PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

DENIS ALOISIO LOPES MEDINA

EFEITO BACTERICIDA DE DIFERENTES SOLUÇÕES PARA *Staphylococcus aureus* e *Staphylococcus epidermidis* EM ESTADO SÉSSIL E PLANCTÔNICO– UM ESTUDO *IN VITRO*

Presidente Prudente - SP
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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal– Área de Concentração: Fisiopatologia Animal.

Orientadora:
Profª. Drª. Gisele Alborghetti Nai

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Presidente Prudente, 30 de março de 2020.

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DEDICATÓRIA

Dedico esse trabalho para minha esposa Flávia e minhas três filhas Ana Beatriz, Ana Luíza e Marina, pois baseado nesse amor é o que me motiva e tudo faz sentido.

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Primeiramente à Deus que é o criador de tudo e de todos.

Aos meus pais Nelson e Urbana que sempre acreditaram e foram a base sólida da minha formação, dedicando suas vidas para proporcionar um bom exemplo pra mim, fica minha eterna e fraternal consideração.

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Aos discentes Bruno Carvalho Henriques, Isadora Delfino Caldeira e Maria Júlia Schadeck Portelinha que colaboraram na execução de muitas etapas desse experimento.

Uma frase que levo comigo há décadas:

“Para um sonho se tornar realidade é preciso duas coisas:
a primeira é a capacidade de sonhar e a segunda é a perseverança.”

Autor desconhecido

RESUMO

Efeito bactericida de diferentes soluções para S*taphylococcus aureus* e S*taphylococcus epidermidis* em estado séssil e planctônico– um estudo *in vitro*

Infecções dos sítios cirúrgicos são comuns, mesmo em pacientes utilizando antibioticoterapia profilática. As infecções do sítio cirúrgico são um dos principais contribuintes para a morbidade e mortalidade nos cuidados pós-operatórios. Os biofilmes são um grupo complexo de células microbianas que aderem a matriz de exopolissacarídeos presente na superfície de dispositivos médicos. As infecções associadas ao biofilme nos dispositivos médicos representam um grave problema para a saúde pública e afetam a função do dispositivo. A infecção no implante mamário de silicone ocorre em 7 a 24% das reconstruções mamárias. Estas infecções ocasionam morbidade e possível diminuição de qualidade de vida para os pacientes, além de altos custos com seu tratamento. Os anestésicos locais são utilizados comumente como agente para analgesia pós-operatória e tem um baixo custo, porém sua ação como agente microbicida ainda é controversa. **Objetivo:** Avaliar o efeito bactericida de diferentes soluções para *Staphylococcus aureus e Staphylococcus epidermidis* associadas infecção de feridas cirúrgicas e próteses de silicone *in vitro*. **Material e métodos:** Para este estudo, foram avaliados os seguintes microrganismos: *S. aureus* e *S. epidermidis*. Foi realizada análise com suspensões em solução salina estéril com os microrganismos. O estudo foi realizado em duas etapas: primeiro o teste de difusão em ágar e posterirormente a análise das próteses de silicone. Na primeira etapa, para o teste de difusão em ágar (avaliação das bactérias em estado planctônico), as suspensões com os microrganismos foram inoculadas com auxílio de *swab* estéril na superfície do ágar sangue. Na sequência, foram confeccionados orifícios equidistantes medindo 3mm de diâmetro e 3mm de profundidade no ágar sangue. Um orifício foi preenchido apenas com 1 gota da solução salina, outro com 1 gota da solução de com antisséptico, outro com 1 gota de Lidocaína pura, outro com 1 gota de solução de Lidocaína e outro com 1 gota da solução com antibiótico. Na segunda etapa, foram utilizadas 36 próteses de silicones (avaliação das bactérias em estado séssil), as quais foram divididos em 3 grupos: próteses contaminadas pelas bactérias e que não receberam tratamento; próteses contaminadas pelas bactérias que receberam tratamento antes da contaminação; e próteses contaminadas pelas bactérias que receberam tratamento após a contaminação. Os tratamentos foram realizados com clorexidina, solução de lidocaína, lidocaína pura e com solução de antibióticos (cefazolina e gentamicina). A incubação foi de 1 semana. As próteses foram semeadas por rolamento em meio de cultura ágar sangue, o qual foi incubado por 48 horas e avaliada a área com formação de colônias através de programa de análise de imagem. **Resultados:** As placas testadas com solução de lidocaína e a lidocaína pura não apresentaram halo de inibição. A solução de antibióticos apresentou os maiores halos de inibição em todas as bactérias testadas. Na pré-lavagem, não houve crescimento de *S. epidermidis* com solução de antibióticos. Na lavagem pós-contaminação, não houve crescimento de nenhuma das bactérias com a solução de antibióticos. Na lavagem pós-contaminação, houve diminuição da densidade de colonização com a clorexidina e ausência de crescimento de *S. aureus* com lidocaína pura e solução de lidocaína. **Conclusão:** A solução de antibióticos se mostrou uma boa alternativa para o controle, principalmente *S. epidermidis*, na lavagem pré e pós-contaminação

nas próteses de silicone. A lidocaína (pura ou em solução) embora não tenham inibido o crescimento bacteriano nas placas com meio de cultura, foi capaz de diminuir a colonização por *S. aureus* na lavagem pós-contaminação, mostrando que pode ser utilizada como tratamento adjuvante nestes casos.

Palavras-chave: Infecção da Ferida Cirúrgica. Próteses e Implantes. Anestésicos. Agentes Bactericidas. Bactérias.

ABSTRACT

Bactericide effect of different solutions for *Staphylococcus aureus* and *Staphylococcus epidermidis* in sessile and planctonic status- an in vitro study

Surgical site infections are common, even in patients using prophylactic antibiotic therapy. Surgical site infections are a major contributor to morbidity and mortality in postoperative care. Biofilms are a complex group of microbial cells that adhere to the exopolysaccharide matrix present on the surface of medical devices. Infections associated with biofilm in medical devices pose a serious public health problem and affect the function of the device. Infection in the silicone breast implant occurs in 7 to 24% of breast reconstructions. These infections cause morbidity and possible decrease in quality of life for patients, in addition to high costs with their treatment. Local anesthetics are commonly used as an agent for postoperative analgesia and have a low cost, but their action as a microbicidal agent is still controversial. **Objective:** To evaluate the bactericidal effect of different solutions for *Staphylococcus aureus* and *Staphylococcus epidermidis* associated with surgical wound infection and silicone prostheses in vitro. **Material and methods:** For this study, the following microrganisms were evaluated: *S. aureus* and *S. epidermidis*. Analysis was carried out with suspensions in sterile saline solution with the microrganisms. The study was carried out in two stages: first the agar diffusion test and then the analysis of silicone prostheses. In the first stage, for the agar diffusion test (evaluation of bacteria in planktonic state), the suspensions with the microrganisms were inoculated with the aid of a sterile swab on the surface of the blood agar. Then, equidistant holes were made measuring 3mm in diameter and 3 mm deep in the blood agar. One orifice was filled with 1 drop of saline only, another with 1 drop of antiseptic solution, another with 1 drop of pure Lidocaine, another with 1 drop of Lidocaine solution and another with 1 drop of the antibiotic solution. In the second stage, 36 silicone prostheses (assessment of bacteria in sessile state) were used, which were divided into 3 groups: prostheses contaminated by the bacteria and which did not receive treatment; prostheses contaminated by bacteria and which received treatment before contamination; and prostheses contaminated by bacteria and which received treatment after contamination. Treatments were performed with chlorhexidine, lidocaine solution, pure lidocaine and antibiotics solution (cefazolin and gentamicin). The incubation was 1 week. The prostheses were sown by rolling in a blood agar culture medium, which was incubated for 48 hours and the area with colony formation was evaluated using an image analysis program. **Results:** The plates tested with lidocaine solution and pure lidocaine did not present an inhibition halo. The antibiotic solution showed the greatest inhibition halos in all the tested bacteria. In the prewash, there was no growth of *S. epidermidis* with antibiotic solution. In the post-contamination wash, none of the bacteria grew with the antibiotic solution. In post-contamination washing, there was a decrease in the density of colonization with chlorhexidine and absence of growth of *S. aureus* with pure lidocaine and lidocaine solution. **Conclusion:** The antibiotic solution proved to be a good alternative for the control, mainly *S. epidermidis*, in the pre and post-contamination washing in silicone prostheses. Lidocaine (pure or in solution) although it did not inhibit bacterial growth on plates with culture medium, was able to decrease colonization by *S. aureus* in post-contamination washing, showing that it can be used as an adjuvant treatment in these cases.

