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EFFECTIVENESS OF DISINFECTING AGENTS IN IMPRESSIONS WITH IRREVERSIBLE HYDROCOLLOID

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ABSTRACT

The purpose: was to determine the effectiveness of two disinfecting agents in impressions with irreversible hydrocolloids. **Methods and Materials:** 10 students of Autonomous University of Tamaulipas-Dentristy Faculty were selected, after the nature of the study was explained and signed the consent form, it was proceeded to obtain dental impressions with irreversible hydrocolloids (Phase Plus®, Zhermak) in the upper arch (n=10). The dental impressions were sectioned and placed in Petri dishes with Trypticasein Soy Broth as culture media. Afterwards, a sample was taken to isolate identify and make bacterial stock and then was divided into three groups: Group A: 0.5ml of bacterial stock + 0.5ml of Saline Solution (PiSA®) Group B: 0.5ml of bacterial stock + 0.5ml Etanol + 2-propanol (Z 7 Spray® Zhermak) Group C: 0.5ml of bacterial stock + 0.5ml of *Morinda Citrifolia, incubation time was* two minutes and was inoculated in culture media, and counted after 24 hrs of incubation. **Results:** A significant relevance of the antimicrobial factor agent (p=<0.0001) was identified. The control group presented the highest values in relation with the bacterial count (media=4.69 ± 0.65 Log10UFCxml) followed by the *Morinda citrifolia* group (media=2.25± 1.76 Log10UFCxml) and the Etanol + 2-propanol group reported a minor growth (media=1.03± 1.93Log10UFCxml). The bacteria that was isolated more times was the Staphylococcus aureus (30%). **Conclusion:** The *Morinda citrifolia* and the Di-ethanol decreased the bacterial growth in irreversible hydrocolloids.

Keywords: Disinfection, Impression, irreversible hydrocolloids, *Morinda citrifolia*, Ethanol.

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

INTRODUCTION

The disinfection systems have to be as simple as possible and effective against most microorganisms, without altering the final result of the dental impression, nor causing volumetric variations or reacting during emptying (Díaz and Cols., 2007). The American Dental Association (Fan, 1996) and the Centers for Disease Control and Prevention have suggested methods to disinfect dental impressions, including immersion or spraying with a disinfectant. For the irreversible hydrocolloid that tends to absorb saliva and blood, the immersion system is preferable, since it ensures the coverage of the entire surface of the dental impression, however, this technique can affect the reproduction of the detail in the dental impression and the superficial hardness of the dental impression. The plasters (Poulos and Antonoff, 1997, Jones and Cols., 1990, Drennon and Cols, 1989, Fan, 1991). Most disinfectants used for spraying and immersion techniques are irritating and, therefore, caution should be exercised when inhaling vapors from disinfectants, especially with their concentrations and their working time (Jones and Cols., 1990; Taylor and Cols., 2002; Tan and Cols., 1993). It has been described that dental impressions that do not receive treatment with a disinfectant present a higher level of contamination (Flanagan and Cols., 1998), while the washing of printing materials with sterile water eliminates 40% of the bacteria (Al Jabrah and Cols. 2007); with reference to dental impressions with irreversible hydrocolloid mixed with the extract of *Morinda citrifolia*, far fewer microorganisms have been found than when only saline was used and it was further demonstrated that the surface characteristics as well as the dimensional stability of the resulting gypsum do not show statistically significant differences (Ahmed and Cols., 2015).

The *Morinda citrifolia* has been used in the disinfection of dentinal tubules infected with Enterococcus faecalis finding a decrease in the number of colony forming units, it has also been compared with chlorhexidine against the same bacteria, obtaining an effectiveness of between 69% to 86%. , 02% (Kandaswamy and Cols., 2010; Bhardwaj and Cols., 2012); It has also been described that *Morinda citrifolia* has difficulty inhibiting the growth of certain bacteria such as *Staphylococcus aureus, Salmonella typhi* and *Mycobacterium phle* (Atkinson, 1956). In this study, we report the antibacterial effect of *Morinda citrifolia* on dental impressions with irreversible hydrocolloid.

