In vitro evaluation of the antimicrobial efficacy of a new silver-triclosan vs a silver collagen-coated polyester vascular graft against methicillin-resistant *Staphylococcus aureus*

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**Objectives:** Vascular graft infection is a rare but serious complication of vascular reconstructive surgery. This in vitro study investigated the antimicrobial efficacy of a new, silver-triclosan collagen-coated polyester vascular graft compared with a silver collagen-coated polyester vascular graft alone during the first 24 hours.

**Methods:** The antimicrobial efficacy of the investigated vascular grafts was assessed by performing a time-kill kinetic assay following Clinical and Laboratory Institute Standards-approved guidelines M26-A. For the purpose of the experimental study, the ATCC 3591 strain of methicillin-resistant *Staphylococcus aureus* (American Type Culture Collection, Manassas, Va) was used. All assays were repeated sixfold. Bacterial survival numbers were obtained at 1, 4, 8, 12, and 24 hours using a standard plate count procedure. Bactericidal activity was defined as a $3 \log_{10}$ reduction factor (logRF), according to the approved guideline M26-A.

**Results:** Both antimicrobial vascular grafts achieved $>3 \log_{10}$ logRF and fulfilled the efficacy criterion for bactericidal activity but performed differently in their speed of antimicrobial action. The silver-triclosan vascular graft achieved 3.37 logRF after 8 hours, and the silver vascular graft showed a 4.19 logRF after 24 hours. The silver-triclosan graft yielded significantly lower colony-forming units/mL counts after 4 hours compared with the silver graft ($4.29 \times 10^4$ vs $1.03 \times 10^5$; $P = .031$).

**Conclusions:** Both antimicrobial collagen-coated polymer vascular grafts showed bactericidal activity against methicillin-resistant *Staphylococcus aureus* in vitro. Although the silver-triclosan vascular graft showed a faster antimicrobial efficacy, the silver graft exhibited its antimicrobial properties after 24 hours. Which concept will protect an implanted vascular prosthetic graft better from bacterial contamination and subsequent infection needs to be investigated further in in vivo animal and clinical studies. (J Vasc Surg 2012;55:S823-9.)

**Clinical Relevance:** Vascular graft infection is a rare but one of the most serious complications of vascular reconstructive surgery. Conservative treatment of prosthetic graft infections is rarely successful and is used only in patients with a high operative risk or apparently limited infection. The most pre-eminent strategy against this severe complication therefore is primary prevention of vascular graft infection. The use of antimicrobial vascular grafts might support prevention of vascular graft infection. Results of a standardized experimental study on the antimicrobial efficacy of the silver-triclosan collagen polyester vascular graft with an identical collagen polyester vascular graft containing silver alone are presented.
will be achieved by early and complete removal of the infected graft, with replacement using an in situ autologous vein or an extra-anatomic prosthetic bypass.\textsuperscript{13-16} Conservative treatment of prosthetic graft infections is rarely successful and is used only in patients with a high operative risk or apparently limited infection.\textsuperscript{12} It appears, therefore, that the most pre-eminent strategy against this severe complication is primary prevention of vascular graft infection.

In vascular surgery, scientific evidence supporting the efficacy of primary prevention of vascular graft infection is scant. Although many are outdated, a number of studies have investigated the utility of systemic prophylactic antibiotics in vascular surgery, and a variety of systemically administered antibiotics, singly or in combination, have been proposed.\textsuperscript{17-20} Only a few studies have investigated antimicrobial graft materials, predominantly containing topical antibiotics and some using topical antiseptics. In recent decades, various concepts for antimicrobial grafts have been developed to prevent vascular graft infection.\textsuperscript{21-24} However, demonstration of the clinical efficacy of antimicrobial graft material remains difficult because of the low incidence of vascular graft infection.

In vitro studies are therefore necessary to evaluate and compare these concepts, preferably under the same test conditions and test methodology. We describe the characteristics of a new antimicrobial polyester vascular graft that combines two topical antiseptic agents, silver acetate and triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol). The aim of this in vitro experimental study was to compare the antimicrobial efficacy of the silver-triclosan collagen polyester vascular graft with an identical collagen polyester vascular graft containing silver alone. A non-antimicrobial collagen polyester vascular graft served as control.

**METHODS**

This study was planned and conducted between September 2010 and October 2010. All tests were performed by NAMSA, an independent laboratory (North American Science Associates, Inc, Northwood, Ohio), following a predetermined study design.

Two antimicrobial vascular grafts and one non-antimicrobial vascular graft were investigated. The three vascular grafts were from MAQUET (La Ciotat, France): (1) a silver-triclosan collagen-coated polyester vascular graft (InterGard Synergy), containing the antiseptic agents silver acetate and triclosan; (2) a silver collagen-coated polyester vascular graft (InterGard Silver), containing silver acetate alone; and (3) a non-antimicrobial collagen coated polyester vascular graft (InterGard), serving as the control.

