serially diluted to obtain  $10^{-3}$  dilutions, and 0.1 ml of this dilution was inoculated on to different agar plates. Schaedler agar was used to determine the total viable count and Mitis Salivarius agar was used to determine the viable count of lactobacilli. The two agar plates were incubated anaerobically at 35°C for 48 h.<sup>[23]</sup> Using a colony counter, the numbers of colonies were determined per sample and expressed as colony forming units (CFU)/ml.

The data thus obtained were tabulated and subjected to descriptive statistical analysis using Wilcoxon signed-rank test and Mann–Whitney U test. Pair wise comparison of mean values was done using Wilcoxon signed-rank test. Mann–Whitney U test was applied to find out the difference between two independent groups.

### RESULTS

At baseline, there was no significant difference between the mean values of different microorganisms (total viable bacteria and lactobacilli) among the dentin samples of the two study groups. The mean values of total viable bacteria and lactobacilli were significantly reduced after complete caries removal using either Carisolv or Papacarie.

Tables 1 and 2, Graphs 1 and 2 show the comparison of mean reduction of different microorganisms (total viable count and *Lactobacillus*) before and after caries removal in group I (Carisolv and Papacarie, respectively). Tables 3 and 4, Graphs 3 and 4 compares the mean percent reduction in different microorganisms between the two tested groups. Mean percent reduction in total viable bacteria in between carisolv and papacarie was  $69.2 \times 10^4$  in group I and  $69.87 \times 10^4$  in group II, with a statistically significant difference between the two study groups

**Table 1: Comparison of mean reduction of different microorganisms before and after caries removal in group I (Carisolv)**

Microorganisms	Mean±(SD)		Wilcoxon signed-rank test	P
	Baseline sample	Second sample		
Total viable bacteria	75±4.97×10 <sup>4</sup>	6.00±1.13×10 <sup>4</sup>	-3.410	0.001
Lactobacilli	18.87±4.21×10 <sup>4</sup>	2.47±1.19×10 <sup>4</sup>	-3.416	0.001

SD=Standard deviation

**Table 2: Comparison of mean reduction of different microorganisms before and after caries removal in group II (Papacarie)**

Microorganisms	Mean±(SD)		Wilcoxon signed-rank test	P
	Baseline sample	Second sample		
Total viable bacteria	75.73±7.55×10 <sup>4</sup>	5.87±1.25×10 <sup>4</sup>	-3.410	0.001 significant
Lactobacilli	17.93±2.96×10 <sup>4</sup>	2.27±0.88×10 <sup>4</sup>	-3.416	0.001 significant

SD=Standard deviation

**Table 3: Percentage reduction of total viable count following usage of Carisolv and Papacarie**

Treatment	CFU per/ml		U	Z	P
	Reduction	% of reduction			
Carisolv	69.2×10 <sup>4</sup>	92.02	96.50	-0.665	0.512 not significant
Papacarie	69.87×10 <sup>4</sup>	92.25			

CFU=Colony forming units

**Table 4: Mean percentage reduction of lactobacillus following caries removal**

Treatment	CFU per/ml		U	Z	P
	Reduction	% of reduction			
Carisolv	16.4×10 <sup>4</sup>	86.93	103.00	-0.397	0.713 not significant
Papacarie	15.67×10 <sup>4</sup>	87.36			

CFU=Colony forming units, LIB=lactobacillus

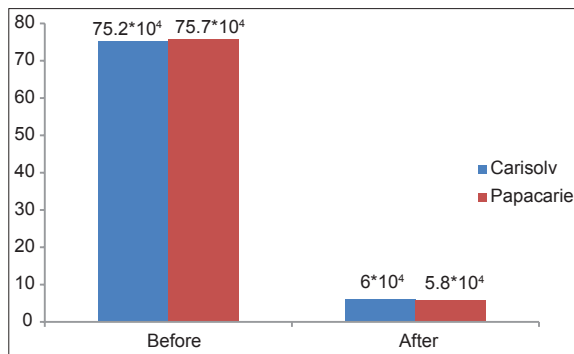
(U value = 96.50, Z value = -0.665, P = 0.512). For lactobacilli, the mean percent reduction following caries removal in group I was 16.4 × 10<sup>4</sup> and in group II was 15.67 × 10<sup>4</sup>, with a significant difference between the two study groups (U = 103.00, Z = -0.397, P = 0.713).