MATERIALS AND METHODS

Using an analytical balance (FX-200I®, AND), lighter (FISHER®), Petri dishes (PYREX®), and 1000ml beakers (PYREX®), and culture media; a) Muller-Hinton (Bioxon®), b) Blood agar base (Bioxon®), c) tripticasein and soy broth. We proceeded to weigh, dissolve and sterilize the culture media at the universal conditions

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

121ºC, 15lbs / 15min, finally it was emptied in Petri dishes and already gelled and stored in refrigeration (Mabe®) until its use. Subsequently, we proceeded to the selection of the 10 patients who met the inclusion criteria and agreed to sign the consent form of the study. All of them performed their brushing 2 hours before the study using the Stillman technique, proceeding to the dental impression of the upper arch with irreversible hydrolocoloid (Phase PLUS® Zhermak), following the manufacturer's instructions. Once the dental impressions were obtained, they were cut in half, using sterile healing tweezers (Tbs®) to remove the dental impression material from the spoon, cutting with sterile scissors.

Upon obtaining the samples they were placed in sterile petri dishes and 20ml of trypticasein broth was added, then incubated at 37 ° C (Shel-lab® model 1510E) for 24 hours and a sample was taken with a sterile swab, seeded on blood agar to start its first isolation, obtaining an aliquot to carry out the bacterial identification and the Gram coloration.

Taking a sample of the bacteria isolated on blood agar, the bacterial stock was prepared in sterile saline at 0.85% in a 16X150 tube. The assay was divided into 3 groups of 10 samples each; group A (control), 0.5ml of bacterial suspension + 0.5ml of saline, group B, 0.5ml of bacterial suspension + 0.5ml of Ethanol + 2-propanol (Z 7 spray® Zhermak) and group C, 0.5ml suspension bacterial + 0.5ml of the extract of *Morinda citrifolia*, incubated for 2 minutes at room temperature and taking 25 micro liters of each group with the pipette (Transferpette® BRAND) were deposited on the blood agar plate; with the help of a glass they were seeded evenly. The same procedure was carried out for Muller-Hinton agar, they were incubated for 24 hours at 37ºC and at the end of the incubation time their bacterial count was carried out.

STATISTIC ANALYSIS

For the exploratory analysis of the qualitative variables, its distribution of frequencies and percentages was obtained. With respect to the quantitative variable (Colony forming unit), the logarithmic transformation was applied in base 10 to obtain descriptive statistics of centrality and variation. In the absence of compliance with the parametric assumptions, normality and homogeneity of variances (Kolmogorov-Smirnov test and Levene test). We opted for the Kruskal-Walis test to contrast the study groups with respect to their bacterial count. Subsequently, multiple post hoc comparisons were carried out using the Mann-Whitney test, adjusting the p-value with the Bonferroni method. The tests were managed at an alpha value of 0.05 using the IBM SPSS statistics 23 statistical program.

RESULTS

Of the total dental impressions with hydrocolloids used for the study, the bacterial species found in them were

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

analyzed statistically in both number and gender.

In Table 1, the distribution of frequencies and percentages of the bacteria identified according to their morphology is shown. (Fig. 1) (Fig. 2).

Table 1: Distribution of frequencies and percentages of bacterial morphologies.