**Test organism.** For the purpose of the experimental study, the ATCC 33591 (American Type Culture Collection, Manassas, Va) strain of methicillin-resistant *Staphylococcus aureus* (MRSA) was used because *S aureus* is the most commonly reported pathogen responsible for vascular graft infection,\textsuperscript{25-29} and the presence of a *mec A* gene counteracts clinical efficacy of perioperative antibiotic prophylaxis with all β-lactam antibiotics.

**Test procedure for assessing antimicrobial efficacy.** The antimicrobial efficacy of the investigated vascular grafts was assessed under static contact conditions, and time-kill assays were performed according to the standard test method M26-A recommended by the Clinical and Laboratory Institute Standards (CLIS).\textsuperscript{30,31}

Briefly, a bacterial suspension was prepared by suspending a loopful of MRSA ATCC 33591 colonies in 9 mL of brain-heart infusion broth, which was incubated at 37°C ± 2°C for 18 to 24 hours. The density of the MRSA target inoculum was set at 1.0 × 10⁷ colony-forming units (CFU)/mL. Standard plate count on trypticase-soy agar plates was performed to determine the initial population of the test organisms.

To reach a power of 80%, six repeated measures of each test sample for each interval (1, 4, 8, 12, and 24 hours) were placed on sterile 60- × 15-mm Petri dishes. The small Petri dishes were placed individually into larger Petri dishes (100 × 15 mm) containing 10 mL of sterile water to create a humid environment.

Each test graft was aseptically cut into 20- × 20-mm pieces and placed with the outer surface facing up in the smaller Petri dish. For the vascular grafts, five sets of six repeated assays each were inoculated with 0.1 mL of the MRSA suspension and tested at static conditions incubated for 1, 4, 8, 12, and 24 hours in an incubator at 37°C ± 2°C. Five sets of six repeated assays each of MRSA suspensions without any test graft were prepared to serve as positive controls. Immediately (zero time, data not shown) and at the selected intervals, which were chosen to investigate the antimicrobial efficacy during the simulated immediate postoperative interval of 1 to 24 hours, aliquots were removed and placed into 100 mL of Dey-Engley neutralizing broth.

**Table I.** Growth of methicillin-resistant *Staphylococcus aureus* is shown for three vascular grafts—two antimicrobial and one non-antimicrobial—compared with the incubated inoculum without any graft material (control)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE (Log₁₀)</th>
<th>Log₁₀</th>
<th>Mean ± SE (Log₁₀)</th>
<th>Log₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
<td></td>
<td>4 hours</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.06 × 10⁶ ± 1.23 × 10²</td>
<td>6.49</td>
<td>6.27 × 10⁶ ± 3.32 × 10³</td>
<td>6.80</td>
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<tr>
<td>IGK</td>
<td>2.06 × 10⁶ ± 9.83 × 10⁴</td>
<td>6.31</td>
<td>1.84 × 10⁶ ± 2.37 × 10³</td>
<td>6.26</td>
</tr>
<tr>
<td>IGS</td>
<td>1.53 × 10⁶ ± 6.84 × 10⁴</td>
<td>6.18</td>
<td>1.03 × 10⁶ ± 1.86 × 10³</td>
<td>6.01</td>
</tr>
<tr>
<td>IGST</td>
<td>1.30 × 10⁶ ± 6.67 × 10⁴</td>
<td>6.11</td>
<td>4.29 × 10⁴ ± 6.90 × 10³</td>
<td>4.63</td>
</tr>
</tbody>
</table>

IGK, Non-antimicrobial InterGard vascular graft; IGS, InterGard Silver vascular graft; IGST, InterGard Silver/Triclosan vascular graft; SE, standard error.

*Data are shown as colony-forming units/mL.
prepared in-house by the NAMSA laboratory, and mixed for 1 minute using a vortex. Bacterial survival numbers were obtained by removing 0.1-mL samples of broth, and subsequent viable counts were determined at 1, 4, 8, 12, and 24 hours using a standard plate count procedure.

**Statistical analysis.** To determine the required number of repeated measurements for each graft at each of the five assessment times (1, 4, 8, 12, and 24 hours), a power analysis using a one-way analysis of variance (ANOVA) was performed. For a significance level of .05, we set the effect size of the antimicrobial grafts at a moderate $\Delta = 2.0$, assuming that an antimicrobial graft will show at least a double decrease in the numbers of test bacteria. Using this conservative assumption, a sixfold repeated measure of each time point reached a power of 80.5%. Therefore, all assays were repeated six times, and the numbers of organisms were averaged as the mean CFU/mL. The averaged means were then transformed and expressed as mean $\log_{10}$ CFU/mL.

