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Does washing medical devices before and after use decrease bacterial contamination?

An *in vitro* study

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Abstract

Surface treatment of medical devices may be a way of avoiding the need for replacement of these devices and the comorbidities associated with infection. The aim of this study was to evaluate whether pre- and postcontamination washing of 2 prostheses with different textures can decrease bacterial contamination.

The following microorganisms were evaluated: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis* and *Enterococcus faecalis*. Silicone and expanded polytetrafluoroethylene vascular prostheses were used and divided into 3 groups: prostheses contaminated; prostheses contaminated and treated before contamination; and prostheses contaminated and treated after contamination. Treatments were performed with antibiotic solution, chlorhexidine and lidocaine. After one week of incubation, the prostheses were sown in culture medium, which was incubated for 48 hours. The area of colony formation was evaluated by fractal dimension, an image analysis tool.

The antibiotic solution inhibited the growth of *S epidermidis* and chlorhexidine decrease in 53% the colonization density for *S aureus* in for both prostheses in the pre-washing. In postcontamination washing, the antibiotic solution inhibited the growth of all bacteria evaluated; there was a 60% decrease in the colonization density of *S aureus* and absence of colonization for *E faecalis* with chlorhexidine; and lidocaine inhibited the growth of *S aureus* in both prostheses.

Antibiotic solution showed the highest efficiency in inhibiting bacterial growth, especially for *S epidermidis*, in both washings. Lidocaine was able to reduce colonization by *S aureus* in post-contamination washing, showing that it can be used as an alternative adjuvant treatment in these cases.

Abbreviations: BHI = brain heart infusion, ePTFE = expanded polytetrafluoroethylene synthetic.

Keywords: blood vessel prosthesis, breast implant, disinfection, prostheses and implants, therapeutic irrigation

1. Introduction

Biofilms are a complex group of microbial cells that adhere to the exopolysaccharide matrix present on the surface of medical devices. Biofilm-associated infections in medical devices pose a serious public health problem and affect device function.[1]

Surface treatment of medical implants by various physical and chemical techniques is attempted to improve their surface properties to facilitate biointegration and prevent bacterial adhesion.[1]

Various forms of surgical infection prevention, such as washing implants with antibiotics and antiseptics, have been reported, but with poor-quality evidence.[2] Among the most commonly used antibiotics, cephalosporins and aminoglycosides are the most commonly used in clinical practice.[2,3] In addition to

Editor: Jorddy Neves Cruz.

This work was funded by research funds from the University of Oeste Paulista (UNOESTE). The authors acknowledge Ligia Maria Delfino Caldeira and Denise Lopes da Costa, from the Laboratory of Clinical Analysis of the Universidade do Oeste Paulista for their support during this research.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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How to cite this article: Nai GA, Medina DA, Martelli CA, Oliveira MS, Portelinha MJ, Henriques BC, Caldeira ID, Almeida Md, Eller LK, Neto FV, Marques ME. Does washing medical devices before and after use decrease bacterial contamination? an in vitro study. Medicine 2021;100:13(e25285).

Received: 19 June 2020 / Received in final form: 1 February 2021 / Accepted: 8 March 2021

http://dx.doi.org/10.1097/MD.0000000000025285

antibiotics, chlorhexidine has proven to be an effective antiseptic agent for surgical infections and is routinely used by surgeons.[4]

The use of many antimicrobial agents for both prophylaxis and the treatment of infected prostheses has resulted in a considerable increase in the number of resistant organisms, such as methicillin-resistant *Staphylococcus aureus*.[5] Thus, new forms of treatment that may be used in place or adjunctive to antibiotics are needed.

There is evidence to suggest that local anesthetics have inherent antimicrobial properties against a broad spectrum of human pathogens. Some studies have shown that at concentrations used in the clinical setting, various local anesthetics, such as bupivacaine and lidocaine, inhibit the growth of various bacteria, such as *S aureus*, and fungi.[6] In studies reporting *in vitro* and *in vivo* antimicrobial effects of local anesthetics, lidocaine is the most studied preparation.[7] The *in vivo* antimicrobial effects of lidocaine still raise doubts as they depend on dose, concentration, temperature of the drug solution, exposure to other diluents and duration of exposure. In addition, wound type and infiltration location, whether subdermal or subcutaneous, may also be factors that interfere with its action.[8] Nevertheless, local anesthetics may contribute to stunting and decreasing the rate of surgical infections, reducing the need for antibiotics.[9] However, existing studies evaluating the efficacy of local anesthetics, especially lidocaine, on bacterial infections were performed with infected tissues rather than prostheses or implants.

Given the lack of consensus on what is the best alternative to avoid bacterial colonization of prostheses and implants, the aim of this study was to evaluate whether washing with different pre- and postcontamination solutions of two prostheses with different textures can decrease bacterial contamination.

2. Materials and methods

This study was evaluated and approved by the Institutional Research Advisory Committee of the Universidade do Oeste Paulista (UNOESTE) (Protocols n° 4603 and 4650).

2.1. Bacterial strains

The bacterial strains (Microbiologics, Inc., St. Cloud, Minnesota, USA) used in the study were *Staphylococcus aureus* subspecies *aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Proteus mirabilis* ATCC 25933 and *Enterococcus faecalis* ATCC 29212™.

2.2. Washing solutions

The following solutions were used for washing:

- Antibiotic solution: 1 g of cefazolin (Fazolon, Blau Pharmaceutical SA, São Paulo, Brazil) and 80 mg of gentamicin sulfate (Gentamicin, Nova Farma Pharmaceutical Ltd., Anapolis, Brazil) diluted in 100 mL of sterile saline solution (0.9% sodium chloride);[10]
- Antiseptic: 0.5% chlorhexidine digluconate (Farmax, Divinópolis, Minas Gerais, Brazil);
- Pure lidocaine (2% lidocaine without vasoconstrictor, Hipo-Labor, Brazil);
- Lidocaine solution: 20 mL of lidocaine (2% lidocaine without vasoconstrictor, HipoLabor, Brazil) diluted in 500 mL of sterile saline solution (0.9% sodium chloride).[11]

2.3. Biofilm formation analysis

For the analysis of biofilm formation, the 96-microtiter plate method was used, as described by Ziuzina et al.[12]

The isolates were cultured in brain heart infusion (BHI) broth at 37°C for 24 hours. Cultures were adjusted to turbidity corresponding to McFarland scale tube 0.5 (1.5×10^8 colony forming units/mL).

