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COMPARATIVE STUDY IN-VITRO ON THE EFFICACY OF GARLIC *(Allium sativum)* AS AN ANTIMICROBIAL AGENT ON *Staphylococcus aureus* AND *Escherichia coli*

Carlos David Quiroz Guerrero[1], Violeta Cecilia Tinoco Cabriales[2], Bertha Luna García[2], Yadira Barreto Orta[2], Rogelio Oliver Parra[2], Mario Alberto Palomares Rodríguez[2]

[1]Graduate in Dental Surgeon, M.D, of the Dentistry Faculty, Autonomous University of Tamaulipas.

[2]Professor of the Dentistry Faculty, Autonomous University of Tamaulipas.

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ABSTRACT

The purpose of this study was to determine and compare the antimicrobial effectiveness of garlic extract *(Allium sativum)* by the Agar method on *Staphyloccoccus aureus* and *Escherichia coli*. Materials and Methods: The bacterias used were *Staphylococcus aureus* and *Escherichia coli*, which were previously purified and identified, then the bacterial population was standardized to tube No. 1 of the Mcfarland nephelometer. We made preparations of the extract of garlic and sodium hypochlorite using sterile 0.9% saline solution, chlorhexidine was used at 0.12% and sterile saline was used as a negative control. Afterwards, both bacterias were seeded in Petri dishes and the sensidiscs were impregnated with 15 ul (microliters) of each of the substances to be studied using the BRAND Transferpette® plunger pipette and were placed in a spaced form inside the Petri dish. Were incubated at 37ºC for 24 hrs and the growth inhibition were observed and reported in mm. Results: A similar effect was observed for *S. aureus* between 5.40% sodium hypochlorite and 100% garlic with a mean of 34 mm and 38 mm inhibition halo, while Sodium Hypochlorite 2.7 %, Chlorhexidine at 0.12% and garlic at 50% obtained means of 20 mm respectively. However for *E.coli*, the garlic in both concentrations as 100% as 50% got it mean higher than Sodium Hypochlorite same as Chlorhexidine. Conclusions: In *Saureus*, the most effective bactericie substance was sodium hypochlorite at 5.40% followed by 100% garlic over the others, while 100% garlic predominated in *E.coli*.

Keywords: Garlic, *Allium sativum,* Antimicrobial, Chlorhexidine, Sodium hypochlorite.

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INTRODUCTION

There are numerous effective chemical agents that help control the amount of bacteria present in the mouth, which act under different mechanisms of action, inhibiting microbial proliferation, of which the most used in Dentistry are undoubtedly Chlorhexidine and Hypochlorite Sodium (1). Currently, an attempt is being made to explore other alternatives such as the use of herbal and ethnobotanical medicine, to solve some ailments, although alternative treatments do not try to replace existing drugs, but rather try to optimize the antimicrobial effect, that is, using both allopathy and alternative medicine together. A substance to consider highly studied in the pharmaceutical field is garlic extract (Allium sativum) for its healing, antimicrobial and antioxidant properties (2). There is research that shows the effectiveness of garlic in all its properties described above, thanks to its main active component, which is allicin, which is obtained during the process of breaking or crushing garlic. Allicin is the conversion of alliin when it is catalysed by the enzyme alliinase (3). The present study seeks to deepen the importance of herbal medicine as an antimicrobial alternative to optimize allopathic treatment, using garlic extract and thus evaluate its efficacy by comparing it with chemical agents used in Dentistry such as Chlorhexidine and Sodium Hypochlorite.

MATERIALS AND METHODS

The bacteria used were *Staphylococcus aureus* and *Escherichia coli*, which were previously purified and identified, but to corroborate this, a Gram stain was performed, observing Gram-positive cocci for *Staphylococcus aureus* and Gram-negative bacilli for *Escherichia*.

