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COMPARISON OF THE EFFICACY OF CHEMOMECHANICAL AND MECHANICAL METHODS OF CARIES REMOVAL IN THE REDUCTION OF *STREPTOCOCCUS MUTANS* AND *LACTOBACILLUS* SPP IN CARIOUS DENTINE OF PRIMARY TEETH

COMPARAÇÃO DA EFICÁCIA DOS MÉTODOS QUÍMICO-MECÂNICO E MECÂNICO DE REMOÇÃO DE CÁRIE NA REDUÇÃO DE STREPTOCOCCUS MUTANS E LACTOBACILLUS SPP DA DENTINA CARIADA DE DENTES DECÍDUOS

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ABSTRACT

The methods of caries removal have been the subject of many studies over the last years. At present, methods involving the least tissue invasion have been outstanding in the field of surgical-restorative treatment. In this context, the Carisolv™ system has appeared as a less traumatic and less invasive approach, particularly in pedodontics. Thus, the objective of the present study was to carry out a comparative analysis of the dentinal structure of primary molars before and after the removal of carious tissue by mechanical (low speed drills and conventional dentinal curettes) and chemomechanical (Carisolv™ kit) procedures based on quantitative culture for cariogenic bacteria to determine the number of bacteria present in the carious dentine after both treatments. Sixty primary molars from children ranging in age from 4 to 8 years, with active occlusal caries in dentine, were divided into two groups (A and B) of 30 teeth each, with group A having been treated by the chemomechanical technique and group B by the mechanical technique. Dentin samples were placed in glass vials containing 1 mL thioglycolate broth and submitted to culture to determine the number of *S. mutans* and *Lactobacillus* per mg of decayed dentine. The results did not reveal significant differences between the two methods of caries removal; however, the chemomechanical method was more efficient in completely eliminating *S. mutans* (p=0.02). In summary, the present results confirm previous studies showing that the two methods are comparable in reducing *Lactobacillus*, but Carisolv™ is more effective in the elimination of *S. mutans*.
Uniterms: Dental caries, therapy; Tooth, deciduous; Microbiology.

RESUMO

Os métodos de remoção de cárie têm sido objeto de muitos estudos nos últimos anos. Atualmente, aqueles que determinam mínima invasão tecidual têm se sobressaído no campo do tratamento cirúrgico-restaurador. Neste contexto, surgiu o sistema Carisolv™ como uma abordagem menos traumática, com destaque na odontopediatria e menos invasiva. Desta forma, este estudo teve como objetivo analisar comparativamente a estrutura dentinária de molares decíduos antes e após a remoção do tecido cariado pelos métodos mecânico (brocas de baixa rotação e curetas dentinárias convencionais) e químico-mecânico (Kit Carisolv™), através de exame microbiológico quantitativo, verificando-se o número de bactérias/mg presentes na dentina cariada após cada tratamento. Foram utilizados 60 molares decíduos de crianças na faixa etária de 4 a 8 anos de idade que apresentavam cárie oclusal ativa em dentina, os quais foram divididos em dois grupos (A e B) de 30 dentes cada, onde o grupo A foi tratado com a técnica químico-mecânica e o grupo B foi tratado com a técnica mecânica. As amostras de dentina foram coletadas e depositadas em frascos de vidro contendo 1mL de caldo Tioglicolato e pérolas de vidro. Em seguida, foram imediatamente encaminhadas para análise microbiológica para que fossem determinadas as concentrações de *S. mutans* e *Lactobacillus* por mg de dentina cariada. Os resultados não revelaram diferenças significantes na comparação dos dois métodos de remoção de cárie; contudo, foi verificado que o método químico-mecânico era mais eficiente na eliminação total de *S. mutans* (p=0,02). Em síntese, os nossos resultados confirmam alguns estudos prévios em que os métodos são comparáveis na redução de *Lactobacillus*, mas o Carisolv™ é mais eficaz na eliminação de *S. mutans*.
Unitermos: Cárie dentária, terapia; Dente decíduo; Microbiologia.

INTRODUCTION

Dental caries is still a challenge to dentists who, through knowledge and exact understanding of its etiology, seek more efficient means of controlling it. Although studies about its etiology have revealed that it is a chronic, invasive and infectious process resulting from the interaction of multiple inter-related factors, the destruction of dental structures does not occur without the localized accumulation of oral bacteria on the tooth surface[24].