Key-words: Surgical Wound Infection. Prostheses and Implants. Anesthetics. Bactericidal Agents. Bacteria.

SUMÁRIO

1 ARTIGO 1

A LIDOCAÍNA TEM EFEITO ANTIMICROBIANO CONTRA PRINCIPAIS PATÓGENOS QUE INFECTAM FERIDAS? UM ESTUDO "IN VITRO"

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Resumo

Introdução: Infecções de locais cirúrgicos são comuns e causam morbidade e diminuição da qualidade de vida dos pacientes. Os anestésicos locais são comumente usados em medicina e odontologia e têm baixo custo, como agente microbicida ainda é controversa. Este estudo teve como objetivo avaliar a atividade antimicrobiana da lidocaína contra bactérias que mais comumente infectam feridas cirúrgicas. **Métodos:** Avaliamos *Staphylococcus aureus* e *Staphylococcus epidermidis*. As soluções testadas foram solução salina, clorexidina, lidocaína (solução e pura) e uma solução antibiótica. O teste de difusão em ágar foi realizado com placas de Petri. As placas de ágar foram feitas em duplicata e incubadas em estufa a 37°C por 48 horas. Posteriormente, os halos de inibição foram medidos. **Resultados:** As placas testadas com lidocaína (pura ou solução) não apresentaram halos de inibição. A solução antibiótica apresentou os maiores halos de inibição para todas as bactérias ($p < 0,05$). **Conclusões:** A lidocaína não apresentou efeito antimicrobiano para nenhuma das bactérias testadas. No entanto, a solução antibiótica e a clorexidina inibiram o crescimento de todas as bactérias.

Palavras-chave: agentes bactericidas, bactérias, microrganismos, feridas, cirurgia.

Declaração de Relevância Clínica

Alternativas às terapias com antibióticos, além de serem mais baratas, podem reduzir os problemas de resistência bacteriana.

As infecções do sítio cirúrgico são comuns, com incidência de 1,5% a 5% para todos os tipos de cirurgia (1). Embora mais de 99% dos pacientes cirúrgicos recebam antibióticos profiláticos, a incidência de infecções pós-operatórias permanece alta, impactando negativamente os resultados dos pacientes e aumentando os custos de saúde de US $ 1 a US $ 10 bilhões por ano (1).

A infecção é prejudicial à cicatrização e a infecção de uma ferida desempenha um papel importante no desenvolvimento da cronicidade, retardando a cura (2). O diagnóstico e o tratamento das infecções de feridas são controversos e variam entre os clínicos (3).

A eficácia de outros tratamentos, além da antibioticoterapia, para infecções bacterianas tem sido avaliada em medicina e odontologia (4), a fim de reduzir sua incidência e repercussões.

Existem evidências que sugerem que os anestésicos locais têm propriedades antimicrobianas inerentes contra um amplo espectro de patógenos humanos. Vários anestésicos locais em concentrações tipicamente usadas em ambientes clínicos inibem o crescimento de várias bactérias e fungos sob uma variedade de condições (5).

A lidocaína, entre as várias formulações de anestésicos locais, é a mais utilizada em uma infinidade de pequenos procedimentos cirúrgicos na prática odontológica, salas de emergência, ambulatórios e centros cirúrgicos (10). A lidocaína é um anestésico barato e de fácil administração, amplamente utilizado pelos cirurgiões (6). Por isso, seu efeito antimicrobiano foi testado em nosso estudo e em estudos anteriores.

Infecções de locais cirúrgicos são comuns, mesmo em pacientes em uso de antibioticoterapia profilática. Isso causa morbidade e uma possível diminuição na qualidade de vida dos pacientes, além de maiores custos associados ao seu tratamento. Os anestésicos locais são comumente usados como agente para analgesia pré-operatória em medicina e odontologia e têm baixo custo, mas sua ação como agente microbicida ainda é controversa.

O objetivo deste estudo foi avaliar se a lidocaína tem um efeito antimicrobiano contra infecções causadas pelas espécies de bactérias que mais comumente infectam feridas cirúrgicas em comparação às terapias usuais.

Materiais e métodos

As seguintes cepas bacterianas foram usadas no estudo (Microbiologics, Inc., St. Cloud, Minnesota, EUA):

- *Staphylococcus aureus* subespécie ATCC® 25923™
- *Staphylococcus epidermidis* subespécie ATCC® 12228™

As suspensões de microrganismos preparadas em solução salina estéril foram ajustadas à turbidez correspondente a 0,5 tubo na escala McFarland (1,5 x 10^8 unidades formadoras de colônias).

A atividade antimicrobiana foi testada para as seguintes soluções: solução salina estéril, solução de antibióticos, clorexidina, lidocaína pura e solução de lidocaína. A solução de lidocaína foi diluída em solução salina (NaCl a 0,9%) na proporção de 20 ml de lidocaína (lidocaína a 2% sem vasoconstritor, HypoLabor, Brasil) para 500 ml de solução salina (7). O antisséptico utilizado foi o digluconato de clorexidina a 0,5% (Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, São Paulo, Brasil). A solução antibiótica foi feita com 1 g de cefazolina sódica (Fazolon®, Blau Pharmaceuticals SA, São Paulo, SP, Brasil) e 80 mg de sulfato de gentamicina (gentamicina, Nova Farma Indústria Farmacêutica Ltda., Anápolis, GO, Brasil) diluído em 100 ml de solução salina (NaCl a 0,9%) (8).

O teste de difusão em ágar foi realizado com 40 placas de Petri de 150x150 mm, contendo aproximadamente 40 mL de ágar de sangue. As suspensões de microrganismos (*S. aureus* e *S. epidermidis*) foram inoculadas com auxílio de swabs estéreis na superfície do ágar. Posteriormente, foram feitos orifícios medindo 3 mm de diâmetro e 3 mm de profundidade no ágar. Placas separadas receberam uma gota de cada solução no orifício (solução salina, clorexidina, solução de lidocaína, lidocaína pura e solução antibiótica) (**Fig. 1**). As placas de ágar foram feitas em duplicata e incubadas em estufa a 37°C por 48 horas. A leitura da placa foi realizada usando uma régua milimétrica para medir o diâmetro dos halos de inibição.

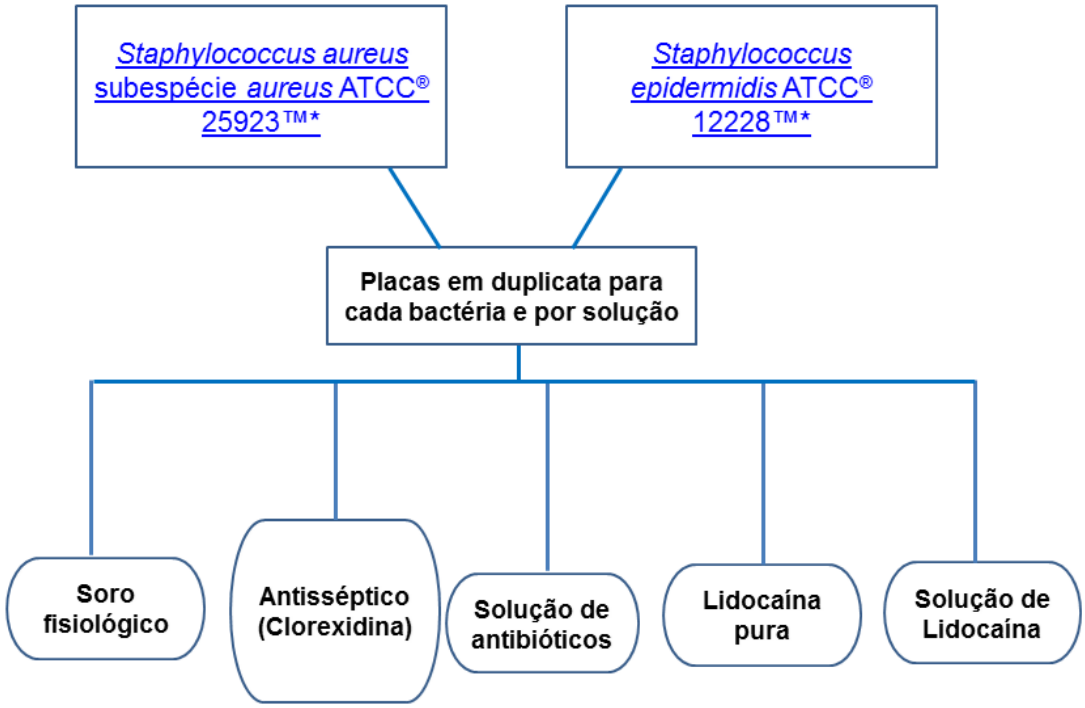

FIGURA 1– Delineamento experimental do estudo.