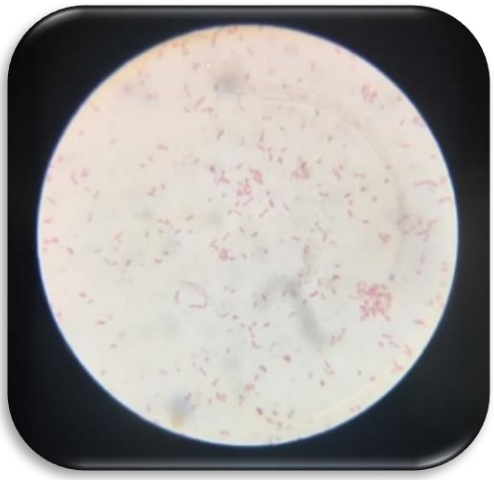

Fig. 1: Escherichia coli viewed under the microscope at 100x with oil immersion.

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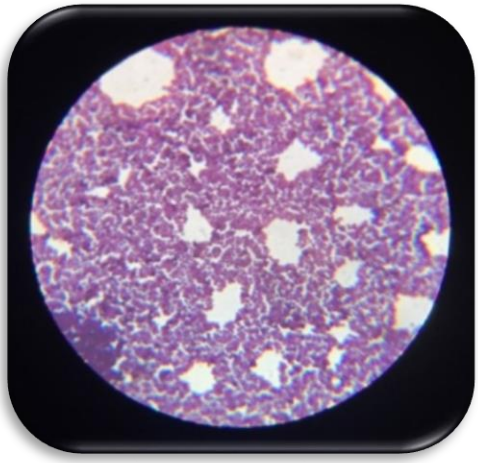

Fig. 2: Staphylococcus aureus viewed under the microscope at 100x with oil immersion.

Each bacterium was standardized to the concentration of tube number 1 of the Mcfarland nephelometer, which was prepared using a sterile 16 x150 tube with a black screw cap which contained 5 ml of sterile 0.9% saline solution. With the sterile bacteriological loop, the bacteria were taken from each corresponding culture, that is, *Escherichia coli and Staphylococcus aureus*, and they were introduced into each tube, mixing with the help of the Vortex; once prepared, the bacteria were seeded with sterile swabs in sterile Petri dishes containing Mueller Hinton Agar, all within the perimeter of sterility provided by the Fisher lighter. Dilutions were made in 10x75 PYREX® test tubes using saline to obtain concentrations of both 50% garlic and 2.7% for Sodium Hypochlorite, mixing 500 microliters of saline solution and 500 microliters of garlic extract and also Sodium Hypochlorite; the concentrations used for this study were 100 and 50% of the garlic extract, 5.40% and 2.7% of the Sodium Hypochlorite and direct Chlorhexidine at 0.12% sterile saline was used as a negative control.

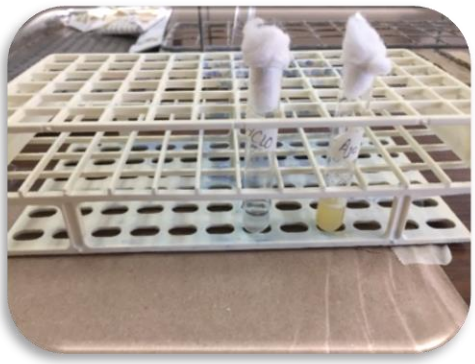

Fig. 3: 50% dilutions of garlic extract (Allium sativum) and Sodium Hypochlorite with 0.9% CS PiSA® saline solution.

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The previously cut and sterilized sensidiscs were loaded with the help of the BRAND Transferpette plunger pipette with 15 microliters for both the 100% garlic concentration, 5.40% Sodium Hypochlorite and 0.12% direct Chlorhexidine, as well as for the 50% concentrations. garlic and 2.7% Sodium Hypochlorite were subsequently placed strategically with the help of sterile straight forceps, leaving enough space for the formation of the inhibition halo in the Petri dishes; once placed, the Petri dishes were closed, labeled and incubated for 24 hours at 37ºC.

Fig. 4: Loading the different concentrations to the sensidiscs.

Subsequently, they were removed from the incubator to observe and measure the inhibition halo that was formed, the measurement of the halo was carried out with the help of a metal millimeter ruler.