Among the cariogenic microorganisms, *Streptococcus mutans* and *Lactobacillus* spp are detected in significant quantities in carious dentine that shows a softened, damp appearance [12,22]. In this context, the presence of microbes on the floor of cavities in primary teeth represents a greater risk due to the high dentinal permeability of these teeth, which makes them vulnerable[13,27].

The clinical management of dental caries has evolved over the last decades. In view of the need for invasive treatment through the removal of carious tissue, the current trend is to select the most suitable method, in which opening of the cavity occurs concurrently with removal of carious tissue[5], preserving remaining healthy dentine structures capable of remineralization[1,17,19]. However, it is evident that further studies are needed to evaluate new methods of removing carious tissue, especially those that aim to reduce or eliminate cariogenic bacteria using a less invasive approach. Thus, the objective of this study was to perform a comparative evaluation of the influence of chemomechanical (Carisolv™ kit) and mechanical (low speed drills and conventional dentinal curettes) methods on the reduction of the number of *S. mutans* and *Lactobacillus* spp. resident in occlusal caries cavities in primary molars.

MATERIAL AND METHODS

After approval by the Research Ethics Committee of the University Hospital of UFMA, the study was carried out using the following protocol: students aged 4 to 8 years were selected from the schools "Educandário Santo Antônio", "Lar de José" and Institute of Education and Day Care Maurício José in São Luis, MA. All subjects were healthy, without a history of systemic diseases, hereditary anomalies or use of medicines, and all presented clinical and radiographic characteristics according to the criteria adopted by Brusco[7] and Pinto[21]:

a) Clinical: at least 2 primary molars with active cavitated carious lesions involving the occlusal face, at the dentine level; without extensive coronal destruction (at most ½ of the coronal structure); absence of spontaneous painful symptoms, with possibility of painful sensitivity caused by cold, passing immediately; absence of edema, fistula and tooth mobility not compatible with chronological age.

b) Radiographic: Deep carious lesion, involving at least the internal half of the dentine; absence of pathological exposure due to caries; absence of periapical or inter-radicular alterations and internal or external resorption, which is not compatible with the exfoliation process.

For the final sample, 88 teeth were selected, of which the first 28 were used in a pilot study to determine the methodology and the remaining 60 were divided into two experimental groups of 30 teeth each. In group A, carious tissue was removed by the chemomechanical method (Carisolv™ kit), and in group B, carious tissue was removed by the mechanical method (low speed drills and conventional dentinal curettes).

Mechanical method

All procedures were carried out under local anesthesia using a rubber dam. Prophylaxis of the selected teeth was done with pumice stone and a low speed Robinson brush. After these procedures, when necessary, access to the carious cavity in dentine was obtained with high speed burs (No. 1011 and 1012), removing enamel without dentine support. Otherwise, the cavity was washed directly with sterile saline with the aid of Luer syringes and the excess was aspirated with metal tubes, drying being completed with sterile cotton balls. The procedures described were carried out in both groups. The efficacy of the methods used to remove carious tissue was compared by bacterial quantification before and after both treatment procedures. Therefore, two collections of dentine were made in the caries cavities as described below[26]:

1st collection. The most superficial portion of carious dentine was removed by performing an excavation with low speed burs No. 2 or 4. After irrigation with sterile saline solution and drying with pieces of sterile cotton, the first sample of carious dentine was collected. With the aid of a No. 17 dentinal curette, the carious dentine surface of the mesial portion of the bottom wall of the cavity was removed and placed in a glass flask containing the transport medium thioglycolate broth and glass beads. Next, the carious tissue was removed according to a previously described method[19], in which the carious tissue was completely removed from the surrounding walls according to visual and tactile clinical criteria, and the softened dentinal tissue was partially removed from the pulp wall with the aid of low speed burs (No. 1, 2 and/or 4) and conventional dental curettes. After these procedures, the treated cavity was irrigated with sterile saline and dried with pieces of sterile cotton.

2nd collection. The surface of the remaining dentinal tissue located in the mesial portion of the bottom wall of the cavity was removed with a low speed No. 4 bur. A dentine sample was then obtained with a No. 17 dentinal curette and placed in the glass flask containing the transport medium. The cavity was then washed with saline, dried with pieces of cotton and lined with calcium hydroxide cement, and the tooth was restored with TPH resin.