Morphology	Frequency	Percentage
Cocci	7	70.0%
Bacilli	3	30.0%
Total	10	100.0%

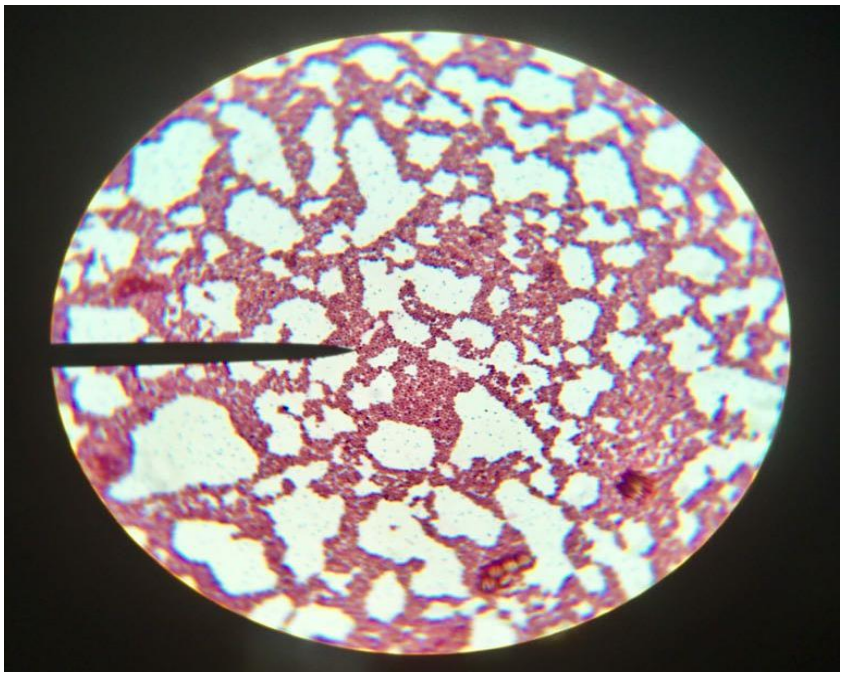

Fig. 1: Microscopic observation of Gram positive cocci. 100x

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

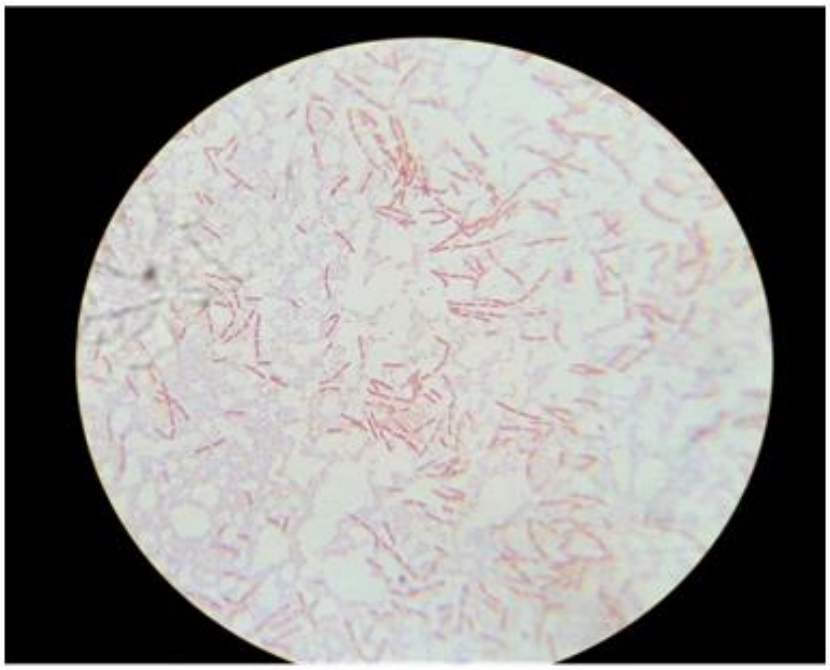

Fig. 2: Microscopic observation of Gram negative bacilli. 100x

Likewise, we proceeded to the bacterial identification, noting that the isolated bacteria most frequently presented were *Staphylococcus aureus* in the case of bacteria with coccus morphology and *Bacillus spp* in bacteria with bacillus morphology. (Table 2)

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

Table 2: Distribution of frequencies and percentages of the morphology of cocci and bacilli.

Bacterias		n	%
cocci	*Staphylococcus aureus*	3	42.8%
	Staphylococcus haemolyticus	1	14.3%
	Streptococcus pseudoporcinus	1	14.3%
	Enterococcus casseliflavus	1	14.3%
	Staphylococcus hominis ssp	1	14.3%
	Total	***7***	***100.0%***
Bacilli	*Bacillus spp*	2	66.7%
	Pseudomonas aeruginosa	1	33.3%
	Total	***3***	***100.0%***

Bacterial count

In accordance with the values of the bacterial count, for which the logarithmic transformation was carried out on base 10, an exploratory analysis was carried out, stratifying the evaluations in a general way into samples with bacteria in the form of coccus and samples with bacteria in the form of bacillus. Identifying a significant relevance of the antimicrobial agent factor ($p < 0.001$). The control group presented the highest values in relation to the bacterial count, followed by the *Morinda citrifolia* group, with the Ethanol + 2-propanol group (Zeta 7 Spray) reporting the lowest growth. Therefore, when carrying out multiple post-hoc comparisons, it was detected that the control group presented a statistically significant difference between the experimental groups ($p < 0.01$). (Table 3) (Fig. 3).

On the other hand, the sample was stratified associating the account with the bacterial morphology.