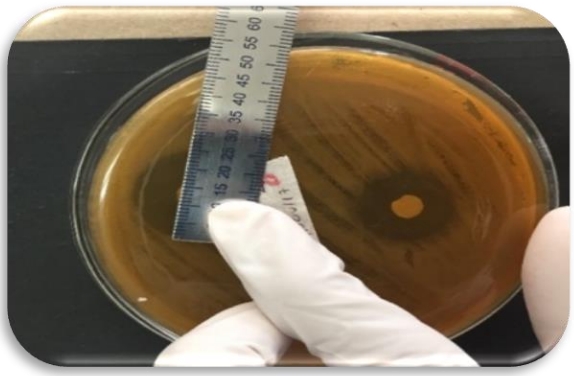

Fig. 5: Measurement of the inhibition halos of the antimicrobial agents used.

RESULTS

The study consisted of 30 samples, which were segmented into 6 groups. The Control Group (Saline Solution) was compared with the 5.40% Hypochlorite, 2.7% Hypochlorite, 0.12% Chlorhexidine Di gluconate, 50% Garlic and 100% Garlic, this in order to determine their

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antimicrobial effectiveness. Gram (+) bacteria When measuring the inhibition halos of Gram + bacteria to evaluate the antimicrobial effect in the six study groups, a statistically significant difference (p <0.05) was observed, where the inhibition values of the Saline, Hypochlorite 2.7 group % and Garlic at 50% remained constant with 0.00 ± 0.00mm, 20.00 ± 0.00mm and 20.00 ± 0.00mm, respectively, while Hypochlorite at 5.40% reported an average of 38 ± 2.74mm, Di Chlorhexidine Gluconate of 20.20 ± 0.45 and 100% Garlic of 34.00 ± 4.18mm. (Table 1).

Table 1: Descriptive of antimicrobial effectiveness in Gram + bacteria.

Grupo		Estadístico							Valor p
		Media	95% de intervalo de confianza para la media		Mediana	Desviación estándar	Mínimo	Máximo	
			Límite inferior	Límite superior					
Gram (+)	Solución Salina*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.0001*
	Hipoclorito al 5.40%	38.00	34.60	41.40	40.00	2.74	35.00	40.00	
	Hipoclorito al 2.7%*	20.00	20.00	20.00	20.00	0.00	20.00	20.00	
	Clorhexidina	20.20	19.64	20.76	20.00	0.45	20.00	21.00	
	Ajo al 100%	34.00	28.81	39.19	35.00	4.18	30.00	40.00	
	Ajo al 50%*	20.00	20.00	20.00	20.00	0.00	20.00	20.00	

Gram bacteria (-)

On the other hand, in the same way, Gram (-) bacteria were analyzed after the application of saline solution, 5.40% hypochlorite, 2.7% hypochlorite, Di Chlorhexidine gluconate 0.12%, Garlic 100% and Garlic 50%, observing a significant difference in the six study groups regarding their antimicrobial effectiveness (p <0.05). (Table 2).

Table 2: Descriptive of antimicrobial effectiveness in Gram (-) bacteria.

Grupo		Estadístico							Valor p
		Media	95% de intervalo de confianza para la media		Mediana	Desviación estándar	Mínimo	Máximo	
			Límite inferior	Límite superior					
Gram (-)	Solución Salina*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	*0.0001*
	Hipoclorito al 5.40%	21.20	19.84	22.56	22.00	1.10	20.00	22.00	
	Hipoclorito al 2.7%	15.80	14.44	17.16	15.00	1.10	15.00	17.00	
	Clorhexidina	13.80	11.76	15.84	15.00	1.64	12.00	15.00	
	Ajo al 100%	24.40	22.73	26.07	25.00	1.34	22.00	25.00	
	Ajo al 50%	20.20	18.84	21.56	20.00	1.10	19.00	22.00	