Chemomechanical method

For the chemomechanical method, the initial stages of the conventional methodology were followed up to collection of the first sample (before treatment) of carious

dentine. Next, the Carisolv™ kit was used according to manufacturer's recommendations (MediTeam Dental AB, Savedalen, Sweden). The process for the use of the Carisolv™ kit was repeated 3 to 5 times, with the cavity being washed with sterile saline between procedures until the caries removal procedure was considered to be complete[19]. When the carious tissue had been removed, the second collection was made in the same way as in group B and the tooth was then restored.

Microbiological analysis

The samples were transported to the Laboratory of Microbiology in glass flasks containing thioglycolate medium and glass beads, and were processed within 2 hours after collection. The dentine samples were submitted to quantitative culture for *Streptococcus* of the *mutans* group and *Lactobacillus* spp.[29]. The flasks were homogenized in a Vortex blender and serial decimal dilutions were prepared in saline. Aliquots of 10 mL of the sample from the thioglycolate broth and from the dilutions in sterile saline solution were inoculated in triplicate into the culture media. Mitis-salivarius agar (Difco, Detroit, MI) supplemented with 0.2 IU/mL bacitracin and 15% sucrose was used for the S. *mutans* count[9] and Rogosa medium (Difco) was used for the *Lactobacillus* spp. count. Both media were incubated at 37°C/48 h under anaerobiosis[29]. Up to 5 colonies were selected from each culture medium and from the selected dilutions, and submitted to biochemical tests to confirm bacterial identification[16]**.** After incubation, the number of colony forming units/mg dentine (CFU/mg) was determined by using the number of colonies in a given dilution, the inverse factor of the dilution selected for the count and the correction factor of the inoculated volume (100).

Statistical analysis

The bacterial counts (CFU + 1) were transformed into decimal logarithm values. The means and the standard deviation were calculated and the mean values compared by the Student t-test. The ability to completely reduce the number of viable bacteria was compared separately for the two groups of bacteria between the two methods of caries treatment by the χ^2 (chi-square) test. The level of significance was set at $p < 0.05$ in all analyses. The data were processed with the BIOESTAT 2.0 program[2].

RESULTS

The mean weight of the dentine samples collected in a pilot study before treatment was 1.35 mg (standard deviation ± 0.157 mg), while the mean weight after treatment was 0.57 mg (standard deviation ± 0.57 mg). The sample weights were corrected to 1 mg in order to express the result in CFU/mg.

Table 1 shows the quantification of *Lactobacillus* in CFU/mg of carious dentine in each tooth before and after treatment. In group A, the selected teeth presented counts ranging from 3.7×10^2 to 2.2×10^7 CFU/mg before treatment with the Carisolv™ kit. After this treatment, the teeth presented counts ranging from 0 to 1.8×10^6 CFU/mg. In group B, the *Lactobacillus* counts ranged from 7.4×10^2 to 2.1×10^7 CFU/mg before treatment by the mechanical method and from 0 to 1.8×10^5 CFU/mg after treatment. Sample 15 of group B was excluded from the statistical analysis for this bacterium, together with the respective paired sample from group A, because it did not present *Lactobacillus* growth before treatment.

Out of 30 teeth treated with Carisolv™, 6 (20%) showed less than 95% reduction of *Lactobacillus* counts (range: 57.4% - 91.2%), whereas of the 29 teeth treated by conventional drilling, 5 (17.2%) revealed bacterial counts of less than 95% (range: 13.6% - 94.4%) (Table 1).

Comparison of the two methods by the Student t-test after transforming the bacterial counts (CFU +1) into decimal logarithm and obtaining the mean values showed that there was no significant difference ($p > 0.05$) for *Lactobacillus*. The same was found using the χ^2 test regarding the capacity of both methods to reduce the *Lactobacillus* counts by 100%.