A análise de variância foi usada para cada uma das soluções para testar a média dos halos de inibição entre as diferentes bactérias e, em seguida, usou-se o teste de comparações múltiplas de Tukey para identificar para quais bactérias as soluções foram mais eficazes. O nível de significância foi estabelecido em 5% e o software SPSS V.22 foi utilizado para realizar as análises.

Resultados

A solução salina, lidocaína pura e solução de lidocaína não formaram halos de inibição para nenhuma das bactérias avaliadas (**Fig. 2**e**Fig. 3**).

Os maiores halos de inibição foram observados para a solução antibiótica (p <0,001) (**Fig. 2**,**Fig. 3** e **Fig. 4**).

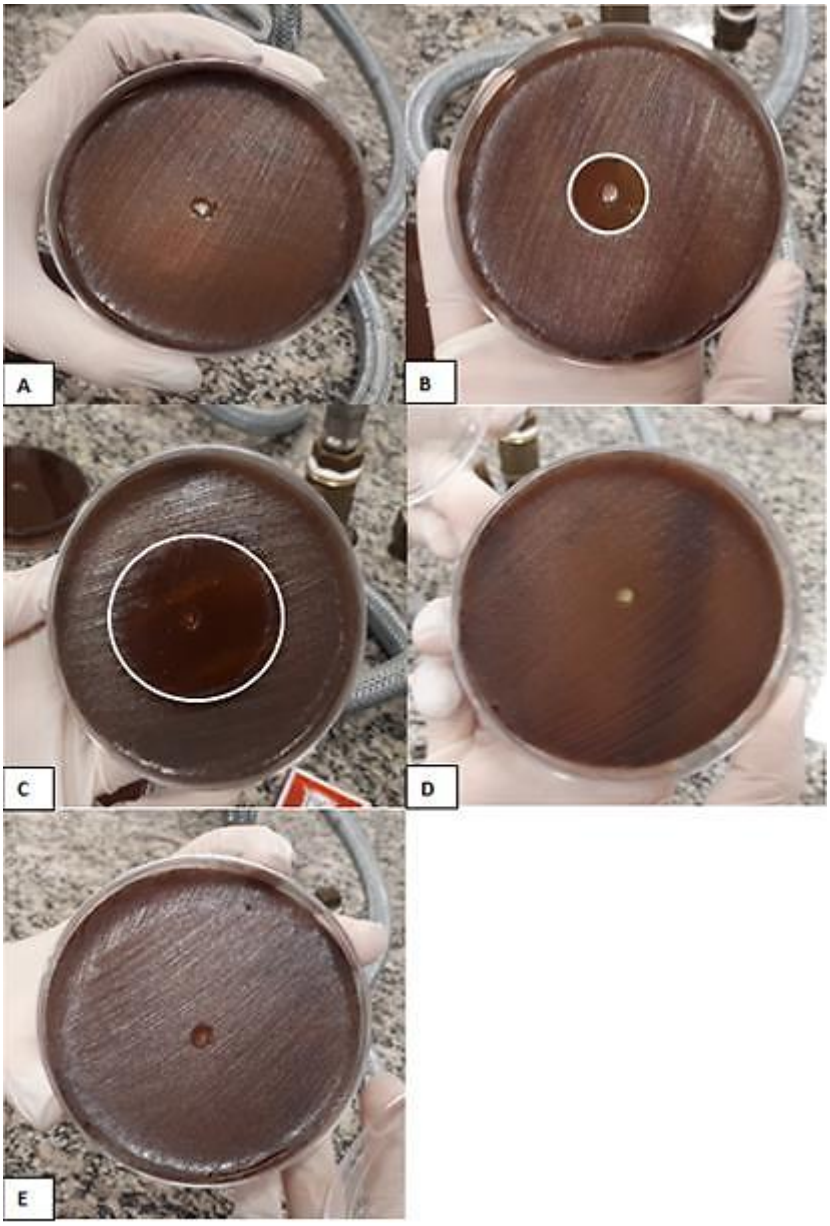

FIGURA 2 - Análise de placas de Petri semeadas com *S. aureus*: A: Solução salina. B: Digluconato de clorexidina. C: Solução de antibióticos. D: lidocaína pura. E: solução de lidocaína. Meio de cultura: ágar-sangue. A marcação branca indica o halo de inibição.

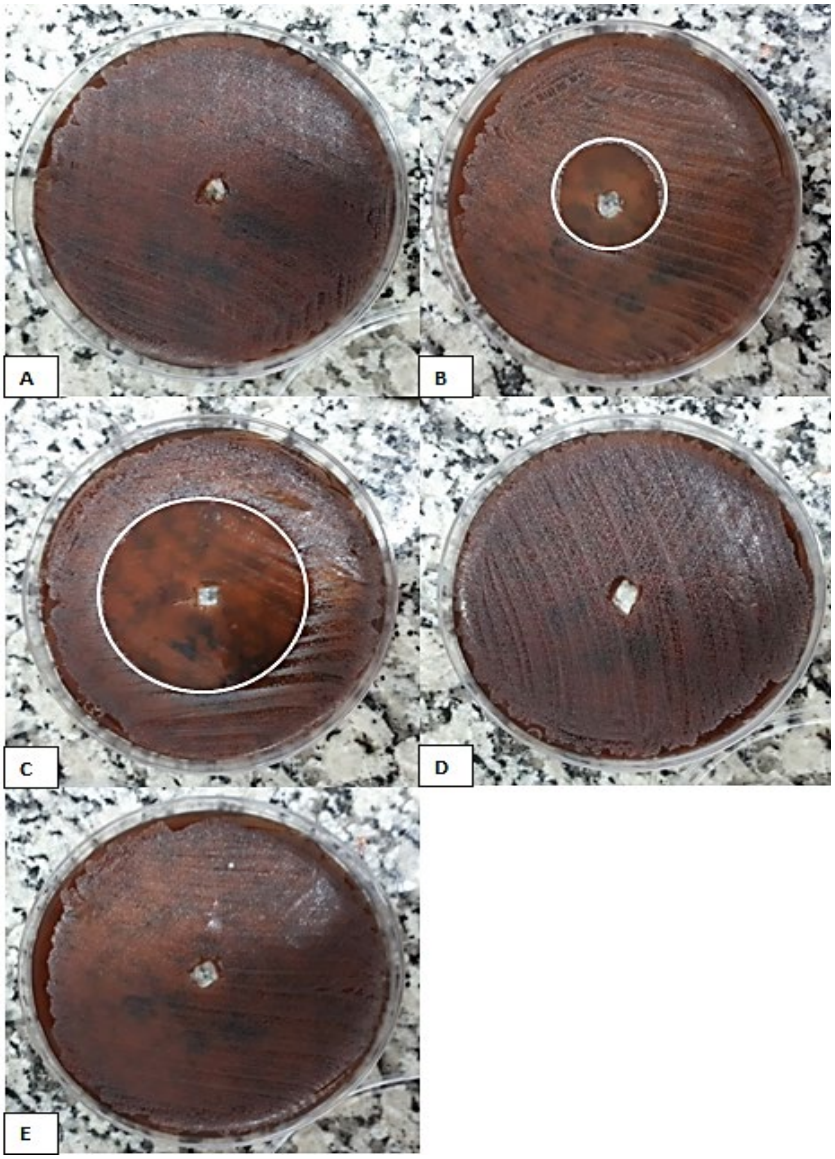

FIGURA 3 - Análise de placas de Petri semeadas com *S. epidermidis*: A: Solução salina. B: Digluconato de clorexidina. C: Solução de antibióticos. D: lidocaína pura. E: solução de lidocaína. Meio de cultura: ágar-sangue. A marcação branca indica o halo de inibição.