Initially, 50 μL of the antibiotic solution, chlorhexidine, pure lidocaine and lidocaine solution were added to the wells of the 96-polystyrene microtiter plate (CRALPLAST, CRAL Laboratory Articles Ltd., Cotia, Sao Paulo, Brazil), which was allowed to dry for a few minutes. Subsequently, 200 μl of BHI broth was placed in the wells of the microplates; 20 μl aliquots of the cell suspension were added from each isolate and then incubated at 37°C for 24 hours. The plates were washed to remove non-adherent cells. The adhered cells were stained with crystal violet.

The optical densities of the solution were read at a wavelength of 600 nm (Microplate reader MR-96A, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).[13,14]

2.4. Prosthesis contamination analysis

Eighty silicone prostheses (Model Forma Compressive Meshes and Hospital Products Ltd. - EPP, Sao Caetano do Sul, Sao Paulo, Brazil) and 80 fragments of the expanded polytetrafluoroethylene synthetic (ePTFE) prosthesis model Exxcel Soft (Maquet Cardiovascular LLC, Wayne, NJ, USA) measuring 1 cm in length each were used.

The prostheses were soaked in 1 mL of tryptic soy broth suspensions of the microorganisms previously incubated at 37°C for 24 hours. The suspensions with the microorganisms were adjusted to the turbidity corresponding to McFarland scale tube 0.5 (1.5×10^8 colony forming units/mL).

The prostheses were contaminated in duplicate by bacteria and divided into three groups (Fig. 1):

- Untreated bacteria-contaminated prostheses: 8 silicone implants and 8 ePTFE fragments that were only soaked in solution with the microorganisms;
- Bacterial contaminated prostheses treated prior to contamination: 8 silicone implants and 8 ePTFE fragments were washed with chlorhexidine, 8 of each were washed with lidocaine solution, 8 of each were washed with pure lidocaine and 8 of were each were washed with the antibiotic solution. After drying for ten minutes, the prostheses were soaked in solution with the microorganisms and incubated in a sterile flask without culture medium in an oven at 37°C for one week;
- Bacterial contaminated prostheses that were treated after contamination: First, the prostheses were contaminated with microorganisms and incubated for 1 week. Subsequently, 8 silicone implants and 8 ePTFE prosthesis fragments were washed with chlorhexidine, 8 of each were washed with lidocaine solution, 8 of each were washed with pure lidocaine and 8 of each were washed with antibiotic solution. After the washes, the prostheses were incubated in sterile flasks without culture medium in an oven at 37°C for one more week.

The washes prior and after contamination were performed with 10 ml of each solution for 1 minute.

At the end of incubations, the prostheses were seeded by rolling in 15x150 mm Petri dishes containing 40 mL of cystine lactose electrolyte deficient agar for evaluation of *P. mirabilis* and 40 mL