When analyzing the samples identified with coccus morphology, a significant relevance of the antimicrobial agent factor was found ($p = 0.001$). The control group showed the

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

highest bacterial growth followed by the *Morinda citrifolia* group and the Ethanol + 2-propanol group (Zeta 7 Spray) showed higher inhibition than the groups. (Table 3) (Fig. 4)

In the case of bacilli, when contrasting the antimicrobial agent as a factor, no significant relevance was presented (p = 0.565). The control group, Ethanol + 2-propanol (Zeta 7 Spray) and *Morinda citrifolia*. (Table 3) (Fig. 4)

Table 3: Evaluación general de los agentes antibacterianos. (Log10UFC/ml)

	Grupos	**Estadístico**					
		Media	**Mediana**	**D. E.**	**Mín.**	**Máx.**	**Valor p**
Total	Control	4.69	4.48	0.65	3.92	5.6	
	Zeta 7 Spray	1.03	0	1.93	0	5.56	*0.001*
n = 10	*morinda citrifolia*	2.25	1.93	1.76	0	5.53	
Cocos	Control	4.61	4.46	0.64	3.92	5.58	
	Zeta 7 Spray	0.15	0	0.28	0	0.6	*< 0.0001*
n = 8	*morinda citrifolia*	1.71	1.77	1.41	0	4.13	
Bacilos	Control	5.02	5.05	0.83	4.43	5.6	
	Zeta 7 Spray	4.54	4.59	1.45	3.52	5.56	*0.565*
n = 2	*morinda citrifolia*	4.37	4.41	1.63	3.22	5.53	

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

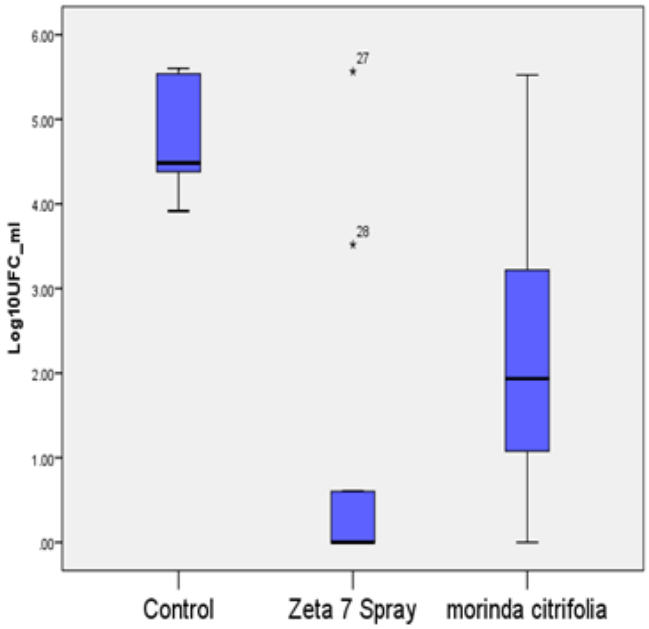

Fig. 3: Efficacy of antimicrobial agents in general.

