Comparison of the Antimicrobial Efficacy of Chemomechanical Caries Removal (carisolv™) with that of Conventional Drilling in Reducing Cariogenic Flora

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Purpose: *The aim of this study was to evaluate the antimicrobial efficacy of chemomechanical caries removal (Carisolv™) in reducing the count of cariogenic flora and compare it to conventional drilling.* ***Materials:*** *The study group consisted of 20 healthy children aged between four and eight years. In each child, two primary molars with broad occlusal cavitated lesions were chosen for caries removal either with Carisolv™ or by conventional drilling. Dentin samples of both groups were taken prior to and following caries removal. They were then processed after suitable dilutions and cultured using Schaedler agar for the Total Viable Bacteria and MRS agar for the lactobacilli. After incubation at 35ºC for 3 days, the Total Viable Count and lactobacilli count was determined and expressed as Colony Forming Units per ml. The two methods of caries removal were then compared and the data was statistically analyzed. Both methods reduced the TVC count by 92% and lactobacilli count by 91%.* ***Results*** *have indicated that the antimicrobial efficacy of Carisolv™ was comparable to that of conventional drilling and can be used as a suitable alternative for caries removal, especially in children.*

Keywords: *Chemomechanical caries removal, Carisolv™, conventional drilling, Total Viable Count, lactobacilli count, Colony Forming Unit*
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INTRODUCTION

Dental caries is a bacterial infection with a demineralization and remineralization process. However, once dentin is involved, as in deep caries lesions, demineralization or decomposition, or both dominate due to the diminished availability of saliva and fluoride. When the organic matrix has been demineralized, the collagen and other matrix components become susceptible to enzymatic degradation, mainly by bacterial proteases and other hydrolases.[1]

Pain during the invasive treatment of dentinal caries is quite a common phenomenon, often inducing fear and anxiety in children. Children and many adults consider caries removal very unpleasant. One major disadvantage of drilling is that it often induces pain and local anesthesia may be needed.[2] The bur also removes both infected and non-infected dentin thereby unnecessarily weakening the sound tooth structure.[3] The minimal removal of infected carious dentin, together with that of a therapeutic restorative material is a fundamental requirement of modern operative dentistry.[4] This also helps to eliminate the pain associated with the removal of carious dentin, thus introducing dental treatment to children in a painless manner.

Developing alternative techniques which are minimally invasive and painless is possible due to several factors such as development of adhesive restorative materials, dispensing with mechanical retention during cavity preparation, use of fluoride releasing restorative materials, biological understanding of the caries process, and the natural defense mechanism of teeth.[5]

In more recent years newer techniques such as chemomechanical caries removal (CMCR) methods were developed for painless removal of caries. The CMCR method by Carisolv™ (Mediteam) has the advantage of selective removal of severely demineralized dentin.[6] It involves the chemical softening of carious dentin followed by its removal with gentle excavation.[1] The mechanism of action of CMCR using Carisolv™ has been described as a first choice treatment alternative for caries removal.[7]

With regard to oral microflora, evidence has shown that

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acidogenic species such as *mutans streptococci (Streptococcus mutans and Streptococcus sobrinus)* and l*actobacilli* are strictly associated with the onset and presence of dental decay.[8] *Mutans streptococci* are mainly implicated with the initiation of enamel caries and gradually increase when the primary dentition is completed and proximal contacts between primary molars are present.[9]

Isolates from both shallow and deep layers of dentin showed an overwhelming majority of microorganisms of obligate anaerobes which are mostly gram positive bacilli such as species of *Eubacterium, Lactobacillus, Bifidobacterium* and *Propionibacterium*.[10] In children aged 5-15 years *lactobacilli* were frequently isolated from dentin lesions.[11] As microflora is one of the main etiological factors in the occurrence of caries, it is essential to reduce the microbial count in a carious lesion.

Several studies have evaluated chemomechanical caries removal with regard to patient comfort, clinical time for caries removal and its effect on healthy tissue and pulp.[5, 12] However, not many studies have been conducted to demonstrate the effect of chemomechanical caries removal on the cariogenic flora in primary teeth.