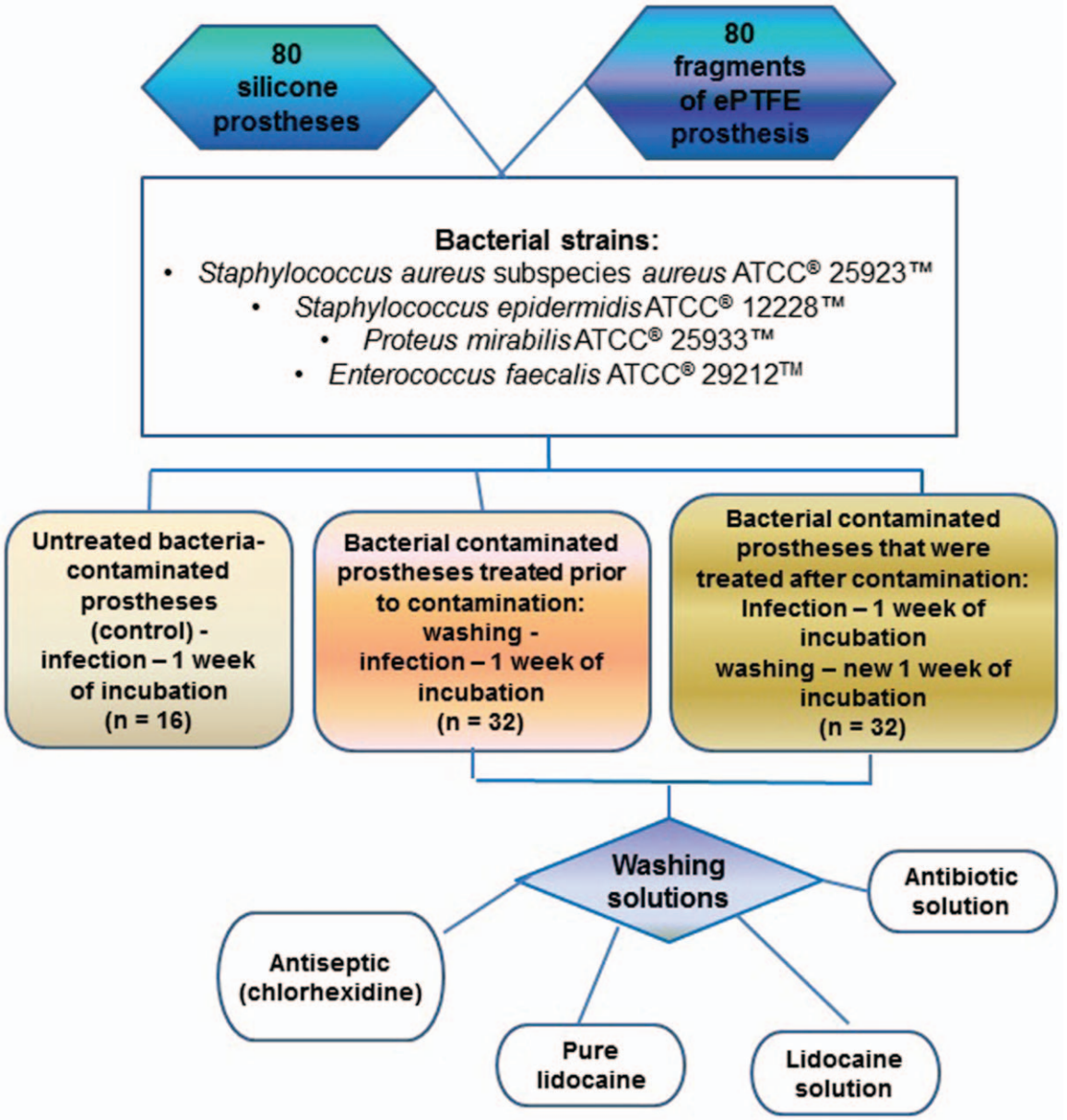

Figure 1. Experimental study design.

of blood agar for evaluation of other bacteria. The plates were incubated in an oven at 37°C for 48 hours.

After incubation, the plates were photographed and analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA, available at http://rsbweb.nih.gov/ij/) using fractal dimension analysis. The fractal dimension analysis was performed by the box-counting method [15].

2.5. Statistical analysis

The analysis of biofilm formation did not show normal distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test), so the Kruskal-Wallis test was applied to compare the positions between the bacterial groups and to compare the treatments using the Dunn test.

For the analysis of the contamination of the prosthesis, the ANOVA test was performed to compare the treatments applied to each of the bacteria using the Games-Howell multiple comparison test because it was not possible to estimate the homogeneity of the variances.

Differences were considered statistically significant when $P < .05$. The tests were performed with SPSS v. 23.0.

3. Results

3.1. Biofilm formation analysis

There was no difference between treatments for *S aureus* ($P = .1461$). For *S epidermidis*, there was a difference ($P = .0000$), and multiple comparisons showed differences between chlorhex-

idine x other treatments, antibiotic x lidocaine solution, and lidocaine x lidocaine solution. For *P mirabilis,* there was also a difference ($P=.0000$), and multiple comparisons also indicated differences between chlorhexidine and other treatments. For *E faecalis,* there was also a difference between treatments ($P=.0000$), and once again, chlorhexidine was different from other treatments and between antibiotic x lidocaine solution. For BHI broth, there was a difference ($P=.0005$), and the differences were between chlorhexidine x antibiotic (Fig. 2).

3.2. Prosthesis contamination analysis

3.2.1. Silicone prostheses. For prior washing of the silicone prostheses with the different solutions, there was no significant difference between treatments for *S aureus* ($P=.110$) or for *E faecalis* ($P=.568$). There were differences for *S epidermidis* ($P=.012$), and the differences were between non-treated prostheses x antibiotic ($P<.05$); for *P mirabilis* ($P<.01$), the differences were between non-treated prostheses x (antibiotic, chlorhexidine and lidocaine) ($P<.05$) (Figs. 3 and 4).