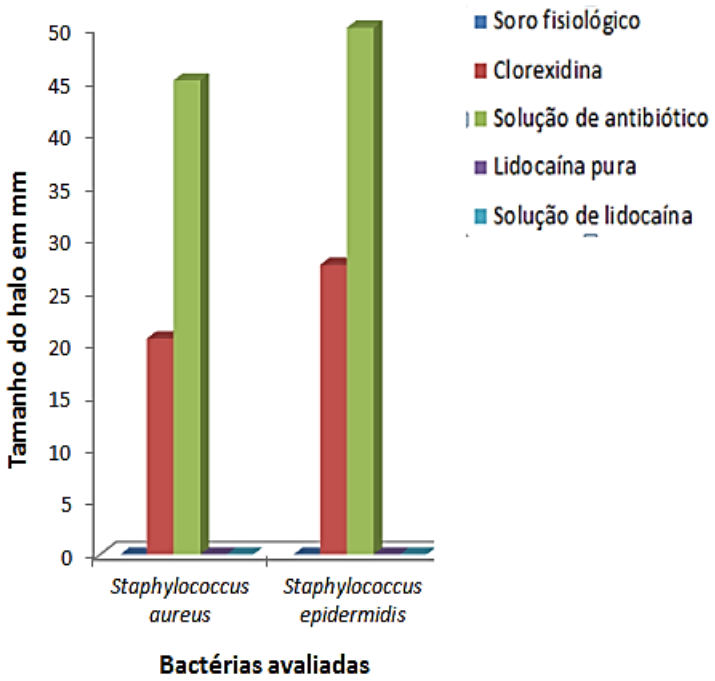

FIGURA 4 - Tamanho médio do halo de inibição em milímetros (mm) de acordo com a bactéria e o agente inibidor utilizado.

Em relação ao halo de inibição produzido pela solução antibiótica, houve diferença entre o halo observado nas análises da placa contendo *S. aureus* e os halos observados na análise das placas contendo *S. epidermidis* (p = 0,017).

Em relação à análise do halo de inibição produzido pela clorexidina, houve diferença entre o halo observado na análise da placa contendo *S. aureus* e o halo observado na placa contendo *S. epidermidis* (p = 0,046).

Discussão

Neste estudo “in vitro”, as placas testadas com a solução de lidocaína e lidocaína pura não apresentaram halos de inibição. A solução antibiótica apresentou os maiores halos de inibição nas duas bactérias testadas.

As bactérias que mais comumente infectam feridas dos mais diversos tipos são *S .aureus*, *Proteus mirabilis*, *Escherichia coli*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Corynebacterium* spp., Estafilococos coagulase-negativos e *Klebsiella* spp. (2,9,10).*S. aureus* e *S. epidemidis* são bactérias mais incidentes, independente

do tipo e localização da ferida e, portanto, também é a bactéria mais avaliada em relação aos agentes antimicrobianos. A incidência de feridas infectadas e a escassez de estudos avaliando os agentes antibacterianos em relação às outras bactérias direcionaram a escolha das bactérias testadas neste estudo.

Modelos "in vivo" de curativo cirúrgico usando lidocaína antes da inoculação de *S. aureus* (6) e com infusão contínua de lidocaína em feridas infectadas por *S. aureus* (11) demonstraram uma diminuição na contagem bacteriana de animais tratados com este anestésico. No entanto, outros estudos não demonstraram atividade antimicrobiana dos anestésicos locais e suas combinações em feridas cirúrgicas de ratos infectados com *S. aureus* (1,12). Em nosso estudo, a lidocaína (pura ou em solução) não teve efeito antimicrobiano para nenhuma das duas bactérias testadas. A lidocaína pode não tem efeito antibacteriano, mas sim efeito tecidual, com atividade vasodilatadora ou mesmo atividade pró-inflamatória. Existem anestésicos, como a ketamina (anestésico dissociativo), que possuem efeitos anti-inflamatórios (13) e influenciam o curso de processos infecciosos.

Um fato que reforça a provável atividade tecidual da lidocaína é o fato de um estudo ter observado uma diminuição significativa na contagem de *S. aureus* de animais tratados com lidocaína, bem como um aumento de 20 vezes com a adição de epinefrina (um vasoconstritor) em comparação a um grupo controle (6). Isso poderia justificar a ausência de ação antimicrobiana em nosso estudo realizado "in vitro". Além disso, também pode justificar a atividade antimicrobiana observada em alguns estudos "in vivo" e não em outros.

A profilaxia antimicrobiana é a principal medida farmacológica eficaz na redução do risco de infecção no local da cirurgia (14). No presente estudo, a solução que combina dois antibióticos (cefazolina sódica e sulfato de gentamicina) apresentou o melhor efeito antimicrobiano, formando grandes halos de inibição, como esperado, mesmo quando os dois antibióticos foram diluídos em solução salina.

Neste estudo, a clorexidina, uma solução antisséptica comumente usada em medicina e odontologia (4,15), mostrou efeito antimicrobiano para todas as bactérias testadas, mas com formação de halo de inibição aproximadamente 40% menor que a da solução antibiótica.

Mais estudos “in vivo” focando as possíveis ações teciduais e sobre citocinas inflamatórias da lidocaína, bem como utilizando diferentes concentrações e doses

deste anestésico, são necessários para que se possa estabelecer se esta pode ou não ajudar na profilaxia das infecções de feridas cirúrgicas.

Conclusões

Com base nos dados obtidos neste estudo, concluímos que a lidocaína não apresenta efeito antimicrobiano “in vitro”. No entanto, a solução antibiótica tem um bom efeito antimicrobiano contra as bactérias testadas, assim como a clorexidina em menor grau, mostrando que essas duas substâncias poderiam ser usadas para prevenir essas infecções.

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Conflito de interesses

Os autores declaram que não há conflito de interesses.

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Referências

1. Sams VG, Lawson CM, Coan P, Bemis D, Newkirk K, Karlstad M, Norwood J, Barlow P, Goldman MH, Daley BJ. Effect of local anesthetic on microorganisms in a murine model of surgical site infection. J Trauma Acute Care Surg. 2012;73:441-5; discussion 445-6. doi: 10.1097/TA.0b013e3182583e4f.
2. Bessa LJ, Fazii P, Di Giulio M, Cellini L. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J. 2015; 12:47-52. doi: 10.1111/iwj.12049.
3. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis. 2004;17:91-6.
4. Davis JM, Maki J, Bahcall JK. An In Vitro Comparison of the Antimicrobial Effects of Various Endodontic Medicaments on *Enterococcus faecalis*. J Endod.

2007;33:567-9. doi:10.1016/j.joen.2007.01.015

5. Johnson SM, Saint John BE, Dine AP. Local anesthetics as antimicrobial agents: a review. Surg Infect (Larchmt). 2008;9:205-13. doi: 10.1089/sur.2007.036.
6. Stratford AF, Zoutman DE, Davidson JS.Effect of lidocaine and epinephrine on *Staphylococcus aureus* in a guinea pig model of surgical wound infection. Plast Reconstr Surg. 2002;110(5):1275-9.
7. Thomas DF, Lambert WG, Williams KL. The direct perfusion of surgical wounds with local anaesthetic solution: an approach to postoperative pain? Ann R Coll Surg Engl. 1983; 65: 226-9.
8. Fernandes TRR, Okada A, Montag E, Almeida PN, Arruda EGP, Ferreira MC. Infecção em reconstrução mamária com expansor/prótese: incidência e correlação com fatores de risco em 120 pacientes. Rev Bras Cir Plast. 2012;27:1-102.
9. Mengesha RE, Kasa BG, Saravanan M, Berhe DF, Wasihun AG. Aerobic bacteria in post-surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. BMC Res Notes. 2014;7:575. doi: 10.1186/1756-0500-7-575.
10. Turtiainen J, Hakala T, Hakkarainen T, Karhukorpi J. The Impact of Surgical Wound Bacterial Colonization on the Incidence of Surgical Site Infection After Lower Limb Vascular Surgery: A Prospective Observational Study. Eur J Vasc Endovasc Surg. 2014;47:411-7. doi: 10.1016/j.ejvs.2013.12.025.
11. Lu CW, Lin TY, Shieh JS, Wang MJ, Chiu KM. Antimicrobial Effect of Continuous Lidocaine Infusion in a *Staphylococcus aureus*-Induced Wound Infection in a Mouse Model. Ann Plast Surg. 2014;73:598-601. doi: 10.1097/SAP.0b013e318276d8e7.
12. Kose AA, Karabaggli Y, Kiremitci A, Kocman E, Cetin C. Do local anesthetics have antibacterial effect on *Staphylococcus aureus* under in vivo conditions? An experimental study. Dermatol Surg. 2010;36:848-52. doi: 10.1111/j.1524-4725.2010.01559.x.
13. Helmer KS, Cui Y, Chang L, Dewan A, Mercer DW. Effects of ketamine/xylazine on expression of tumor necrosis factor-alfa inducible nitric oxide synthase, and cyclo-oxygenase-2 in rat gastric mucosa during endotoxemia. Shock. 2003;20:63-9.

14. Young PY, Khadaroo RG. Surgical site infections. Surg Clin North Am. 2014;94(6):1245-64. doi: 10.1016/j.suc.2014.08.008.
15. Staneviciute E, Na'amnih W, Kavaliauskas P, Prakapaite R, Ridziauskas M, Kevlicius L, Kirkliauskiene A, Zabulis V, Urboniene J, Triponis V. New in vitro model evaluating antiseptics' efficacy in biofilm-associated *Staphylococcus aureus* prosthetic vascular graft infection. J Med Microbiol. 2019;68:432-9. doi: 10.1099/jmm.0.000939.