## DISCUSSION

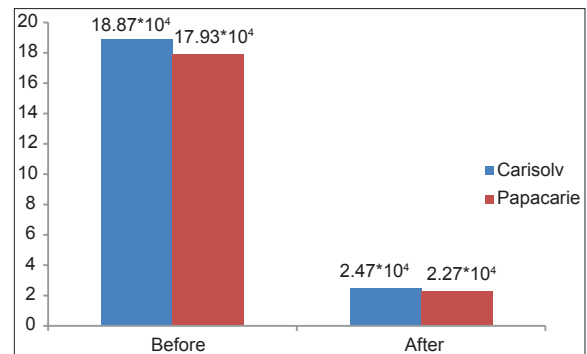
Out of all the advanced methods, CMCR agents are gaining interest because of their unique property to differentiate affected and infected dentin; thus, it is a minimally invasive technique which avoids unnecessary removal of sound tooth structure, lessens or eliminates the use of local anesthesia, is economical, and the armamentarium required is minimal compared to all other advanced methods.<sup>[24-26]</sup> Because of all these advantages, it is quite commonly used, particularly in the field of pediatric dentistry.

The ideal requirements for a chemomechanical agent used for caries removal include the following:

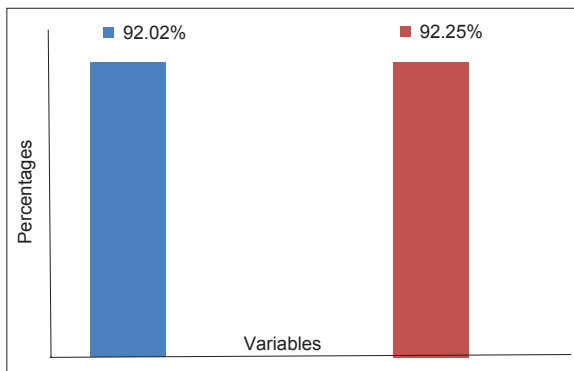
It should have tissue-specific action<sup>[27]</sup> (able to differentiate between infected and affected dentin); it should have good antibacterial activity in order to facilitate the eradication of bacteria, if at all they gain access into the affected dentin which will be preserved during cavity preparation;<sup>[28]</sup> it should be effective in removing the smear layer; it should be non-irritant to the pulp and the surrounding hard and soft tissues;<sup>[3]</sup> it should neither discolor the tooth structure nor interfere with the properties of the restorative materials; it should have long shelf life; it should be easy to manipulate and less expensive. Although extensive research has been conducted in the field of CMCR technique, only two commercially available CMCR agents (Carisolv and Papacarie) were found to meet most of the above-mentioned requirements, if not all. However, the antimicrobial activity was not tested enough to attribute bacteriostatic/bactericidal properties to these agents. Thus, the aim of the present study is to identify and compare the antimicrobial activity of these agents.



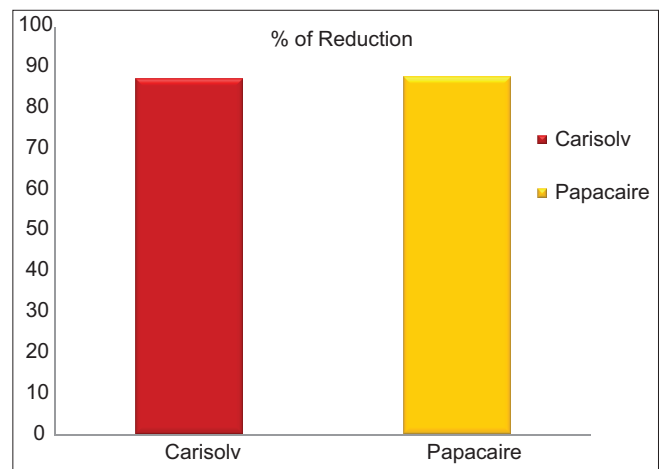
**Graph 1:** Mean total viable count before and after application of Carisolv and Papacarie