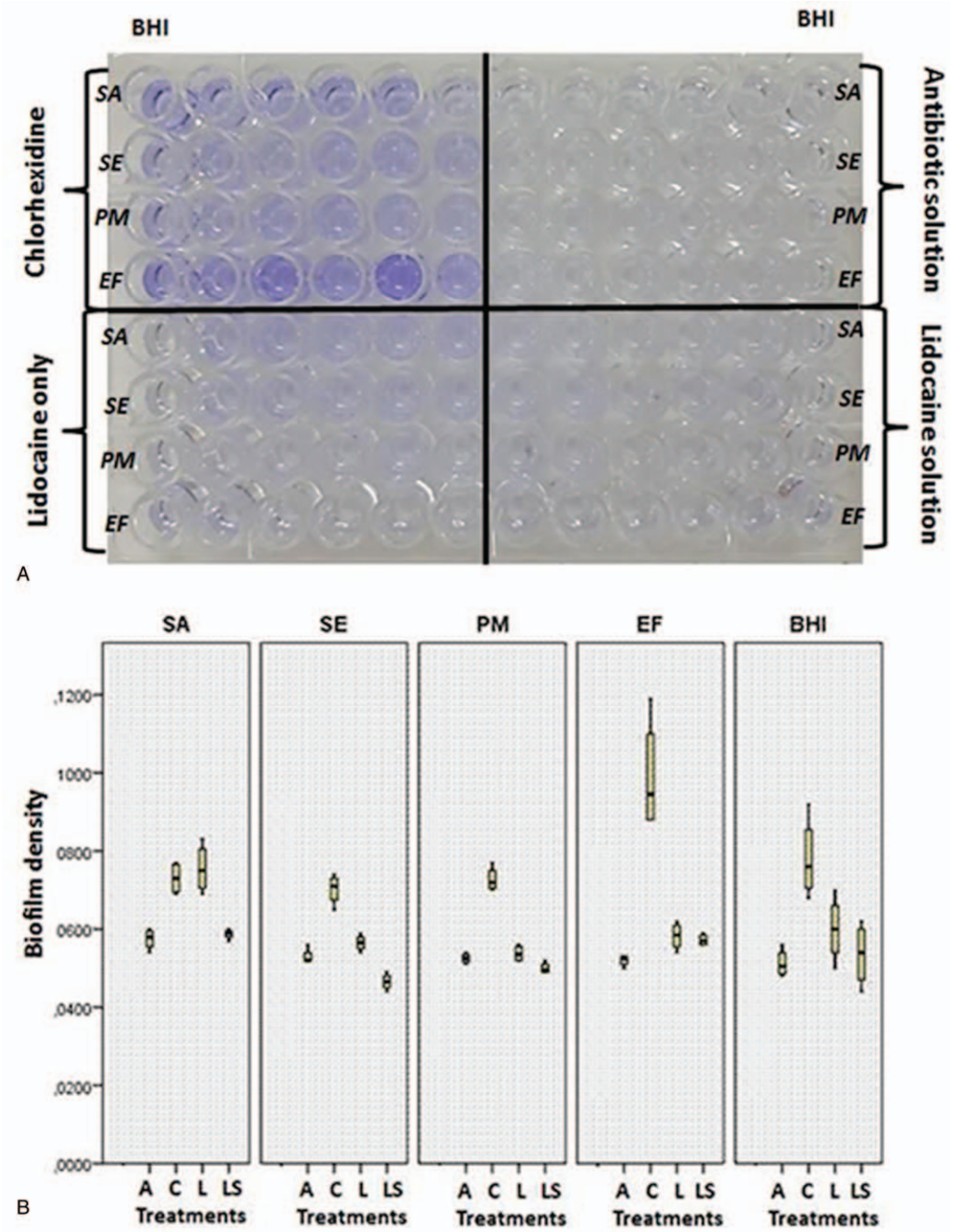

Figure 2. A - Microtiter plate showing biofilm formation of different bacteria with different solutions. B - Biofilm formation density for each bacterium studied according to the treatment used (median and interquartile range). SA: *S aureus*; SE: *S. epidermidis*; PM: *P mirabilis*; EF: *E faecalis*; BHI: *brain heart infusion*.

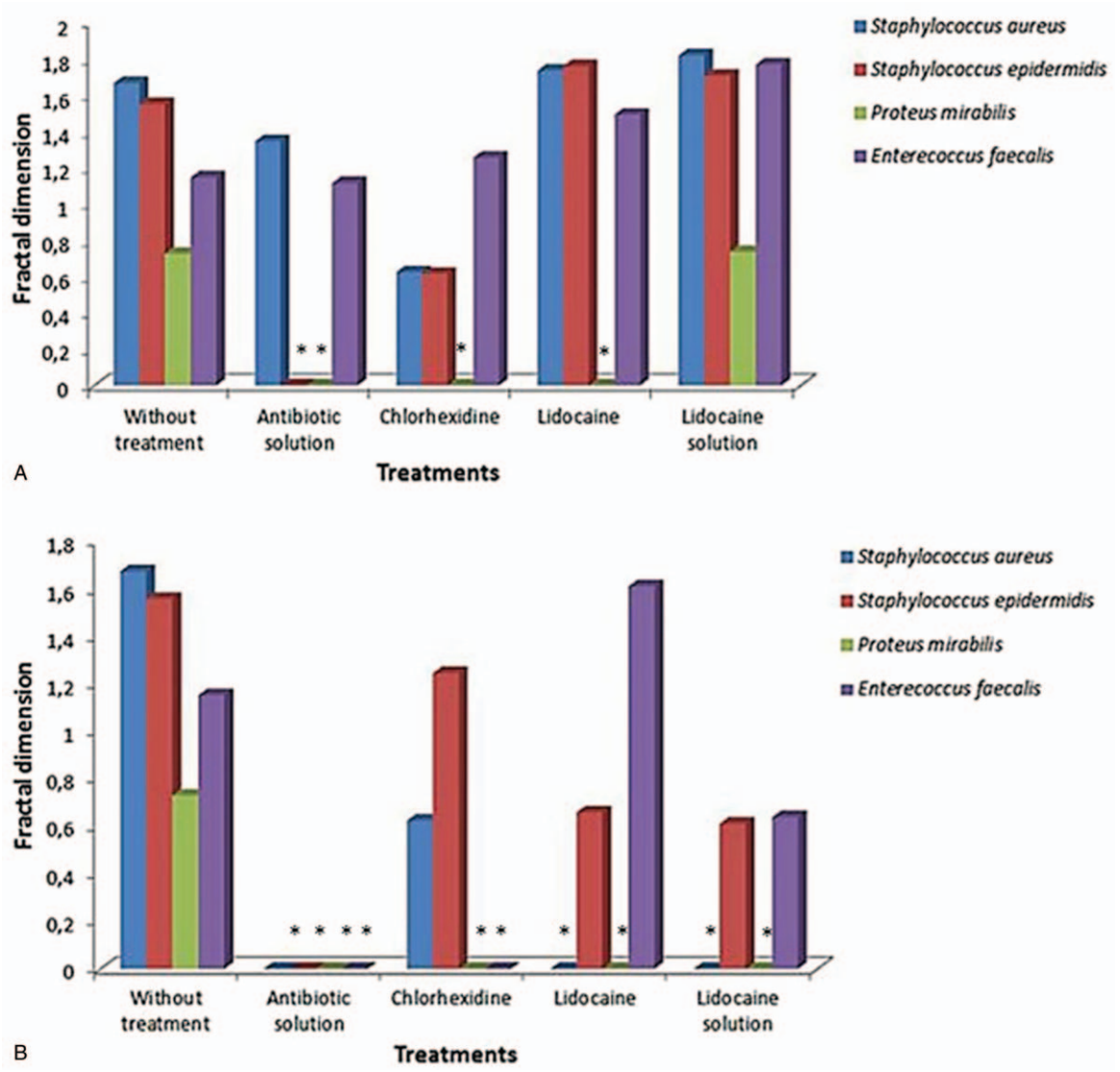

Figure 3. A - Fractal dimension of Petri dishes inoculated with prewashed silicone prostheses with solutions compared to untreated prostheses. B - Fractal dimension of Petri dishes inoculated with silicone prostheses washed with solutions after contamination compared to untreated prostheses. *: $P < .05$, when compared to non-treated prostheses.

Regarding the washing of silicone prostheses after infection, there was a difference between treatments for *S aureus*, and the differences were non-treated prostheses x (antibiotics, lidocaine and lidocaine solution); for *S epidermidis*, the differences were between non-treated prostheses x antibiotics; and for *E faecalis*, the differences were between non-treated prostheses x (antibiotics and chlorhexidine) ($P < .05$). There was no growth of *P mirabilis* after all treatments, which differentiated it from other bacteria ($P = .0001$) (Figs. 3 and 4).