2 ARTIGO 2

EFEITO DA LAVAGEM DE PRÓTESE DE SILICONE SOBRE A COLONIZAÇÃO POR *Staphylococcus aureus* e *Staphylococcus epidermidis* – UM ESTUDO *IN VITRO*

Título resumido: Efeito da lavagem de próteses de silicone

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RESUMO

Introdução: Os biofilmes são um grupo complexo de células microbianas que aderem à matriz de exopolissacarídeos presente na superfície de dispositivos médicos. As infecções associadas ao biofilme nos dispositivos médicos representam um grave problema para a saúde pública e afetam a função do dispositivo. A infecção no implante mamário de silicone ocorre em 7 a 24% das reconstruções mamárias.
Objetivo: Avaliar se a lavagem pré e pós-contaminação de próteses de silicone diminui a colonização bacteriana por *Staphylococcus aureus e* S*taphylococcus epidermidis*.
Métodos: Foram utilizadas próteses de silicones, as quais foram divididos em 3 grupos: próteses contaminadas pelas bactérias que não receberam tratamento; próteses contaminadas pelas bactérias que receberam tratamento antes da contaminação; e próteses contaminadas pelas bactérias que receberam tratamento após a contaminação. Os tratamentos foram realizados com clorexidina a 0,5%, solução de lidocaína, lidocaína pura e solução de antibióticos (cefazolina e gentamicina). A incubação foi de 1 semana. As próteses foram semeadas em meio de cultura, o qual foi incubado por 48 horas e avaliada a área de formação de colônias por meio da ferramenta digital de análise de dimensão fractal.
Resultados: Na pré-lavagem, não houve crescimento de *S. epidermidis* com solução de antibióticos. Na lavagem pós-contaminação, não houve crescimento de nenhuma das bactérias com a solução de antibióticos. Na lavagem pós-contaminação, houve diminuição da densidade de colonização com a clorexidina e ausência de crescimento de *S. aureus* com lidocaína pura e solução de lidocaína.
Conclusões: A lavagem pré e pós-contaminação com solução de antibióticos se mostrou eficaz para diminuir a colonização bacteriana, principalmente para *S. epidermidis* nas próteses de silicone. A lidocaína (pura ou em solução) foi capaz de diminuir a colonização por *S. aureus* na lavagem pós-contaminação, mostrando que pode ser utilizada como tratamento adjuvante nestes casos.

Palavras-chave: infecção da ferida cirúrgica, próteses e implantes, anestésicos, agentes bactericidas, bactérias.

Introdução

O *Staphylococcus epidermidis* (*S. epidermidis*) e o *Staphylococcus aureus* (*S. aureus*) são as causas mais comuns de infecções associadas a dispositivos médicos, infecções nosocomiais e adquiridas podem produzir biofilme como fator de virulência. Estafilococos constituem a microbiota da pele humana e podem contaminar os dispositivos internos. Quando microrganismos expostos a condições de estresse, a expressão gênica do biofilme é induzida como uma resposta a este estresse [1].

Estafilococos aderem, colonizam e infectam superfícies bióticas, como tecidos, ou superfícies abióticas, como superfícies protéticas, que podem atuar como substrato para adesão microbiana e causar disseminação bacteriana em todo o corpo, formando biofilme que é um glicocálice semelhante a lodo [1].

Próteses mamárias são materiais de uso frequente no arsenal terapêutico do cirurgião plástico para finalidades estéticas e reparadoras. Infecções pós-operatórias e a formação de biofilme trazem inúmeros transtornos ao tratamento, podendo resultar na retirada cirúrgica do implante nas infecções agudas ou proporcionar uma ambiente favorável ao aparecimento das contraturas capsulares e linfoma anaplásico de grandes células nas infecções subclínicas tardias [2].

Quanto às características do implante mamário, sua superfície poderá ter diferentes apresentações quanto à texturização, podendo ser alta, intermediária, baixa e mínima texturização. Quanto maior a texturização, maior a adesão do implante mamário ao tecido do paciente apresentando menos rotação e deslocação do implante e nas de mínima texturização as próteses apresentam mobilidade dando naturalidade ao resultado estético. Porém essa rugosidade na superfície do implante pode ser um fator de favorecimento de formação do biofilme e irritação tecidual [2].
S. aureus e *S. epidemidis* são bactérias que se apresentam como cocos gram positivos, normalmente agrupadas em forma de cachos e estão entre os mais frequentes patógenos envolvidos em infecções cirúrgicas pós-implante mamário [3,4]. O comportamento destas bactérias sobre os implantes mamários e o biofilme ainda é pouco conhecido.

Várias formas de prevenção de infecção cirúrgica, como a lavagem dos implantes com antibióticos e antissépticos tem sido relatadas, porém com resultados de baixa qualidade de evidência [5]. Dentre os antibióticos mais empregados, as cefalosporinas e amiglicosídeos são os mais comumente utilizados na prática clínica

[5,6]. Além dos antibióticos, a clorexedina se mostrou um agente antisséptico eficaz para infecções cirúrgicas e é usada rotineiramente pelo cirurgião [7].

Há evidências que sugerem que os anestésicos locais possuem propriedades antimicrobianas inerentes contra um amplo espectro de agentes patogênicos humanos [8]. Porém, os resultados ainda são controversos [9].

O objetivo deste estudo foi avaliar se a lavagem pré e pós-contaminação de próteses de silicone diminui a colonização bacteriana por *S. aureus e S. epidermidis*. Isto poderá oferecer ao cirurgião informações para uma boa conduta cirúrgica, ou seja, quando for utilizar próteses de silicone poderá escolher uma possível substância de prevenção infecciosa sobre os implantes, seja antes da implantação no organismo ou como tratamento de uma infecção instalada.

Métodos

Foi realizado um estudo experimental *in vitro* no Setor de Microbiologia do Laboratório de Análises Clínicas da Universidade do Oeste Paulista (UNOESTE), Presidente Prudente - SP.

Foram utilizadas 36 próteses de silicones (base do conformador para umbigo anatômico, Model Form Malhas Compressivas e Produtos Hospitalares Ltda. – EPP, São Caetano do Sul, São Paulo – Brasil) (Figura 1).

Figura 1 – A - Conformador para umbigo anatômico. B - Base do conformador para umbigo anatômico utilizada no estudo.

As próteses foram embebidas em suspensões em 1ml de caldo TSB (*Tryptic Soy Broth* - Caldo Soja Tripticaseína) com microrganismos previamente incubados a 37°C por 24 horas. As suspensões com microrganismos foram ajustadas à turvação correspondente ao tubo 0,5 da escala de McFarland ($1,5 \times 10^8$ unidades formadoras de colônias). Posteriormente, as próteses foram incubados em estufa a 37°C por 1 semana.

As cepas bacterianas avaliadas neste estudo foram: *Staphylococcus aureus* subespécie ATCC® 25923™ e *Staphylococcus epidermidis* subespécie ATCC® 12228™(Microbiologics, Inc., St. Cloud, Minnesota, USA).

Para as lavagens foram utilizadas as seguintes soluções:

- Solução com antibiótico: 1 g de cefazolina (Fazolon®, Blau Farmacêutica S.A., São Paulo, SP, Brazil) e 80 mg de sulfato de gentamicina (Gentamicin, Nova Farma Indústria Farmacêutica Ltda., Anápolis, GO, Brasil) diluídas em 100 ml de soro fisiológico (NaCl a 0,9%) estéril [10].
- Antisséptico: digluconato de clorexidina 0,5% (Farmax, Divinópolis, Minas Gerais, Brasil);
- Lidocaína pura (Lidocaína 2% sem vasoconstritor, HipoLabor, Brasil);
- Solução de lidocaína: 20 ml de lidocaína (Lidocaína 2% sem vasoconstritor, HipoLabor, Brasil) diluída em 500 ml de soro fisiológico estéril [11];

As próteses foram contaminadas em duplicata por bactéria e divididas em três grupos (Figura 2):

- Grupo G1 (controle): Próteses contaminadas pelas bactérias e que não receberam tratamento: 4 implantes de silicone que foram apenas embebidos em solução com os microrganismos;
- Grupo G2: Próteses contaminadas pelas bactérias e que receberam tratamento antes da contaminação: 4 implantes de silicone foram lavados com solução com antisséptico, 4 foram lavados com solução de lidocaína, 4 foram lavados com lidocaína pura e 4 com a solução de antibióticos. Após a secagem por 10 minutos, as próteses foram embebidas em solução com os microrganismos e incubadas em frasco estéril sem meio de cultura em estufa a 37°C por uma semana;
- Grupo G3: Próteses contaminadas pelas bactérias e que receberam tratamento após a contaminação: primeiramente as próteses foram contaminadas com os microrganismos e incubadas por uma semana. Posteriormente 4 implantes de silicone foram lavados com solução com antisséptico, 4foram lavados com solução de lidocaína, 4 foram lavados com lidocaína pura e 4 com a solução de antibióticos. Após as lavagens,

as próteses foram incubadas em frasco estéril sem meio de cultura em estufa a 37°C por mais uma semana.