Thus, the purpose of this study was to evaluate the antimicrobial efficacy of the chemomechanical agent (Carisolv™) for caries removal from primary molars using a bacteriological evaluation and to compare it to that of conventional drilling.

MATERIALS AND METHODS

Twenty healthy children aged between 4 and 8 years (Seven boys and thirteen girls) and who had at least two primary molars with broad cavitated occlusal lesions showing brown and softened dentin were chosen for the study. For ethical reasons, every child had one carious primary molar treated with chemomechanical caries removal method (Carisolv™) and the other primary molar using the conventional drilling method. Hence, no child was withheld from the benefit of both treatment methods. Since both the molars in every child were exposed to a similar oral environment, this study was more suitable to compare the two treatment modalities. A brief history was recorded and the teeth were subjected to clinical examination and radiographic evaluation prior to study.

Intra oral periapical radiographs with lesions clearly visible as radiolucency extending into, but confined to the outer dentin of the occlusal surface were included and teeth with interproximal caries were excluded. Patients on antibiotic regimen either on the day of treatment or for at least two weeks prior to the study were also excluded.

CLINICAL PROCEDURE

Both chemomechanical caries removal and the conventional drilling method of caries removal were carried out under rubber dam isolation in order to obtain moisture control and avoid microbial contamination.

CHEMOMECHANICAL METHOD OF CARIES REMOVAL

According to the manufacturer's instructions, both syringes of Carisolv™ were removed from the refrigerator approximately 1 hour before treatment, and their contents were mixed shortly before use.

The first sample from the superficial carious lesion was removed with the help of a sterile Carisolv™ hand instrument (Carisolv™4) and transferred into a sterile vial containing 1 ml of saline for microbiological evaluation.

With the aid of Carisolv™2 hand instrument the gel was applied to cover the dentinal caries. Following 20 seconds, the carious dentin was gently scraped using light pressure with Carisolv™2 hand instrument to remove softened carious tissue. On initial application the fresh gel was clear, but gradually became opaque or cloudy due to debris removed from the carious lesion. The debris saturated gel was removed with a cotton pellet and fresh gel applied. The procedure was repeated until the gel was no longer cloudy. Hardness upon application of gentle pressure with a WHO periodontal probe was considered as the tactile criteria for assessing complete caries removal. The second sample was then taken from the cavity floor with a sterile Carisolv™ hand instrument (Carisolv™4) and transferred to another sterile vial containing 1ml of saline for microbiological evaluation.

CONVENTIONAL DRILLING METHOD OF CARIES REMOVAL

The conventional method of caries removal was carried out using a No.16 sterile round bur on a micromotor hand-piece at slow speed, without water spray. Dentin samples were taken, prior to and after drilling, using the same sampling procedure, as with the chemomechanical caries removal.

In both groups, after caries removal, all teeth were restored with glass ionomer cement. (Ketac Molar Easy Mix).

MICROBIAL CULTIVATION AND EVALUATION

The dentin samples of both groups were processed in the microbiological laboratory within one hour of collection. Each sample was vortexed for about 30 seconds in order to dislodge the bacteria from the dentin. The samples were then serially diluted to obtain a 10^{-3} dilution and 0.1 ml of this dilution was inoculated on two different agar plates. Schaedler agar was used to determine the Total Viable Counts and MRS agar was used to determine the viable counts of lactobacilli. The agar plates were incubated anaerobically (Gaspak- Anaerogas pack® HIMEDIA) at 35ºC for 3 days. Then, using a colony counter, the number of colonies was determined per sample and expressed as CFU/ml.

The data obtained were tabulated and subjected to statistical analysis using Wilcoxon Sign rank test for pair wise comparison and Mann Whitney U test to find out the significant difference between two independent groups.

Table 1. Mean reduction in Total Viable Count and *lactobacilli* count following the Chemomechanical caries removal method (CarisolvTM) and the conventional drilling.