3.2.2. ePTFE prostheses. For previous washing of ePTFE prostheses with different solutions, there was no difference between treatments for *S aureus* and *P mirabilis* ($P > .05$). There were differences between treatments for *S epidermidis*, and the differences were between non-treated prostheses x antibiotics; for *E faecalis* the differences were between non-treated prostheses x antibiotics ($P < .05$) (Figs. 5 and 6).

Regarding the washing of ePTFE prostheses after infection, there were differences between the treatments for *S aureus*, and the differences were between non-treated prostheses x (antibiotics and lidocaine); for *S epidermidis*, the differences were between non-treated prostheses and antibiotics; and for *E faecalis*, the difference was between non-treated prostheses x (antibiotics, chlorhexidine and lidocaine solution) ($P < .05$). There was no *P mirabilis* growth after all treatments ($P < .05$) (Figs. 5 and 6).

4. Discussion

We have evaluated silicone prostheses and vascular prostheses because they are two medical devices widely used in clinical practice. In addition, they have different textures, which may influence the biofilm formation. Also we evaluated four different bacteria (*S aureus* subspecies *aureus*, *S epidermidis*, *P mirabilis*

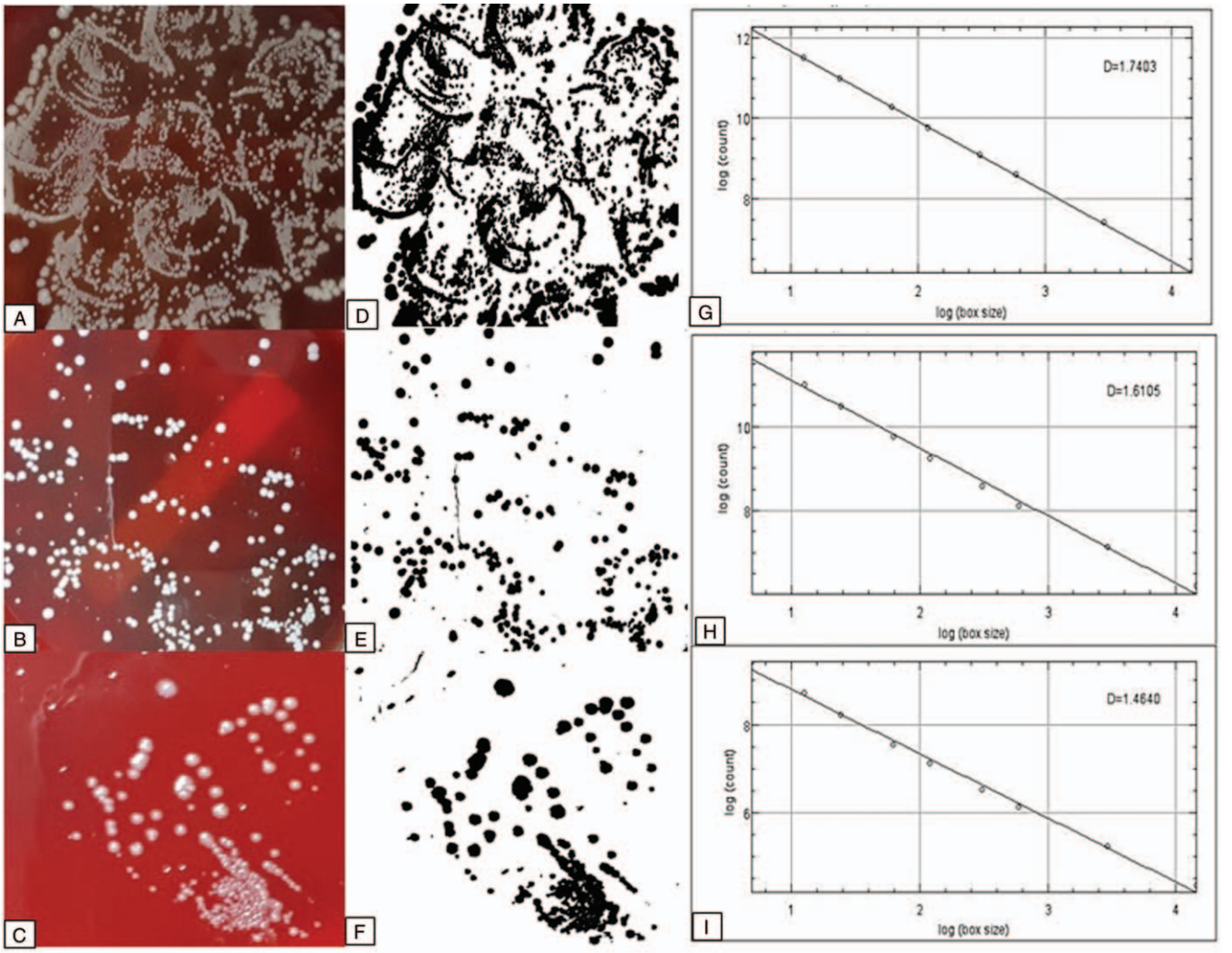

Figure 4. A - Original image of the Petri dishes inoculated with silicone prosthesis contaminated with *S. aureus*. B - Original image of the Petri dishes inoculated with silicone prosthesis contaminated with *S aureus* and previously washed with chlorhexidine. C - Original image of the Petri dishes inoculated with silicone prosthesis contaminated with *S aureus* and later washed with chlorhexidine. D, E and F- Binarized image. G, H and I - Box-counting of the fractal dimension analysis.

and *E faecalis*) that are the ones that most commonly infect wounds and devices.[16,17]

In the present study, we observed that the antibiotic solution decreased biofilm formation for all bacteria in the biofilm formation test. In the pre contamination washing of both prostheses, the antibiotic solution was efficient, especially for *S epidermidis*. In postcontamination washing, the antibiotic solution inhibited the growth of all bacteria evaluated in both prostheses. Still, in the postcontamination washing with chlorhexidine, there was no growth of *E faecalis*, and there was a decrease in *S aureus*; with lidocaine, there was no growth of *S aureus*, and there was a decrease in *S epidermidis*; however, with lidocaine solution, there was a decrease in *S epidermidis* in both prostheses. All solutions prevented *P. mirabilis* growth in postcontamination washing in both prostheses.