The *S. mutans* counts in carious dentine samples before and after each treatment are reported in Table 2. Before treatment, group A presented carious dentine counts between 1.1×10^2 and 2.2×10^7 CFU/mg. After treatment, the teeth presented counts that ranged from 0 to 6.1×10^5 CFU/mg. Before treatment, the *S. mutans* counts of the teeth in group B ranged from 7.4×10^1 to 2.2×10^7 CFU/mg, while after treatment the variation in the count ranged from 0 to 3.2×10^4 CFU/mg. Teeth 11 and 12 did not present growth of these bacteria before treatment and were excluded from the statistical evaluation for *S. mutans*. Comparison of the methods by the Student t-test showed that they are comparable, since there were no significant differences between them ($p>0.05$). However, the chemomechanical method was found to be more efficient ($p=0.02$) than the mechanical one when the teeth in which the initial *S. mutans* population presented a 100% reduction after treatment were compared. Of the 27 teeth included in the analysis, 22 no longer presented the bacteria after treatment by the chemomechanical method, while in the mechanical method the elimination of *S. mutans* was observed in 14 teeth (Table 3).

Of these 27 teeth, only 1 (3.7%) showed a bacterial count lower than 95% (80%) after treatment with Carisolv (tooth 25). A similar result was also obtained by the conventional method, since 1 of the 28 teeth (3.6%) showed a count of 92.3% (tooth 29) (Table 2).

DISCUSSION

Studies about carious dentine have indicated the presence of two layers: a more external, infected one, and necessarily removable, and another more internal, presenting as affected, softened, but capable of remaining and being remineralized[17]. In addition, it has been shown that the use

of rotary instruments in the conventional mechanical method most of the time involves the removal of healthy dental tissue. This is not considered satisfactory, because there is an over-reduction of the dentinal tissue softened by the demineralization that precedes the bacterial invasion which would be able to be mineralized. Thus, current knowledge about the process of caries disease development determines changes in its treatment, not only establishing a preventive approach, but looking for less invasive methods to treat infected dentinal tissue[8].

However, a question that should be raised is whether these methods are efficient in reducing or eliminating microbial flora from the tooth cavity. The clinical impact of bacterial persistence in caries-free dentine is not clear, but some authors agree that elevated bacterial counts remaining after a caries removal procedure can be considered clinically significant because they cause further disease progression[12].

In this comparative study, the two methods of caries removal proved to be similar in their capacity of reducing *Lactobacillus* levels, but the chemomechanical method had a more significant effect on the *S. mutans* counts by apparently eliminating this microorganism in many cases. This might be due to the antimicrobial action of chloramines present in Carisolv gel. On the other hand, if this is true one

TABLE 1- *Lactobacillus* counts before and after treatment of carious dentine of primary molars with Carisolv™ and the mechanical method

	***Lactobacillus* CFU/mg of dentine**						
Tooth	**Group A (Carisolv)**		**Reduction (%)**	**Tooth**	**Group B (mechanical)**		**Reduction (%)**
No.	**Before**	**after**		**No.**	**Before**	**after**	
1C	1.5×10^5	0	100	1M	9×10^4	0	100
2C	7.4×10^4	3.5×10^3	95.3	2M	3×10^4	0	100
3C	2.2×10^4	0	100	3M	7.4×10^3	0	100
4C	1.5×10^5	0	100	4M	1.9×10^5	1.8×10^3	99.2
5C	1.6×10^4	0	100	5M	1.1×10^4	0	100
6C	3.1×10^5	0	100	6M	2.2×10^5	0	100
7C	1.5×10^4	0	100	7M	7.4×10^3	2.1×10^3	71.6
8C	2.4×10^5	1.8×10^3	99.3	8M	1.5×10^5	0	100
9C	7.4×10^4	0	100	9M	1.1×10^5	0	100
10C	3.7×10^4	1.8×10^3	95.1	10M	3.2×10^4	1.8×10^3	94.4
11C	7.4×10^4	1.8×10^4	75.7	11M	$7,4 \times 10^4$	1.8×10^2	98.8
12C	2.6×10^5	0	100	12M	1.1×10^5	0	100
13C	4.3×10^6	1.6×10^4	99.6	13M	3.5×10^6	0	100
14C	4.1×10^5	0	100	14M	5.3×10^5	0	100
15C	2.2×10^7	1.8×10^6	-	15M	0	0	-
16C	5.9×10^5	7×10^4	88.1	16M	6.8×10^6	1.8×10^5	97.4
17C	1.1×10^5	0	100	17M	7.4×10^2	0	100
18C	8.5×10^6	0	100	18M	2.7×10^6	0	100
19C	9.2×10^6	2.6×10^4	99.7	19M	8.5×10^6	1.2×10^5	98.6
20C	7.4×10^4	1.8×10^3	97.6	20M	2.1×10^7	1.2×10^5	99.4
21C	5.2×10^6	1.3×10^4	99.8	21M	8.9×10^5	0	100
22C	9.6×10^5	0	100	22M	2.6×10^5	1.8×10^3	99.3
23C	2.3×10^5	9.8×10^4	57.4	23M	3×10^5	0	100
24C	3.1×10^6	5.3×10^5	83.0	24M	8.7×10^6	9.8×10^3	99.9
25C	4.7×10^5	1.8×10^4	96.2	25M	9.6×10^6	0	100
26C	3.9×10^5	0	100	26M	1.6×10^4	1.6×10^4	0
27C	3.7×10^2	0	100	27M	1.1×10^3	0	100
28C	1.5×10^5	0	100	28M	1.1×10^4	1.8×10^3	83.6
29C	4.4×10^4	1.4×10^4	68.2	29M	2×10^6	5.4×10^4	97.3
30C	8.7×10^4	0	100	30M	8.1×10^3	7×10^3	13.6