	CMCR (CARISOLV™)					CONVENTIONAL DRILLING				
MICRO FLORA	Before Treatment CFU/ml	After Treatment CFU/ ml	Reduction CFU/ ml	Z Value	P Value	Before Treatment CFU/ml	After Treatment CFU/ml	Reduction CFU/ ml	Z Value	P Value
MEAN TOTAL VIABLE COUNT	73.25 ± 5.78 × 10^4	5.85 ± 1.14 × 10^4	67.4 × 10^4	-3.925	<0.001**	74.15 ± 7.68 × 10^4	5.8 ±1.20 × 10^4	68.35 × 10^4	-3.927	<0.001**
MEAN LACTO BACILLI COUNT	18.65 ± 4.38 × 10^4	1.6 ± 0.82 × 10^4	17.05 × 10^4	-3.926	<0.001**	18.05 ± 3.19 × 10^4	1.55 ± 0.51 × 10^4	16.5 × 10^4	-3.923	<0.001**

(p <0.001** is highly significant)

RESULTS

In the present study, 40 primary molars selected from 20 children aged between four and eight years (seven boys and thirteen girls) were evaluated. The Total Viable Count (TVC) and viable count of *lactobacilli* was determined and expressed as Colony Forming Units (CFU) *per* sample.

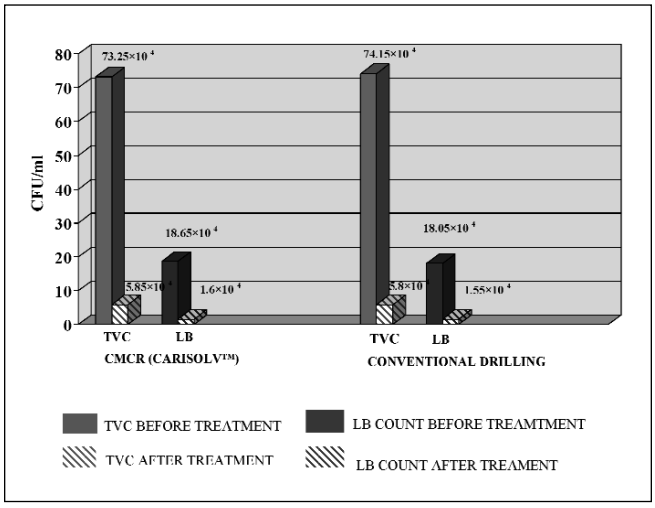
The mean TVC were observed to be 73.25 × 10^4 CFU/ml and 74.15 × 10^4 CFU/ml before caries removal with chemomechanical caries removal (CMCR) (Carisolv™) and conventional methods, respectively. The TVC was reduced to 5.85 × 10^4 CFU/ml following CMCR (Carisolv™) and 5.8 × 10^4 CFU/ml after conventional drilling. This corresponds to a mean reduction of TVC of 67.4 × 10^4 CFU/ml and 68.35 × 10^4 CFU/ml in CMCR and conventional drilling methods, respectively. The reduction in TVC by both methods of caries removal was highly significant. (p <0.001) **(Table 1, Graph 1).**

The *lactobacilli* count decreased from 18.65 × 10^4 CFU/ml before treatment to 1.6 × 10^4 CFU/ml after the CMCR (Carisolv™) method. There was a reduction from 18.05 × 10^4 CFU/ml before treatment to 1.55 × 10^4 CFU/ml after treatment using the conventional drilling method. **(Table 1, Graph 1).** The reduction in the mean *lactobacilli* count by both methods of caries removal was also highly significant (P<0.001).

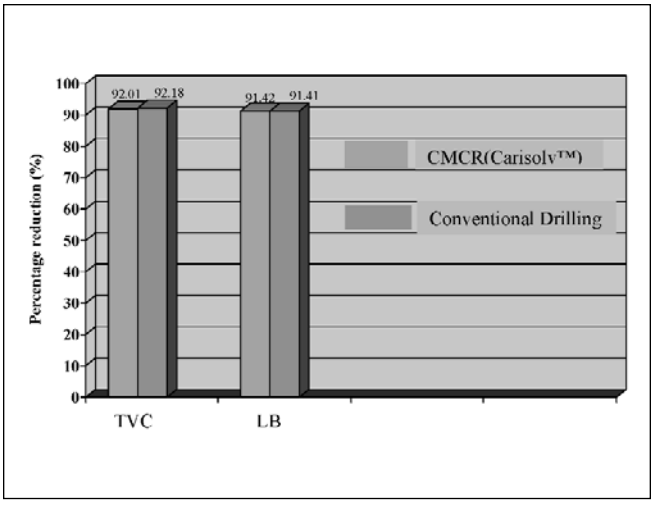
Table 2. Percentage reduction of Total Viable Count and lactobacilli count with the chemomechanical caries removal method (Carisolv™) and the conventional drilling method.