When infection is present, removal of the infected graft is usually the most appropriate approach; however, this approach may have high morbidity. For this reason, administration of antimicrobial agents may be beneficial in cases of mild infection.[18] In our study, the antibiotic solution had the expected effect due to its intrinsic bactericidal activity. The bactericidal action of cefazolin, a first-generation cephalosporin, is through the inhibition of bacterial cell wall synthesis, also cefazolin is active *in vitro* and in clinical infections against *S aureus* (including penicillinase producing strains) and *S epidermidis*. The bactericidal action of gentamicin, an antimicrobial of the aminoglycoside class, occurs through inhibition of bacterial protein synthesis.[19] Even when diluted in saline, the combination of these two antibiotics had an excellent effect on the biofilm formation test, with decreased biofilm formation, and especially on postcontamination washing of both prostheses. Washing the prostheses with this antibiotic solution might be sufficient to avoid the need for implant replacement and the possibility of using the same implant in cases of contamination. In addition, the antibiotic solution proved to be efficient regardless of the texture of the prostheses, a factor that may provide greater adherence of bacteria to the medical device.[20]

The mammoplasty for breast implants is one of the most commonly performed aesthetic plastic surgery procedures.[21] Breast implants are commonly used by plastic surgeons for

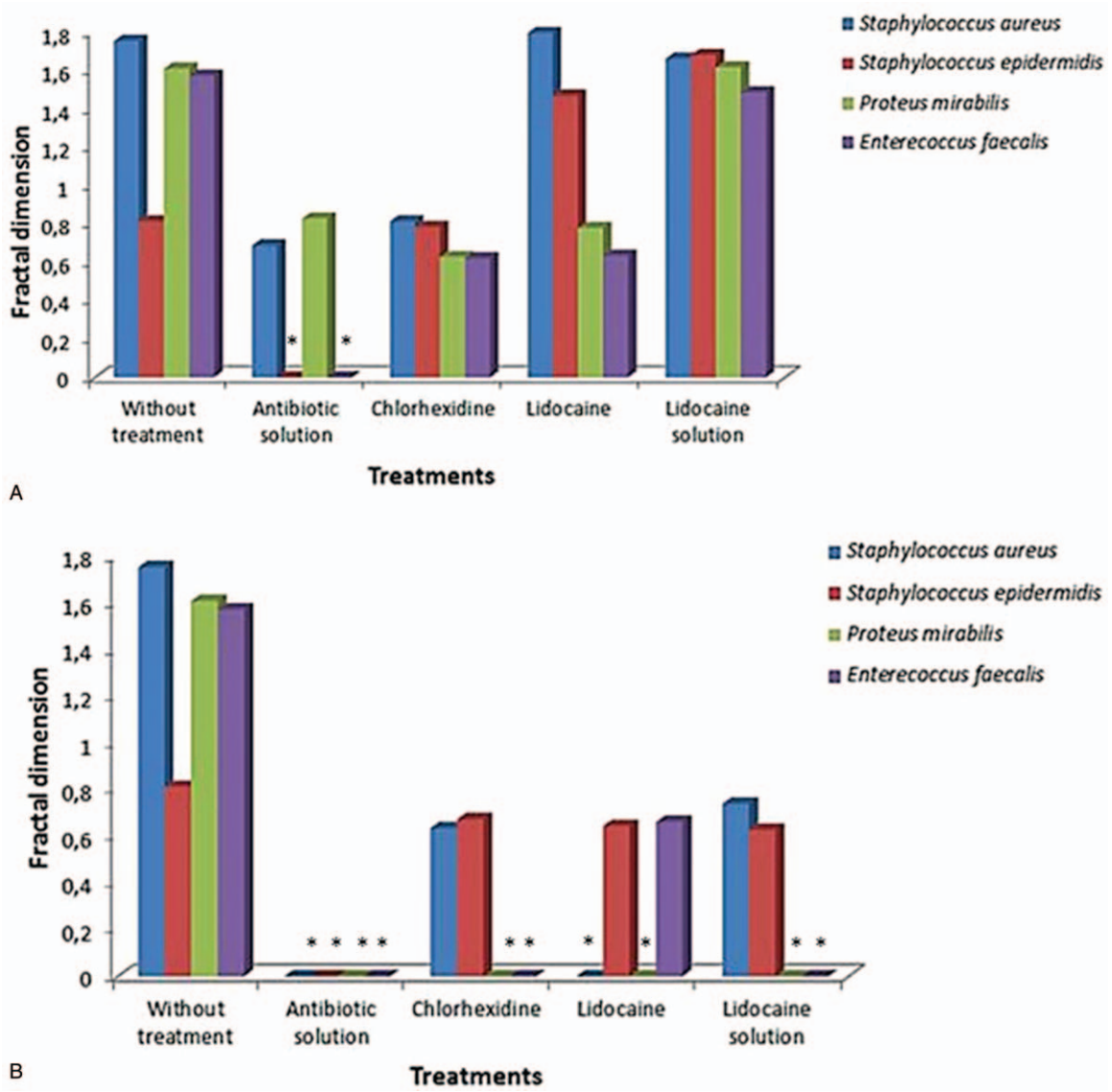

Figure 5. A - Fractal dimension of Petri dishes inoculated with ePTFE prostheses prewashed with solutions compared to untreated prostheses. B - Fractal dimension of Petri dishes inoculated with ePTFE prostheses washed with solutions after contamination compared to untreated prostheses. *: $P < .05$, when compared to non-treated prostheses.

aesthetic and restorative purposes. Postoperative infections and biofilm formation disrupt treatment and may result in surgical removal of the implant in acute infections or provide a favorable environment for the appearance of capsular contractures and large cell anaplastic lymphoma in late subclinical infections.[22] Strategies to prevent biofilm infiltration in breast implant mammoplasty involve the use of strict aseptic techniques, triple antibiotic irrigation (bacitracin-cefazolin-gentamicin) and additional strategies to prevent exposure to common breast microbiota (e.g., *S epidermidis* or other bacteria).[21] In the case of silicone prostheses, prewash with antibiotic solution inhibited the growth of *S epidermidis* and *P mirabilis*, and postcontamination wash was effective for all bacteria. These data show that washing pre- and postcontamination with antibiotic solution prevents the contamination of silicone prostheses by one of the main bacteria that contaminate these prostheses, *S epidermidis*.