**Graph 2:** Mean total *Lactobacillus* count before and after application of Carisolv and Papacarie



**Graph 3:** Percentage reduction of total viable count following application of Carisolv and Papacarie



**Graph 4:** Percentage reduction of lactobacilli by carisolv and papacarie

It is a well-established fact that bacteria are the prime etiological factor in the development and progression of dental caries.<sup>[29]</sup> Two bacterial genera are of special interest in cariogenesis – the mutans streptococci (MS) and lactobacilli (LB).<sup>[30]</sup> MS are now considered to be major pathogenic bacterial species involved in the initiation of caries process because of their acidogenic and aciduric potential along with their ability to produce dextrans. But the count of MS drastically decreases at the deeper layers of the carious lesion because of their aerobic properties indicating their role limited only to initiate the caries process. The second commonest organisms involved in the caries process are lactobacilli; these organisms are both acidogenic and aciduric.<sup>[31,32]</sup> Because of their strong aciduric and anaerobic properties, the count of *Lactobacillus* predominates in the deeper portion of the caries lesion, indicating their strong role in the progression of the lesion.<sup>[33,34]</sup>

Minimal carious lesions were not considered in the study as caries is restricted to enamel where in CMCR can dissolve only dentinal caries structure. Proximal caries lesions were also not considered, as they do not allow placement of rubber dam and may lead to seepage of saliva into the cavity resulting in contamination

of sample.<sup>[35]</sup> To avoid bias in the study, split-mouth technique was followed where Carisolv and Papacarie were used in the opposite arches or contralaterally.

In the present study, difference in the bacterial count before and after application of CMCR agent was considered as the measure for its antibacterial efficacy.<sup>[8,29,36,37]</sup> The first dentin sample taken before the application of CMCR agents contained increased number of microorganisms and degraded collagen (infected dentin).<sup>[19,38]</sup> In the second dentin sample, which was taken following the application of CMCR agents, the microbial count was reduced (affected dentin). The color change and change in turbidity of Carisolv and Papacarie also give an indication that the softening process is complete and the cavity is caries free. Loss of turbidity with Carisolv<sup>[39,40]</sup> and change in color from blue to cloudy with Papacarie act as indicators.

Carisolv excavation resulted in a significant reduction of all tested viable bacteria. Other bacteriological studies have reported similar results.<sup>[19,22,39,40]</sup> Lager *et al.*

reported that the composition of Carisolv (contains NaOCl as the main ingredient) is responsible for its antibacterial activity. In accordance with our data, this has been proved by Silva *et al.*,<sup>[41-43]</sup> Korb,<sup>[44]</sup> Motta *et al.*,<sup>[38]</sup> and Bussadori *et al.*<sup>[45]</sup> In case of Papacarie, the main ingredients are papain, chloramines, and toluidine blue. Papain acts as a debris removing agent and exhibits antimicrobial activity because of the presence of proteolytic cysteine enzymes. El-Tekeya *et al.*<sup>[45]</sup> concluded that Papacarie is significantly more effective in reducing the residual cariogenic bacteria in the dentin of primary teeth versus Carisolv and hand excavation methods. The studies of Motta<sup>[46]</sup> and Matsumoto<sup>[47]</sup> inferred Papacarie as an excellent option for minimally invasive removal of caries tissue, achieving significant reduction in total bacteria and *Streptococcus mutans*.

The microbiological and clinical results of this study have vital implications in the operative management of caries in children. Both cmcr strategies softened dentinal caries for effective mechanical removal criteria. From bacteriological point of view, these strategies additionally removed cariogenic bacterium to a good extent. The small amount of residual microorganisms remains negligible because it does not exceed the clinically accepted level.<sup>[33,48]</sup> Moreover, with the new adhesive restorations providing utterly sealed margins and with the recently introduced antimicrobial cavity cleaners, this touch extent of demineralization bacteria can be minimized.

## ACKNOWLEDGMENT

Financial interests, direct or indirect, for any of the authors do not exist for this article. Sources of outside support of the project are none for this article.

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**How to cite this article:** Reddy MC, Sai Shankar AJ, Pentakota VG, Kolli H, Ganta H, Katari PK. Efficacy of antimicrobial property of two commercially available chemomechanical caries removal agents (Carisolv and Papacarie): An *ex vivo* study. *J Int Soc Prevent Communit Dent* 2015;5:183-9.

**Source of Support:** Nil, **Conflict of Interest:** None declared.