Treatment	TOTAL VIABLE COUNT			LACTOBACILLI COUNT		
	Percentage reduction (%)	Z value	p value	Percentage reduction (%)	Z value	p value
CMCR (CARISOLV™)	92.01	-0.495	0.867	91.42	-0.298	0.779
CONVENTIONAL DRILLLING	92.18			91.41		

The results also indicated that both methods of caries removal showed almost similar percentage of reduction in TVC (92%) and *lactobacilli* counts (91%). **(Table 2, Graph 2)**



Graph 1. Mean reduction in Total Viable Count (TVC) and lactobacilli (LB) count following chemomechanical caries removal (Carisolv™) and conventional drilling.



Graph 2: Percentage reduction of Total Viable Count (TVC) and lactobacilli (LB) count using the chemomechanical caries removal (Carisolv™) and conventional drilling technique.

DISCUSSION

Conventional drilling is the most common clinical procedure for caries removal, but it generates pain, fear, discomfort and anxiety in children.[5] The new chemomechanical caries removal system by Carisolv™ is desirable in Pediatric dentistry since it allows a minimal invasive technique to be applied.[13] This system also eliminates the use of anesthesia, painful symptoms and unnecessary removal of sound tooth structure since only carious dentin is removed and the painful removal of sound dentin is avoided.[1,7]

Studies conducted on the antimicrobial efficacy of Carisolv™ differ in their selection criteria, sampling procedures, culture media and culture technique. Some of these are *in-vitro* studies and mostly done on permanent teeth.

Carisolv™ consists of two basic components. One contains the active ingredient sodium hypochlorite while the other comprises three aminoacids namely leucine, lysine and glutamic acid. When mixed with aminoacids it generates chloramines. This results in the chlorination and further disruption of collagen cross linkage in the matrix of the carious dentin. Due to its high pH, only the organic phase of dentin is affected. Sodium hypochlorite solution alters the configuration or removes the organic components of dentin, especially the collagen fibrils. [14]

The three natural aminoacids used in this gel solution, are known for their effective interaction with dentin, since they act on different protein chains of denaturalized collagen, thus enhancing the effect of sodium hypochlorite on carious dentin. They also neutralize the action of the reagent on healthy tissue and prevent degradation of healthy collagen, which can be remineralized. In the gel form, the active molecules have prolonged access to dentin, when compared to the irrigation solution of the Caridex® system. It is also highly probable that the gel provides lubrication to the action of hand instruments, which aid in the removal of softened tissue. It is said to be an alternative system to avoid or at least minimize, the adverse effects produced by drilling instruments[3,7]

While *mutans streptococci* (MS) are mainly implicated with the initiation of enamel caries, they are rarely the predominant species isolated from carious dentin. The composition of the microflora is known to become more complex as the lesions progress, and obligate anaerobes, mainly Gram positive rods predominate, accounting for 70% of the total Colony Forming Units per milliliter. Among these, Lactobacillus species are the principal isolates and play an important role in the progression of dental caries. [2,15]

It is essential to determine both the Total Viable Count and the *lactobacilli* count, from the infected dentin as well as from the top layers of residual dentin, following caries removal. The number of microorganisms isolated from a site can also be influenced by the sampling method.[2]

Certain studies have used sterile burs of definite size for taking dentin samples.[16,17] In our study, all dentin samples were carefully removed with a Carisolv™ excavator in order to reduce the risk of accidental pulp exposure, especially when sampling hard dentin. Dislodging bacteria from the dentin samples was done by the vortex method. This might yield an overall lower bacterial count because some bacteria remain behind. However, this would occur in both samples and the reduction expressed as a percentage would not be affected.