Synthetic vascular grafts have provided life preservation and limb salvage for millions of patients worldwide, both through arterial revascularization grafts and hemodialysis access grafts. Although this technology has been used for many years, one of the most frequent causes of failure is bacterial colonization and infection.[23] Studies have shown that antibiotic therapy associated with partial or total preservation of the vascular graft in peripheral revascularization has a higher graft patency and lower amputation rate when compared to explants with extra-anatomical reconstruction.[24] Prostheses impregnated with antibiotics such as rifampicin, daptomycin, vancomycin and bacteriophage Endolisin HY-133 are in the process of being studied *in vitro*, as they present a viable alternative to reduce the infection of synthetic vascular grafts. However, cytotoxicity to endothelial cells, caused by the necessary concentration of these substances for bactericidal effects, causes necrosis of the

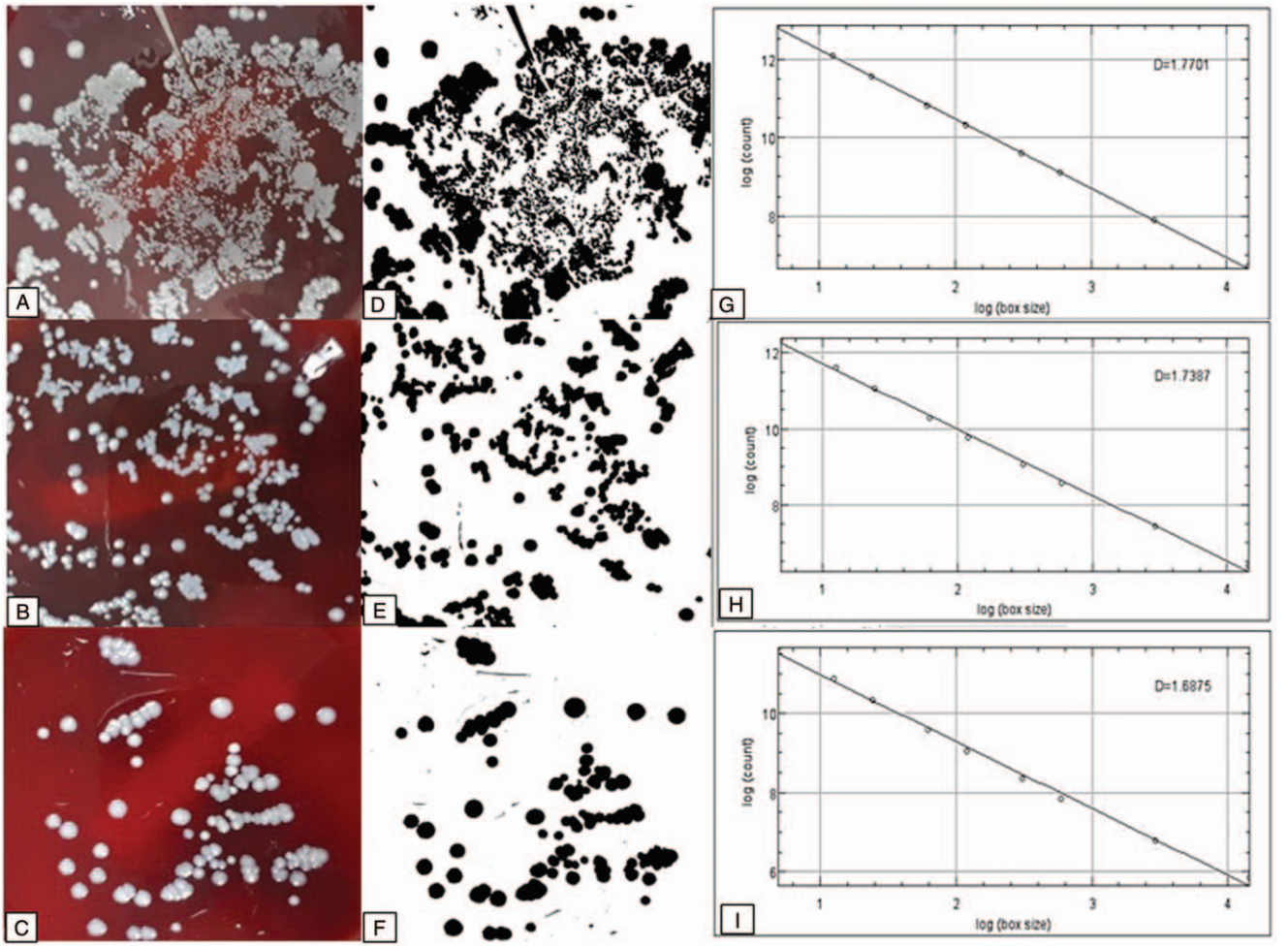

Figure 6. A - Original image of the Petri dishes inoculated with ePTFE prosthesis contaminated with *S aureus*. B - Original image of the Petri dishes inoculated with ePTFE prosthesis contaminated with *S aureus* and previously washed with chlorhexidine. C - Original image of the Petri dishes inoculated with ePTFE prosthesis contaminated with *S aureus* and later washed with chlorhexidine. D, E, and F- Binarized image. G, H, and I - Box-counting of the fractal dimension analysis.

anastomotic site, which constitutes a barrier to the development of these new materials.[25] In our study, prewashing with the antibiotic solution inhibited the growth of *S. epidermidis* and *E. faecalis* and decreased the number of *S. aureus* colonies. Additionally, for ePTFE prostheses, washing with antibiotic solution postcontamination were effective for all bacteria.