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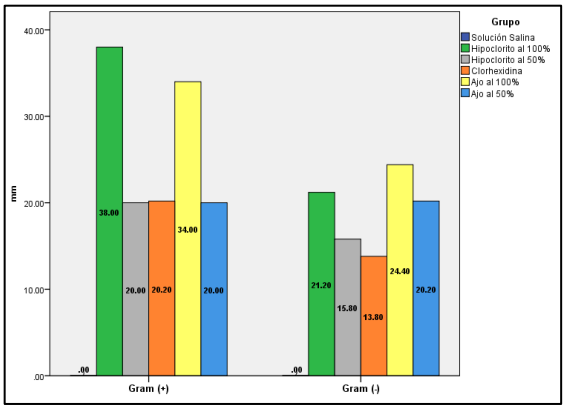
Therefore, when carrying out multiple post hoc comparisons, it was detected that the control group presented a significant difference between the experimental groups in both Gram (+) and Gram (-) bacteria (p <0.05). (Table 3)

Table 3: Multiple comparison in Gram + and Gram- bacteria.

Comparaciones Múltiples			
Grupo		Valor p	
		Gram(+)	Gram(-)
Solución Salina	Hipoclorito al 5.40%	.008	.008
	Hipoclorito al 2.7%	.008	.008
	Clorhexidina	.008	.008
	Ajo al 100%	.008	.008
	Ajo al 50%	.008	.008
Hipoclorito al 5.40%	Hipoclorito al 2.7%	.008	.008
	Clorhexidina	.008	.008
	Ajo al 100%	.151	.016
	Ajo al 50%	.008	.222
Hipoclorito al 2.7%	Clorhexidina	.690	.095
	Ajo al 100%	.008	.008
	Ajo al 50%	.999	.008
Clorhexidina	Ajo al 100%	.008	.008
	Ajo al 50%	.690	.008
Ajo al 100%	Ajo al 50%	.008	.008

Finally, Graph 1 shows the general comparison of the six study groups regarding their antimicrobial effectiveness in both Gram (+) and Gram (-) bacteria, observing that in In the case of Gram (+), it was 5.40% Hypochlorite who reported the highest effectiveness, followed by 100% garlic, while 100% Garlic was the most effective in Gram (-) bacteria.



Graph 1: General comparison of the antimicrobial effectiveness of the study groups.

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DISCUSSION

In our study, we used the Staphylococcus aureus bacteria, finding a very similar effect between 5.40% Sodium Hypochlorite and 100% garlic, while 2.7% Sodium Hypochlorite, 0.12% direct Chlorhexidine and garlic at 50% they had a mean of 20 mm, thus achieving a statistical significance of ($p < 0.05$). (Table 1). Reviewing the literature, we find that it has been demonstrated by different authors (4,5) that Staphyloccocus aureus cultures are sensitive to the antimicrobial action of garlic extract. Regarding the Gram-negative bacteria Escherichia coli, both 100% and 50% garlic obtained a higher average than Sodium Hypochlorite at 5.40% and 2.7% respectively, as well as Chlorhexidine at 0.12%, yielding a statistical significance de ($p < 0.05$) (Table 2).

Our data on Escherichia coli coincide that the garlic suspensions (Allium sativum) that they had prepared presented an inhibitory effect on this bacterium (6,7). On the other hand, it has already been cited (8,9) who in their research demonstrated that the antimicrobial spectrum of Chlorhexidine is very broad for both Gram positive and Gram-negative bacteria. Sodium Hypochlorite has been used for decades as an antiseptic and disinfectant. In our study we verified its bactericidal effectiveness on bacteria such as S. aureus and E. coli, as the results demonstrated this.

For this reason, we agree with Anderson (10) who corroborated the effectiveness of Sodium Hypochlorite by demonstrating that the chlorinated compounds were bactericidal for Escherichia coli and Salmonella typhimurium. Furthermore, isolated Gram-positive bacteria, including Staphylococcus cohnni, Staphylococccusaureus and Staphylococcus haemolyticus and verified the effectiveness of Sodium Hypochlorite when using it as a bactericidal agent on these bacteria. It is important to remember that Staphylococcus aureus is capable of producing oral infections including angular cheilitis, endodontic infections, mumps and more frequently a form of oral mucositis in older people. in immunosuppressed patients and with bone marrow transplantation (11,12).