The chemomechanical caries removal (Carisolv™) is less painful, when compared to the rotary method. This may be due to the special design of Carisolv™ instruments for safe scraping action. They have a 90 degree edge and not a sharp cutting profile, which allows working in two or more directions, reducing dentin shattering. Regular round excavators are shown to cause more discomfort as they invite the operator to 'dig' into the carious dentin and break off dentin pieces, thereby opening more dentin tubules.[4,18]

The efficacy of new methods of excavation such as Carisolv,™ which selectively removes carious dentin, can be assessed by studies on the bacteriological content of the dentin caries lesion.

In a microbiological study, the proportions of *lactobacilli* and *mutans streptococci* in the initial sample represented 13.7% and 4.9% of the total cultivable flora, respectively. In our study, lactobacilli count represented 24-25% of the Total Viable Count, prior to caries removal by either method. Before caries removal, the Total Viable Count ranged from 60×10^4 to 84×10^4 CFU per ml. Bjorndal *et al*[15] reported the total CFU/ml after first excavation to range from 1.2×10^3 to 9.5×10^5.

In a comparison of the two methods of caries removal, Azrak *et al* [2] showed that the Total Viable Count was reduced to less than 10^2 CFU in 90.5% of the samples after treatment with rotary instruments and in 95.2% of the samples after the application of Carisolv.™ The viable count of *lactobacilli* was also reduced to less than 10^2 CFU in 95.2% of the samples after treatment using either method.

In the present study, the mean Total Viable Count after caries removal was reduced to 5.85×10^4 CFU per ml using chemomechanical caries removal (Carisolv™) and 5.8×10^4 CFU per ml with conventional drilling. These reductions were highly significant. Similarly, the *lactobacilli* count was significantly reduced by 91.42% following the use of Carisolv™ and by 91.41% in the conventional method. Moreover, this represented 27.35% to 26.72% of the Total Viable Count after caries removal; further emphasizing the predominance of *lactobacilli* in deeper layers of the carious lesion.

Kneist *et al* [19] concluded that the microflora on the cavity floor of primary molars was nearly the same after both chemomechanical (Carisolv™) and mechanical caries

removal. Similarly, in our study there was no significant difference between the two methods in reducing the Total Viable Count and *lactobacilli* count.

It was observed that following dentin caries removal by conventional drilling and chemomechanical caries removal, the total number of Colony Forming Units (CFU) was reduced with increasing lesion depth. Also, both methods of caries removal were effective showing equal numbers of colonies in the caries free dentin.[20]

As reported in an earlier study,[2] both methods of caries removal produced a statistically significant reduction in the Total Viable Counts and in the viable counts of *lactobacilli*. While comparing caries excavation using a conventional rose bur or Carisolv,™ Lager *et al* [17] reported no significant differences except in the case of blood agar (aerobic), which showed that Carisolv™ was more effective in reducing CFU. In the present study, both methods of caries removal reduced the Total Viable Count by 92%. Such reduction in microbial flora can be attributed to the antibacterial properties of Carisolv,™ which contains chloramines and sodium hypochlorite.

Guida[21] reported that sodium hypochlorite causes a biosynthetic alteration in cellular metabolism and phospholipid destruction, and formation of chloramines, which interferes in cellular metabolism, an oxidative action with irreversible enzymatic inactivation in bacteria, and lipid and fatty acid degradation. Small amounts of sodium hypochlorite may remain in the cavity after Carisolv™ treatment on subsequent removal of carious dentin.

From the present study, the efficacy of the CMCR removal of carious dentin in cavitated primary molars by means of Carisolv™ was comparable to that of the conventional drilling method. Although the use of the CMCR is limited to cavitated lesions, it was effective against microflora present in carious dentin of primary molars. This shows that Carisolv™ could be a suitable alternative method for caries removal and has great potential for use in Pediatric operative dentistry.

CONCLUSION

The following conclusions were drawn from the present study:

The mean reduction in the TVC was 67.4×10^4 CFU/ml using the CMCR (Carisolv™) method and 68.35×10^4 CFU/ml using the conventional drilling technique.

The mean reduction in *lactobacilli* count were 17.05×10^4 CFU/ml and 16.5×10^4 CFU/ml using the CMCR (Carisolv™) and conventional drilling methods, respectively.

The antimicrobial efficacy of both methods of caries removal was comparable and did not differ significantly.

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