Thus, for both silicone and ePTFE prostheses, the use of antibiotic solution may be an alternative to prevent the loss of these implants and to avoid more serious complications associated with biofilms.

There was a decrease in bacterial colonization by *S aureus* in the precontamination wash, and bacterial colonies of *P mirabilis* and *E faecalis* were absent and *S. aureus* decreased in the chlorhexidine postcontamination washing for both prostheses. This can be explained by the strong action that chlorhexidine has on the destruction of bacterial biofilms.[26] Chlorhexedine has the function of displacing divalent cations (Mg^{2+} and Ca^{2+}) associated with phospholipid groups causing changes in the cell wall fluidity. At high concentrations the bacterial cell membrane adopts a liquid crystalline state leading to a rapid loss of cell content.[27] In silicone prostheses, chlorhexidine, in addition to reducing bacterial colonization by *S aureus* and *S epidermidis*, prevented colonization by *P mirabilis*. In ePTFE vascular prostheses, chlorhexidine prewash also decreased colonization by *E faecalis*. In an *in vitro* study, the antimicrobial effect of 0.02% chlorhexidine digluconate against *S aureus* biofilms in vascular grafts was observed.[26] In the biofilm formation analysis, chlorhexidine was the least effective solution in decreasing biofilm formation. However, there was an impregnation of the crystal violet in the wells where there was only BHI broth without bacteria (control). We believe that chlorhexidine favored the impregnation of the crystal of violet, falsifying the biofilm formation in the wells with bacteria and treated with this antiseptic. Therefore, chlorhexidine solution may be an alternative, albeit less effective, to minimize contamination by these bacteria.

Lidocaine is the most commonly used local anesthetic for small surgical procedures in Medicine, because it is inexpensive and easily administrable.[28] Although the use of local anesthetics appears to have an antimicrobial effect, this topic remains controversial. There are *in vivo* studies that used lidocaine in *S aureus*-infected wounds and have shown decreased bacterial counts in animals treated with this anesthetic,[28,29] and other studies showed that this anesthetic did not have antimicrobial

activity.[30,31] The mechanisms of action considered for lidocaine bactericidal activity are cell wall disruption, altered DNA synthesis and cellular respiration dysfunction.[29] In precontamination washing, pure lidocaine inhibited colonization by *P mirabilis* in silicone prostheses and decreased *E faecalis* colonization in ePTFE prostheses, while lidocaine solution decreased colonization by *P mirabilis* in silicone prostheses but had no effect in ePTFE prostheses. In postcontamination washing, silicone and ePTFE prostheses washed with pure lidocaine showed no colonization by *P mirabilis* and *S. aureus,* and there was a reduction in *S epidermidis*. As in the lidocaine solution, there was no colonization by *P mirabilis,* and there was a decrease in *S epidermidis*. Lidocaine has a fleeting bactericidal effect,[29] and in precontamination washing, possibly the lidocaine bactericidal effect had already ceased, so its efficacy was lower. Postcontamination washing with lidocaine was effective in eliminating contamination with *S. aureus*, the bacterium that most often infects silicone and ePTFE prostheses, and can be added to the therapeutic arsenal for preventing prostheses infections. In addition, postcontamination washing with lidocaine and lidocaine solution was also effective in reducing contamination with *S epidermidis*, one of the bacteria that most often contaminate both prostheses studied and is an alternative to control tissue infection in these cases, even without the possibility of preservation of the prostheses.

P mirabilis has an impressive arsenal of virulence factors. Urease is a critical feature of this species; however, it also expresses a number of fimbriae and adhesins, as well as a variety of potent toxins and proteases.[32] The process of adhesion and formation of biofilms can also be considered a virulence factor. Extracellular matrix (ECM) is a very important component of biofilms. It is composed of water (97%) and exopolymers, which are a mixture of polysaccharides (EPS), proteins, nucleic acids, glycoproteins and phospholipids. Bacterial EPS play an important role in biofilm development; they participate in the adhesion process, where they intensify cells that attach to solid surfaces as in prostheses.[33]

In the present study, *P mirabilis* adhesion was inhibited in both the postwash test of both contaminated prostheses. This fact may be associated with interference of the tested conditions on extracellular matrix formation and adhesion capacity of *P mirabilis* on surfaces. The conditions evaluated (antibiotic solution, chlorhexidine and lidocaine) are known to be poor in nutrients, and this fact may have been primordial in the expression of genes related to the adhesion of this microorganism. Mory et al.[34] evaluated the effect of nutrients and stress factors on polysaccharide synthesis in *P mirabilis* biofilms. In that work, it was observed that after biofilm cultivation in medium with reduced nutrient content, polysaccharide synthesis was inhibited by approximately 48% for twelve *P mirabilis* strains, which was associated with a reduction in adhesion by those microorganisms.made in our study

Postcontamination washing that we made in our study is similar to clinical practice, where it is sought to avoid replacement of the prosthesis. Thus, the washing of the infected prosthesis intraoperatively, mainly with an antibiotic solution (which inhibited the growth of all bacteria in both prostheses in postcontamination washing) should be considered by surgeons.

Although the data from our study have shown a good efficiency of pre- and postcontamination washes to decrease bacterial contamination, *in vivo* studies and prospective clinical trials are still needed to better clarify the real effectiveness of this therapeutic proposal.

With the data from this *in vitro* study, we can conclude that the pre- and postcontamination washes of the silicone and ePTFE prostheses showed efficiency in reducing bacterial contamination. *P mirabilis* was the most sensitive bacteria to postcontamination washing treatments. The antibiotic solution was the one that showed the most effectiveness in inhibiting the growth of bacteria in both washes. Lidocaine was able to reduce colonization by *S aureus* in post-contamination washing, showing that it can be used as an adjuvant treatment in these cases.

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Writing – review & editing: Fausto Viterbo Oliveira Neto, Mariângela Esther Alencar Marques.

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