On the other hand, Thuler (13) have shown a marked increase in the virulence of oxacillin resistant Staphyloccocus aureus among cancer patients. Finally, it is convenient to point out to the strains of Staphylococcus aureus associated to the community which are resistant to methicillin (CA-MRSA), since it has been demonstrated by Vandenesch (14) their wide dissemination in severe diseases generally correlates with strains presenting Panton-Valentine leukocidin (PVL). Such toxin is strongly associated with necrotizing pneumonia and skin and soft tissue infections; the ability to cause necrosis has been linked to the lysis of neutrophils, which are the primary target of Panton-Valentine leukocidin (PVL).

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As for Escherichia Coli, contamination by this bacterium has been found, a consequence of the prolonged stagnation of the water due to the periods of inactivity of the clinics during the holidays, which contributes greatly to the increase in contamination by total coliform bacteria and fecal coliforms, among which Escherichia coli is mainly found.

In this regard Cazar (15) reported in their research a colonization of the maxillary sinus by Escherichia coli which can easily cause infections and put the patient's health at risk, since it comes into direct contact with the water used by the clinician, representing sinusitis of dental origin in 10 to 25% of cases. As dental sources of infection to the maxillary sinus, periapical abscesses, periodontal lesions, teeth or dental materials present in the maxillary sinus have been considered, the most common accidents also occur in the endodontic area due to root perforations, instrument fractures and extrusion of gutta-percha cones towards the maxillary sinus. Similarly, Montes (16) reported in their study the presence of this bacterium in dental root canals.

It should be mentioned that garlic has bactericidal and virucidal capacity and, given the severity of the infection by the coronavirus disease 2019 (COVID-19), the manifestations vary from an asymptomatic disease to a severe acute respiratory infection. Fever, dry cough, dyspnea, myalgia, fatigue, loss of appetite, olfactory and taste dysfunctions are the most prevalent general symptoms. Decreased immune system cells, such as suppressed regulatory T cells, cytotoxic and helper T cells, natural killer cells, monocytes / macrophages, and increased cytokines proinflammatory are the characteristic features. Compounds derived from Allium sativum (garlic) have the potential to decrease the expression of pro-inflammatory cytokines and reverse immunological abnormalities to more acceptable levels. Allium sativum is suggested as a beneficial preventive measure before becoming infected with the SARS - CoV - 2 virus. Most current antiviral drugs have some limitations including toxic side effects, drug resistance, and poor bioavailability thus, the discovery of new antiviral drugs could play a critical role in a pandemic response, as is the case in the current COVID-19 pandemic. Taking into account the resistance of bacteria and viruses today, we undertook the task of investigating alternatives to Ethnobotany that help us fight pathogens that are harmful to the human body such as viruses and bacteria, but without trying to replace them. Existing drugs used in medical practice but trying to optimize and contribute to a better antimicrobial and virucidal effect; It is for this reason that we used garlic as a probable antimicrobial and virucidal finding as a final result that it was more efficient on Gram Negative bacteria, while Sodium Hypochlorite at 5.40% was more effective on Gram positive bacteria and it is for this reason that We conclude that garlic (Allium sativum) is also targeting the microbial cell wall.

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CONCLUSIONS

The mean antimicrobial effect of garlic at 100% and 50% was 34 and 20 mm respectively in Staphylococcus aureus. The mean antimicrobial effect of 0.12% Chlorhexhydine was 20.20 mm in Staphylococococcus aureus. The mean antimicrobial effect of 5.40% and 2.7% Sodium Hypochlorite in Staphylococcus aureus was 38 mm and 20 mm, respectively. The mean antimicrobial effect of garlic at 100% and 50% was 24.40 and 20.20 mm respectively in Escherichia coli. The mean antimicrobial effect of 0.12% Chlorhexhydine was 13.80 mm in Escherichia coli. The mean antimicrobial effect of 5.40% and 2.7% Sodium Hypochlorite in Escherichia coli was 21.20 mm and 15.80 mm respectively. It was determined that the antimicrobial effect of 5.40% Sodium Hypochlorite was greater followed by 100% garlic extract over the other groups in Staphylococcus aureus, as for Escherichia coli it was determined that 100% garlic had a greater antimicrobial effect on the other groups, followed by Sodium Hypochlorite at 5.40%.