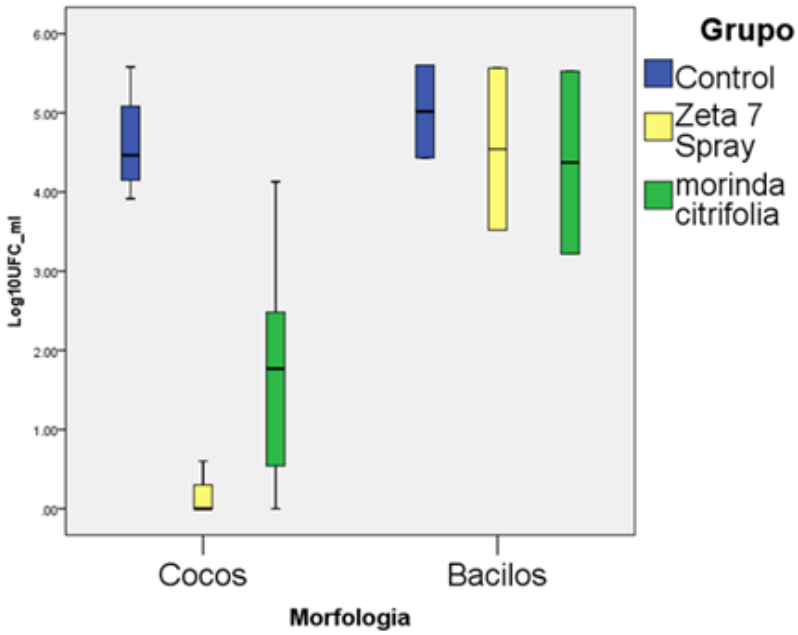

Fig. 4: Evaluation of bacterial agents according to their morphology

ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

DISCUSSION

The dental impressions are one of the tools that are commonly used in the area of oral rehabilitation since they allow to visualize the structures to be restored and thus make an adequate treatment plan, however, one of its disadvantages is the bacterial growth that occurs in the instruments that come into contact with the patient, since once the dental impression is taken saliva, blood, detritus, oral bacteria, fungi and viruses remain on the surface of the dental impressions, for which, the American Dental Association (Fan, 1996) recommends the use of disinfectants and antiseptics.

In another study (Al-Jabrah and Cols., 2007) they report that the dental impressions of alginate when treated with some type of disinfectant transport less microorganisms than those that do not receive any treatment with disinfectant ($p < 0.05$), reporting that the bacteria that Commonly found in the mouth and causing pathologies are: *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Candida albicans, Actinomyces viscosus* (Jafari and Cols., 2013).

In the present study, in dental impressions with irreversible hydrocolloid, bacteria such as *Staphylococcus aureus* were identified in 30%, *Staphylococcus haemolyticus* in 10%, *Bacillus spp* in 20%, *Streptococcus pseudoporcinus* in 10%, *Pseudomonas aeruginosa* in 10%, *Enterococcus casseliflavus* in 10% and *Staphylococcus hominis ssp hominis* in 10%.

Regarding the bacterial count, it was demonstrated that the alginate specimens that were treated with *Morinda citrifolia* showed a decrease of the microorganisms compared to those that were treated with saline solution, obtaining a p value <0.01; in this way we agree with the results of (Ahmed and Cols., 2015) who mentioned that using the juice of the *Morinda citrifolia* with the alginate found a smaller amount of microorganisms compared with those that used saline solution, in addition they did not find modifications in the surface characteristics and the dimensional stability of the plaster

On the other hand, (Atkinson, 1956) using bacteria such as Staphylococcus aureus and disinfectants such as *Morinda citrifolia* reported that it did not find any inhibition of bacterial growth, we did not agree with Dr. Atkinson since we found a marked decrease in bacterial growth, said The difference could be due to the fact that Atkinson used parts of the plant, while in our research we used fruit juice, whose antibacterial effect is produced by its phenolic compounds that contain fruit such as acubin, alizarin, scopoletin and other anthraquinones. dissolve the polar and non-polar components (Zhang and Cols., 2016). On the other hand, we must remember that there are strains of Staphylococcus aureus that are more

resistant to antibacterials than others such as MRSA (Meticillin Resistant *Staphylococcus aureus*) (*Staphylococcus aureus* resident to methicillin) which are resistant to penicillins, cephalosporins, ami nopenicillins associated with inhibitors of beta-lactamases, carbapenems as well as the macrolides azalides erythromycin, clarithromycin (Tamariz and Cols., 2010).

CONCLUSIONS

From the development of the present study we can conclude the following:

1. *Morinda citrifolia* and Ethanol + 2-propanol decreased bacterial growth on irreversible hydrocolloids ($p < 0.001$).

2. There was a statistically significant difference between the disinfectants Ethanol + 2-propanol (Zeta 7 Spray) and *Morinda citrifolia* in alginate specimens, with greater efficacy in bacteria with coccus morphology.

3. Disinfection of the specimens with alginate exposed to *Bacillus spp* bacteria was ineffective when using Ethanol + 2-propanol (Zeta 7 Spray) and *Morinda citrifolia*.

4. Ethanol + 2-propanol (Zeta 7 Spray) was the one that obtained the highest efficiency in the disinfection of dental impressions with alginate.

5. The values of the colony forming unit (FMU) of the alginate specimens treated with *Morinda citrifolia* were lower than those treated with saline.

6. The highest bacterial growth occurred in specimens treated with saline.

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ISSN: 2456-1851

Volume:03, Issue:01 "January-February 2018"

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