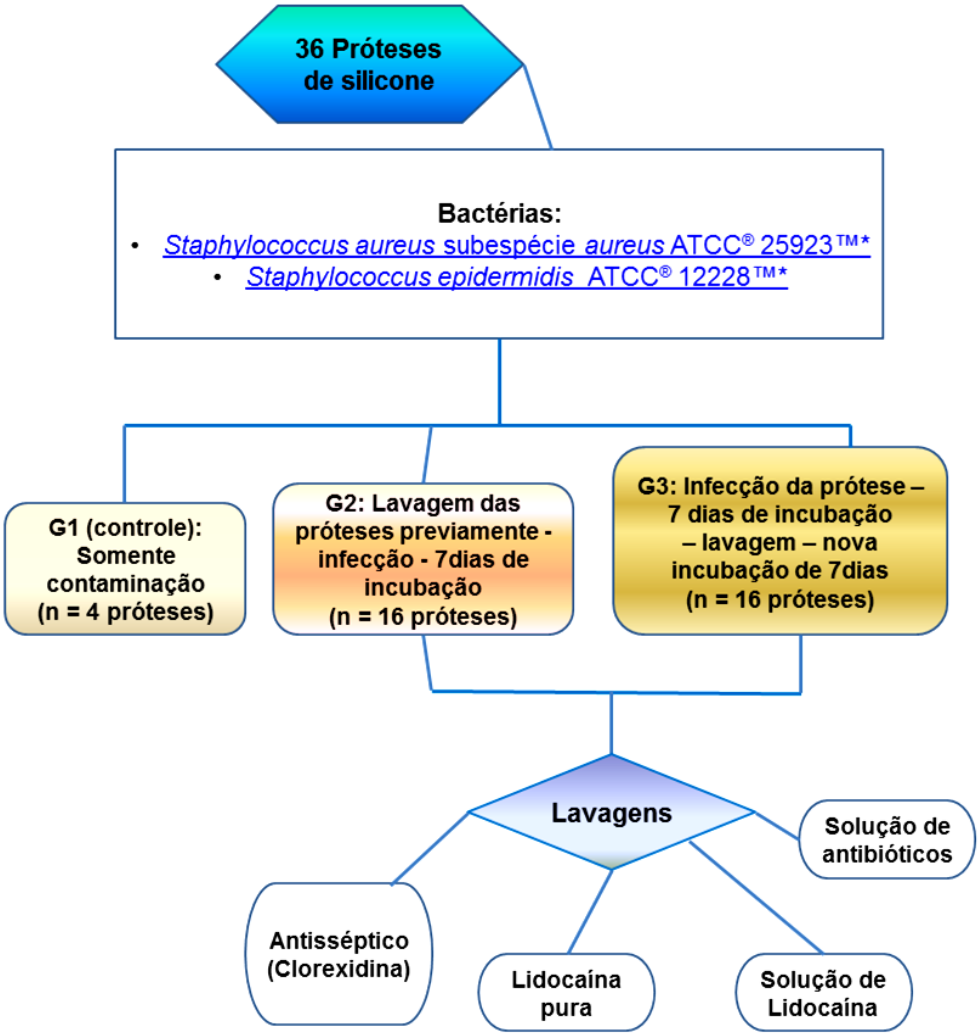

Figura 2 – Delineamento experimental do estudo.

As lavagens pré e pós contaminação bacteriana foram realizadas com 10 ml de cada solução durante 1 minuto.

Ao final das incubações, as próteses foram semeadas por rolamento em placas de Petri de 150x150 mm contendo 30 mL de ágar sangue. As placas foram incubadas em estufa a 37°C por 48 horas.

Após incubação, as placas foram fotografadas e analisadas com o software Image J® (*National Institutes of Health* – NIH, Bethesda, Maryland, EUA). A análise da dimensão fractal foi realizada pelo método de *box-counting.* A dimensão fractal calculada fica sempre entre 0 e 2, não distinguindo texturas diferentes [12].

Análise estatística

Foi realizado o teste de Anova para comparar os tratamentos aplicados para cada uma das bactérias, usando o teste de comparações múltiplas de Games-Howell, pois não foi possível estimar a homogeneidade das variâncias.

As diferenças foram consideradas estatisticamente significantes quando $p<0,05$. Os testes foram realizados com o pacote SPSS v. 23.0.

Resultados

Houve diferença significante para lavagem prévia com a com clorexidina, em relação às demais soluções de lavagem ($p<0,05$). Além disso, houve diferença entre a solução de antibióticos para as duas bactérias estudadas ($p<0,05$) (Figuras 3 e 5).

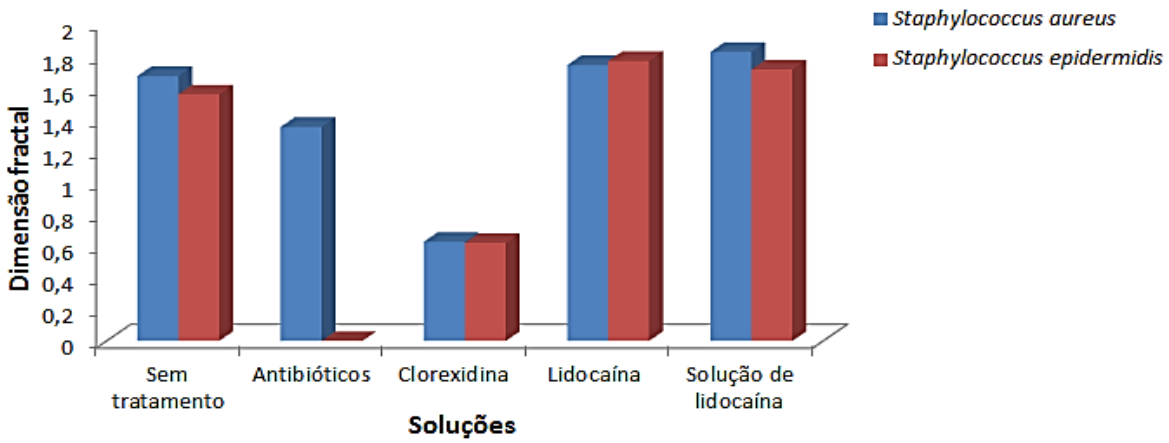

Figura 3 – Dimensão fractal das placas de Petri inoculadas com próteses de silicone pré-lavadas com as soluções comparadas as não tratadas.

Houve diferença significante para lavagem após a contaminação com o a solução de antibióticos, com lidocaína pura e solução de lidocaína em relação às demais soluções de lavagem para *S. aureus* ($p<0,05$). Houve diferença entre a solução de antibióticos em relação às demais soluções de lavagem para *S. epidermidis* ($p<0,05$) (Figura 4). Além disso, houve diferença entre a clorexidina para as duas bactérias estudadas ($p<0,05$) (Figuras 4 e 5).