C = chemomechanical method
M = mechanical method

may question why *Lactobacillus* was not eliminated so significantly by this method. A possible explanation may be related to the evolution of the cariogenic process, which is generally initiated by *S. mutans*, but progression of the disease is associated with other cariogenic bacteria, including *Lactobacillus* spp.[6]. According to van Stripp, et al.[26], *Lactobacillus* spp. are positively correlated with advanced lesions of carious dentine. Thus, in these situations, most *Lactobacillus* bacteria would be protected in an inner location in the carious dentine, and the antimicrobial action of chloramines influences the elimination of this group of microorganisms to a lesser extent than *S. mutans*. The latter microorganism, in turn, would be more exposed to the antimicrobial action of Carisolv.

In contrast, in the mechanical method many microorganisms are removed together with the necrotic dentine, but this does not assure that the excavated tooth is bacteria-free or, at least, that a significant reduction in bacterial colonization has occurred. Furthermore, many tooth samples displayed a less significant level of reduction in *Lactobacillus* counts (<100%) after treatment with both methods, which further supports the hypothesis of protected localization of this microorganism in advanced lesions.

TABLE 2- *S. mutans* counts before and after treatment of carious dentine of primary molars with Carisolv™ and the mechanical method

	***S. mutans* CFU/mg of dentine**						
Tooth	**Group A (Carisolv)**		**Reduction (%)**	**Tooth**	**Group B (mechanical)**		**Reduction (%)**
No.	**Before**	**after**		**No.**	**Before**	**after**	
1C	7.4×10^3	0	100	1M	7.4×10^3	0	100
2C	2.4×10^5	0	100	2M	1.5×10^5	0	98.8
3C	2.6×10^5	0	100	3 M	3×10^5	0	100
4C	2.8×10^6	0	100	4 M	1.6×10^7	1.8×10^4	99.9
5C	4.4×10^5	0	100	5M	7.4×10^4	0	100
6C	2.5×10^5	0	100	6 M	1.5×10^5	0	100
7C	1.7×10^5	0	100	7M	7.4×10^5	3.5×10^3	99.5
8C	7.4×10^4	0	100	8M	1.3×10^5	1.8×10^3	98.6
9C	2.6×10^4	0	100	9M	5.6×10^4	0	100
10C	1.7×10^4	0	100	10M	4.1×10^4	0	100
11C	0	0	-	11M	0	0	-
12C	0	0	-	12M	0	0	-
13C	7.4×10^5	2.3×10^4	96.9	13M	3.7×10^6	0	100
14C	4.1×10^5	0	100	14M	5.5×10^5	0	100
15C	2.2×10^5	0	100	15M	1.5×10^3	0	100
16C	1.1×10^2	0	100	16M	1.7×10^4	3.5×10^3	99.9
17C	1.9×10^5	0	100	17M	7.4×10^1	0	100
18C	0	0	-	18M	8.9×10^2	0	-
19C	6.7×10^5	0	100	19M	2.4×10^6	8.8×10^3	99.6
20C	1.6×10^6	0	100	20M	1×10^6	1.2×10^4	98.8
21C	5×10^5	7.9×10^3	98.4	21M	3×10^5	0	100
22C	1.5×10^5	0	100	22M	5.2×10^5	1.8×10^4	96.5
23C	2.2×10^7	6.1×10^5	97.2	23M	1×10^7	1.8×10^3	99.9
24C	1.5×10^4	0	100	24M	1.3×10^4	0	100
25C	9×10^5	1.8×10^5	80	25M	3.3×10^5	1.8×10^2	99.9
26C	1.6×10^4	0	100	26M	2.2×10^7	1.3×10^4	99.9
27C	3.7×10^2	0	100	27M	1.1×10^2	0	100
28C	6.4×10^5	0	100	28M	9×10^3	0	100
29C	2.3×10^6	0	100	29M	8.9×10^4	7×10^3	92.3
30C	5.1×10^6	2.4×10^4	99.5	30M	4.3×10^6	3.2×10^4	99.3