REFERENCES

[1] Kennedy HF, Morrison D, Kaufmann ME, Jackson MS, Bagg J, Gibson BES, Gemmell CG, Michie JR, 2000. Origins of *Staphylococcus epidermidis* and *Streptococcus oralis* causing bacteraemia in a bone marrow transplant patient. Journal of Medical Microbiology. 49 (1) p.367-370.

[2] Arroyo-LA, Landin LA, Alonso BA, Sánchez AM, Suarez FG, 2015. Actividad inhibitoria de *Allium cepa* y *Allium sativum* sobre cepas de *Escherichia coli* y *Salmonella enteritidis*. Biológico Agropecuaria Tuxpan. 3 (5) p.1045-1052.

[3] González Ma, González JA, Robles E, Martínez B, Sainz MG, Tolosa J, Salas A, 2007. Calidad Bacteriológica del Agua Utilizada en Clínicas Odontológicas. Acta Odontológica Venezolana. 45 (1) p.1-6.

[4] Jenkinson HF., 2011. Beyond the oral microbiome. Environmental Microbiology. 13 (12) p.3077-3087.

[5] Álvarez KH., 1998. Dental Hygiene Handbook. Baltimore: Williams & Wilkins. p.211-212.

[6] Porcel FND., Ureña R., Gaudioso AC., Ureña R., Cecilia ME., 2013. Bactericidia de Hipoclorito de Sodio sobre *Staphylococcus cohnii* productor de biofilm en una fábrica. Acta Bioquímica clínica Latinoamericana. 47 (4) p.693-700.

[7] Mercado MP., Arevalo CL., 2013. Sensibilidad de cultivos de *Staphylococcus aureus, Staphylococcusepidermis* y *Pseudomona aeruginosa* frente a la acción antibacteriana del extracto de de *Allium sativum* "Ajo". REBIOL. 33 (1) p.2-13.

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[8] Chalar V, Moya MJ, Vargas AE, Sejas RM, Romero B, 2014. Función Antimicrobiana de la Alicina de Ajo en cultivos de *Staphylococcus aureus, Pseudomonas aeruginosa* y *Escherichia coli.* Revista Científica Ciencia Médica. 17(1) p.26-28.

[9] Morales LJ., 1999. Efecto Bacteriostático de aceites esenciales de ajo *(Allium sativum*) y cebolla (*Allium cepa)* sobre dos microorganismos presentes en carnes. Tesis de Maestría en Biotecnología. Universidad Autónoma de Iztapalapa. México.

[10] Anderson RC, Harvey RB., Byrd JA, Callaway TR, Genovese KJ, Edrington TS, et al., 2005. Novel Prejarvest Strategies Involving the Use of experimental chlorate Preparations and nitro- based compounds to prevent Colonization of Food-Producing Animals by Foodborne Pathoghens. Poultry Science. 84:649-654.

[11] Morcillo EJ., 1993. Antisépticos. En: Esplugues J, Morcillo EJ, De Andres- Trelles F, editores. Farmacología en Clínica Dental. Barcelona: JR Prous Editores. p.235-249.

[12] López PJ., 2011. Observación de la actividad antimicrobiana del ajo *(Allium sativum)* en el laboratorio de Educación Secundaria. Eureka sobre Enseñanza y Divulgación de las Ciencias. 8; p.491-494.

[13] Thuler LCS., Velasco E., Martins CA., D'Assunção MV., 1999. Determinants of mortality in oncology patients colonized or infected with *Staphylococcus aureus.*Revista do Hospital das Clínicas. 54 (2) p.47 -52.

[14] Vandenesch F., Naimi T., Mark C., Lina G., 2003. Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Carrying Panton-Valentine Leukocidin Genes: Worldwide Emergence. Emerging.

[15] Cazar AM., Miranda VJ, Palacios VD, Cedillo, 2005. Infección del seno maxilar por *Escherichia coli:* Revisión de la literatura y reporte de caso. Acta odontológica venezolana. 53; p.25-36.

[16] Montes LS., Estrada RS., 2012. *Escherichia coli* en conducto radicular dentario. Investigaciones Médico-quirúrgicas. 4 (2) p.214-220.