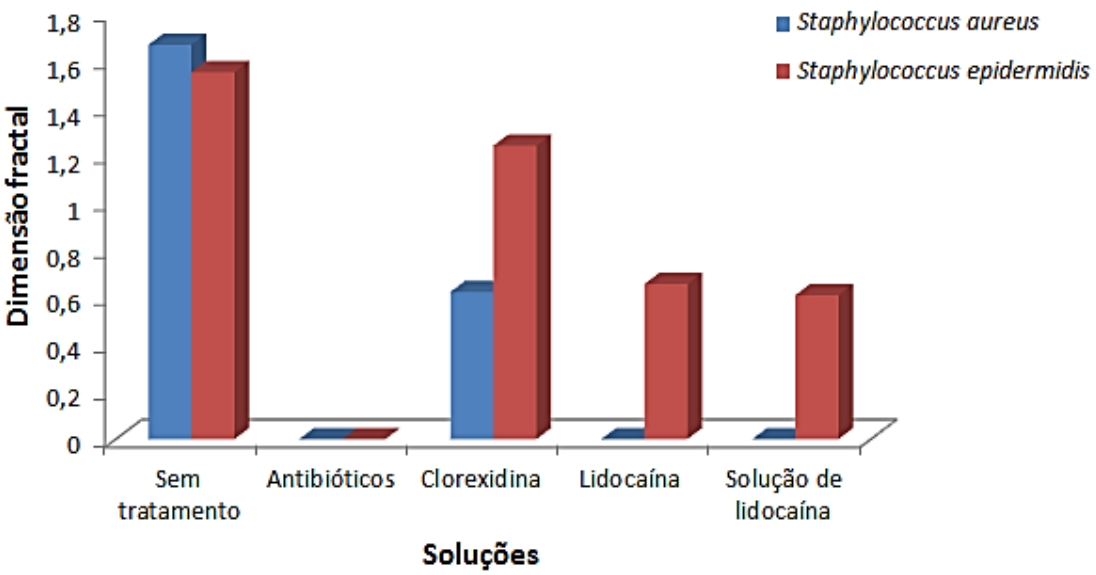

Figura 4 – Dimensão fractal das placas de Petri inoculadas com próteses de silicone lavadas com as soluções após a contaminação comparadas as não tratadas.

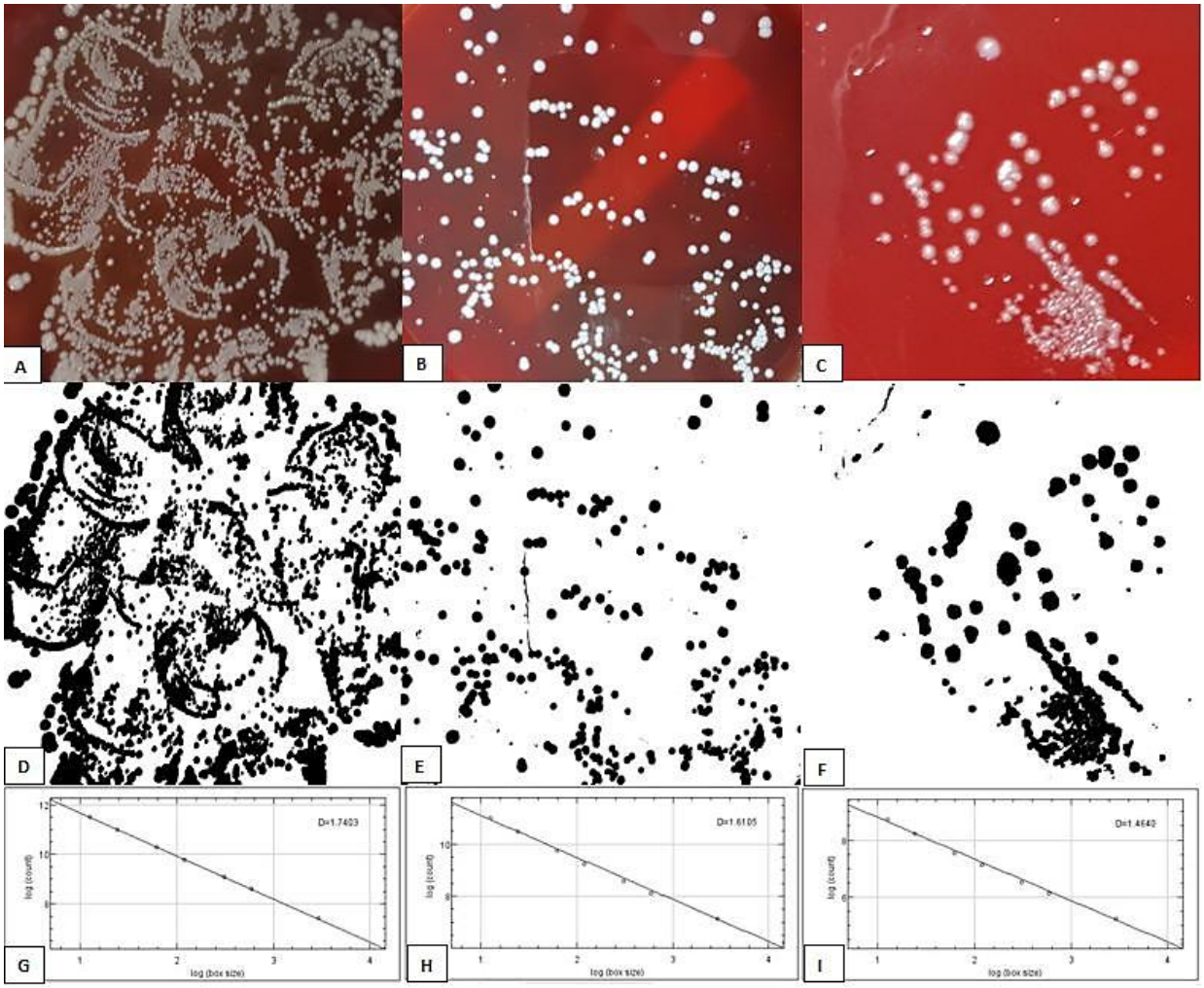

Figura 5 – A – Imagem original da placa de Petri inoculada com prótese de silicone contaminada com *S. aureus*. B – Imagem original da placa de Petri inoculada com prótese de silicone contaminada com *S. aureus* e lavada previamente com clorexidina. C – Imagem original da placa de Petri inoculada com prótese de silicone contaminada com *S. aureus* e lavada posteriormente com clorexidina. D, E e F- Imagem binarizada. G, H e I – *Box-counting* da análise da dimensão fractal.

Discussão

Métodos preventivos para infecção em próteses mamárias são estudados a fim de evitar complicações dessa natureza. A lavagem da prótese de silicone com substâncias bactericidas é uma conduta realizada com resultados não totalmente esclarecidos [13] e dentre as soluções testadas, existem vários mecanismos de ação distintos.

A clorexidina tem como função o deslocamento de cátions divalentes (Mg^{2+} e Ca^{2+}) associados a grupos fosfolipídeos causando alteração da fluidez da parede celular. Em altas concentrações a membrana celular bacteriana adota um estado líquido cristalino levando a uma rápida perda do conteúdo celular [14]. A Cefazolina

é uma cefalosporina de primeira geração, com ação através da inibição da síntese da parede celular bacteriana mostrando-se ativa “in vitro” e em infecções clínicas contra algumas cepas *S. aureus* (incluindo cepas produtoras de penicilinase) e *S. epidermidis*. A ação bactericida da gentamicina, antimicrobiano da classe dos aminoglicosídeos, ocorre através da inibição da síntese proteica bacteriana [15]. Quanto à lidocaína, os mecanismos de ação cogitados para a atividade bactericida podem ocorrer pela ruptura da parede celular, alteração da síntese de DNA e disfunção da respiração celular [16].

No grupo G2, onde as próteses de silicone foram lavadas antes da inoculação bacteriana, a clorexedina conseguiu reduzir o número de colônias para as ambas as bactérias; essa comprovação era esperada, pois clorexedina é um agente bactericida usado rotineiramente para prevenção infecciosa, comprovando assim sua eficácia [7]. Com o uso da solução de antibióticos houve diferença de crescimento das colônias nas próteses de silicone entre *S. aureus* e o *S. epidermidis*. Apesar de serem bactérias com alta virulência comprovadas, pode haver diferença de comportamento de crescimento bacteriano e formação de biofilme pela diferença de virulência entre as cepas, onde existe diferença na expressão gênica e mecanismos de adesão à prótese nos diferentes microrganismos [1,17]. Em relação à lidocaína, não houve redução do crescimento bacteriano nas próteses de silicone provavelmente pelo poder bactericida fugaz da lidocaína, ou seja, quando as próteses foram inoculadas com as bactérias após alguns minutos provavelmente não havia mais efeito bactericida dessa substância [16].

No grupo G3, em que as próteses foram lavadas com as soluções após a inoculação bacteriana, a solução de antibióticos inibiu totalmente o crescimento bacteriano, demonstrando ser uma solução eficaz para a diminuição de colônias bacterianas sobre a prótese de silicone [18]. Em relação à clorexedina, houve diferença estatística entre as duas bactérias, demonstrando um efeito bactericida mais eficaz contra *S. aureus*, pois a clorexidina possui um maior poder de destruir a integridade do biofilme desta bactéria [19] e as bactérias apresentam diferentes comportamentos de crescimento bacteriano quando submetidas a um ambiente de estresse [1]. A lidocaína e a solução de lidocaína apresentaram diferença estatística para *S. aureus* em relação ao controle, demonstrando um importante efeito

bactericida; e para *S. epidermidis* houve redução intermediária do número de colônias demonstrando efeito bactericida intermediário frente a este patógeno.