C = chemomechanical method
M = mechanical method

Some authors have compared the efficacy of Carisolv™ with the mechanical method in removing cariogenic microbiota; however, the results of the studies are controversial and cannot be considered to be conclusive because of some methodological limitations. In this context, Yazici, et al.[28] observed that conventional drilling was more efficient than Carisolv™ in removing caries. However, evaluation of the efficacy was performed by microscopic observations of bacteria in tooth sections, which does not permit to state that the microorganisms observed after treatment with Carisolv™ were *Lactobacillus*, *S. mutans* or other bacteria, or even whether the observed bacteria are viable for growth.

On the other hand, other authors have demonstrated that Carisolv™ can be an efficient alternative method compared to drilling to remove bacteria, even though there are also limitations in the methods used in the evaluation. According to Larger, et al.[14], Carisolv™ is capable of promoting a significant reduction in bacterial growth in Mitis Salivarius agar, a selective culture medium for *S. mutans*, but not in Rogosa agar, a selective culture medium for *Lactobacillus*. Even though the culture media are selective for each of the two bacteria, the authors did not perform biochemical identification of some bacterial colonies to confirm their findings, a fact that may have influenced the results of their study. Similar data were also obtained by Okida[18] in the analysis of the remaining dentine after the removal of carious dentine. These investigators found a larger amount of bacteria in the group treated by the mechanical method than in that one treated by the chemomechanical method.

Recently, Azrak, et al.[3] verified that Carisolv is comparable to the conventional method regarding the action on *Lactobacillus*. However, the authors did not include *S. mutans* in the study, a microorganism that is frequently found in early carious lesions. In our study, bacterial isolates were identified by biochemical tests, which supported our conclusion that Carisolv™ is at least as efficient as the conventional method of caries removal regarding the potential of cariogenic bacteria reduction. In general, our data are in good agreement with those obtained by Brusco[7] who also found few bacteria in the dentine after using the same methods for removal of carious dentine. However, in our case, Carisolv™ was more efficient in the complete removal of *S. mutans* than the conventional method.

Although both methods are comparable in reducing the bacterial population from the excavated cavity, the chemomechanical method has other advantages also observed by us, such as selective removal of the carious dentine[23]; applicability to child care[20], with the children attended remaining relaxed and at ease and some even falling asleep during the operating procedure; a painless method[10,15], and consequently a decrease in the use of anesthesia[4, 7, 11]. Furthermore, during the operation with the chemomechanical method, none of the children reported any sensitivity.

CONCLUSIONS

In conclusion, the results of this study indicate that:

1. The chemomechanical (Carisolv™ kit) and mechanical methods of caries removal are comparable in the reduction of the population density of *S. mutans* and *Lactobacillus* spp. in the carious dentine of primary molars.

2. The chemomechanical method is more efficient in the total elimination of *S. mutans* from the carious dentine of primary molars than the mechanical method of caries removal.

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TABLE 3- Reduction of *S. mutans* and *Lactobacillus* in carious dentine of primary molars after treatment with Carisolv and by the mechanical method of caries removal

METHOD	S. mutans reduction * N = 27 teeth		Lactobacillus reduction ** N = 29 teeth	
	less than 100% N (%)	equal to 100% N (%)	less than 100% N (%)	equal to 100% N (%)
Carisolv	5 (18.5)	22 (81.4)	13 (44.8)	16 (55.1)
Mechanical	13 (48.1)	14 (51.8)	13 (44.8)	16 (55.1)

*$\chi^2 = 5.33$, $p = 0.02$
**$\chi^2 = 0$, $p = 1$

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