O grande valor desse trabalho foi poder demonstrar o efeito bactericida da lidocaína contra *S. aureus* nas próteses de silicone infectadas, já que este patógeno é muito frequente em infeções cirúrgicas e biofilmes.

Os dados coletados neste trabalho poderão ajudar o cirurgião na escolha de uma boa conduta cirúrgica intraoperatória ou pós-operatória nas lavagens de próteses de silicone com as substâncias analisadas visando prevenção ou tratamento de infecções nos implantes. Porém, mais estudos abordando esse tema deverão ser realizados para comprovação científica e poderão servir de base para estudos “in vivo” e correlação dos resultados.

Conclusão

A lidocaína (pura ou em solução) diminuiu a colonização por *S. aureus* na lavagem das próteses de silicone pós-contaminação, demonstrando um poder antimicrobiano importante frente *S. aureus* sendo, portanto, uma possibilidade de complementação terapêutica para biofilme em materiais aloplásticos. A lavagem pré e pós-contaminação com solução de antibióticos se mostrou eficaz para diminuir a colonização bacteriana, principalmente para *S. epidermidis*, nas próteses de silicone.

Conflito de interesses

Os autores declaram que não há conflito de interesses.

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Referências

1. Kırmusaoğlu S. *Staphylococcal* biofilms: pathogenicity, mechanism and regulation of biofilm formation by quorum-sensing system and antibiotic resistance mechanisms of biofilm-embedded microorganisms. 2016. Available from: http://dx.doi.org/10.5772/62943. Accessed in 30 January 2020.

2. Mempin M, Hu H, Chowdhury D, et al. The A, B and C's of silicone breast implants: anaplastic large cell lymphoma, biofilm and capsular contracture. Materials (Basel). 2018; 11(12). pii: E2393. doi: 10.3390/ma11122393.
3. Gil Conesa M, Climent Martínez NM, Del Moral Luque JA, et al. Evaluation of compliance with the antibiotic prophylaxis protocol in breast surgery and its effect on the incidence of surgical infection. An Sist Sanit Navar. 2019; 23;42(2):139-146. doi: 10.23938/ASSN.0637.
4. Walker JN, Poppler L, Pinkner CL, et al. Establishment and characterization of bacterial infection of breast implants in a murine model. Aesthet Surg J. 2019; pii: sjz190. doi: 10.1093/asj/sjz190
5. Frois AO, Harbour PO, Azimi F, et al. The role of antibiotics in breast pocket irrigation and implant immersion: a systematic review. Plast Reconstr Surg Glob Open.2018; 6(9):e1868. doi: 10.1097/GOX.0000000000001868
6. Dang T, Yim N, Tummala S, et al. Povidone-Iodine versus antibiotic irrigation in breast implant surgery: Revival of the ideal solution. J Plast Reconstr Aesthet Surg. 2019; pii: S1748-6815(19)30403-6. doi: 10.1016/j.bjps.2019.09.007.
7. Privitera GP, Costa AL, Brusaferro S, et al. Skin antisepsis with chlorhexidine versus iodine for the prevention of surgical site infection: A systematic review and meta-analysis. Am J Infect Control. 2017; 45(2):180-189. doi: 10.1016/j.ajic.2016.09.017
8. Johnson SM, Saint John BE, Dine AP. Local anesthetics as antimicrobial agents: a review. Surg Infect (Larchmt). 2008; 9(2): 205-213. doi: 10.1089/sur.2007.036.
9. Stratford AF, Zoutman DE, Davidson JS. Effect of lidocaine and epinephrine on *Staphylococcus aureus* in a guinea pig model of surgical wound infection. Plast Reconstr Surg. 2002; 110(5):1275-1279.
10. Fernandes TRR, Okada A, Montag E, et al. Infecção em reconstrução mamária com expansor/prótese: incidência e correlação com fatores de risco em 120 pacientes. Rev Bras Cir Plast. 2012; 27(supl):1-102.
11. Thomas DF, Lambert WG, Williams KL. The direct perfusion of surgical wounds with local anaesthetic solution: an approach to postoperative pain?Ann R Coll Surg Engl. 1983; 65(4): 226–229.

12. Morris BA, Sadana A. A fractal analysis of pathogen detection by biosensors. Biophys Chem. 2005; 113(1):67-81. doi:10.1016/j.bpc.2004.07.041
13. Lynch JM, Sebai ME, Rodriguez-Unda NA, et al. Breast Pocket Irrigation with Antibiotic Solution at Implant Insertion: A Systematic Review and Meta-Analysis. Aesthetic Plast Surg. 2018; 42(5):1179-1186. doi: 10.1007/s00266-018-1166-2.
14. Touzel RE, Sutton JM, Wand ME. Establishment of a multi-species biofilm model to evaluate chlorhexidine efficacy. J Hosp Infect. 2016; 92(2):154-160. doi: 10.1016/j.jhin.2015.09.013.
15. Trissel L. Handbook on injectable drugs. 15th ed. American Society of Health-System Pharmacists; 2009.
16. Lu CW, Lin TY, Shieh JS, et al. Antimicrobial effect of continuous lidocaine infusion in a *Staphylococcus aureus*-induced wound infection in a mouse model. Ann Plast Surg. 2014; 73(5):598-601.
17. Chessa D, Ganau G, Spiga L, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* virulence strains as causative agents of persistent infections in breast implants. Plos one. 2016; 11(1): e0146668. doi:https://doi.org/10.1371/journal.pone.0146668
18. CampbellAJ, DotelR, BlythCC, et al. Adjunctive protein synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal. J Antimicrob Chemother. 2019; 74(1): 1-5. doi:https://doi.org/10.1093/jac/dky387
19. Staneviciute E, Na'amnih W, Kavaliauskas P, et al. New *in vitr*o model evaluating antiseptics' efficacy in biofilm-associated *Staphylococcus aureus* prosthetic vascular graft infection. J Med Microbiol. 2019; 68(3):432-439. doi: 10.1099/jmm.0.000939.

ANEXOS

ANEXO A– APROVAÇÃO DO TRABALHO PELO COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) DA UNIVERSIDADE DO OESTE PAULISTA - UNOESTE

22/06/2018 Certificado

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PPD - Programa de Pesquisa Docente
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Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"AVALIAÇÃO DO EFEITO BACTERICIDA DA LIDOCAÍNA FRENTE AOS PRINCIPAIS PATÓGENOS QUE INFECTAM FERIDAS CIRÚRGICAS E PRÓTESES DE SILICONE"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **4650** e tendo como participante(s) **CESAR ALBERTO TALAVERA MARTELLI (discente), DENIS ALOISIO LOPES MEDINA (discente), BRUNO CARVALHO HENRIQUES (discente), MARIA JULIA SCHADECK PORTELINHA (discente), ISADORA DELFINO CALDEIRA (discente), MARIÂNGELA ESTHER ALENCAR MARQUES (participante externo), MERCIA DE CARVALHO ALMEIDA (docente), GISELE ALBORGHETTI NAI (orientador responsável)**, foi avaliado e **APR. COM RECOMENDAÇÃO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Presidente Prudente, 22 de Junho de 2018.

Prof. Dr. Jair Rodrigues Garcia Jr.
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ANEXO B– NORMAS DE PUBLICAÇÃO DAS REVISTAS CIENTÍFICAS AS QUAIS OS ARTIGOS SERÃO SUBMETIDOS

Artigo 1

J Endodontics

GUIDE FOR AUTHORS

The *Journal of Endodontics* is owned by the American Association of Endodontists. Submitted manuscripts must pertain to endodontics and may be original research (eg, clinical trails, basic science related to the biological aspects of endodontics, basic science related to endodontic techniques, case reports, or review articles related to the scientific or applied aspects of endodontics). Clinical studies using CONSORT methods (http://www.consort-statement.org/consort-statement/) or systematic reviews using meta-analyses are particularly encouraged. Authors of potential review articles are encouraged to first contact the Editor during their preliminary development via e-mail at *JEndodontics@UTHSCSA.edu*. Manuscripts submitted for publication must be submitted solely to *JOE*. They must not be submitted for consideration elsewhere or be published elsewhere.

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a. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section “c,” below), or sentences with little

to no transition within a paragraph.

b. Keep to the point. The subject of the sentence should support the subject of the paragraph For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al, reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al, 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.

c. Sentences are stronger when written in the active voice, that is, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

d. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus, or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)

e. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.

f. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

g. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

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Introduction, Methods, Results, Conclusions
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Acknowledgments

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Artigo 2

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