To present the data in the format of a “time-kill curve,” the obtained viable mean $\log_{10}$ counts (CFU/mL) at each investigated time point were plotted for each graft and the control without any graft material at single time point, a nonparametric Wilcoxon signed-rank test was calculated. To determine one graft’s overall difference in the mean $\log_{10}$ CFU/mL over the five tested time points, a one-way ANOVA was computed. To minimize a type I error, one-way and two-way ANOVA were calculated with a Bonferroni correction. For all statistical tests, a value of $P < .05$ was considered statistically significant.

### RESULTS

The mean CFU/mL, together with standard error and the corresponding $\log_{10}$ transformations of the time-kill study, are summarized in Table I and plotted as time-kill curves in the Fig. As expected, no antibacterial activity was seen the incubated inoculum without any graft material (control; $P = .0964$, one-way ANOVA) and the non-antimicrobial collagen-coated polyester vascular graft (InterGard). The control curve (Fig) showed a typical growth curve for incubated bacteria in cultivation medium. The non-antimicrobial graft showed no increase, but also no decrease, in the number of viable organisms at the five time points during 24 hours of incubation ($P = .0798$ one-way ANOVA).

The antimicrobial vascular grafts both achieved $>$3 logRF and fulfilled the efficacy criterion for bactericidal activity; yet, they performed differently in their speed of antimicrobial action. The silver-triclosan vascular graft (InterGard Synergy) exhibited a mean 3.37 logRF after 8 hours, and the silver vascular graft (InterGard Silver) showed a mean 4.19 logRF after 24 hours of incubation (Table II) compared with the non-antimicrobial graft.

For the InterGard Silver graft, one-way ANOVA with Bonferroni correction showed a significant difference over time ($P = .0224; F = 3.45; df = 4$) with a significance between all five time points from 1 to 24 hours; for the InterGard Synergy, the difference was also significant ($P < .0001; F = 362.44; df = 4$; one-way ANOVA with Bonferroni correction) with significant differences between 1 vs 4 hours, 1 vs 8 hours, 1 vs 12 hours, and 1 vs 24 hours.

InterGard Silver yielded significantly lower mean CFU/mL counts at 24 hours than the non-antimicrobial control graft ($1.04 \times 10^4$ vs $6.47 \times 10^3$; $P = .031$ Wilcoxon rank-sum test). InterGard Synergy, however, achieved significant lower mean CFU/mL counts than the non-antimicrobial control graft already at 4 hours of incubation ($4.29 \times 10^4$ vs $1.84 \times 10^6$; $P = .031$ Wilcoxon-rank-sum test).

Comparing the two antimicrobial vascular grafts, InterGard Synergy yielded significantly lower mean CFU/mL counts at 4 hours vs InterGard Silver ($4.29 \times 10^4$ vs $1.03 \times 10^6$; $P = .031$ Wilcoxon rank-sum test), but not at 1 hour ($1.30 \times 10^6$ vs $1.53 \times 10^6$; $P = .437$ Wilcoxon rank-sum test).

#### Table I. Continued.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE</th>
<th>Log$_{10}$</th>
<th>Mean ± SE</th>
<th>Log$_{10}$</th>
<th>Mean ± SE</th>
<th>Log$_{10}$</th>
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<tbody>
<tr>
<td>Control</td>
<td>$2.35 \times 10^2 \pm 2.74 \times 10^2$</td>
<td>7.37</td>
<td>$3.20 \times 10^2 \pm 5.42 \times 10^2$</td>
<td>7.51</td>
<td>$1.60 \times 10^2 \pm 3.53 \times 10^2$</td>
<td>8.20</td>
</tr>
<tr>
<td>IGK</td>
<td>$2.30 \times 10^2 \pm 2.65 \times 10^2$</td>
<td>6.36</td>
<td>$2.64 \times 10^2 \pm 9.83 \times 10^2$</td>
<td>6.42</td>
<td>$6.47 \times 10^2 \pm 2.67 \times 10^2$</td>
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<tr>
<td>IGS</td>
<td>$4.27 \times 10^2 \pm 1.19 \times 10^2$</td>
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<td>$3.75 \times 10^2 \pm 9.16 \times 10^2$</td>
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<td>$1.04 \times 10^2 \pm 4.97 \times 10^2$</td>
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</tr>
<tr>
<td>IGST</td>
<td>$9.96 \times 10^2 \pm 7.52 \times 10^2$</td>
<td>4.00</td>
<td>$1.58 \times 10^2 \pm 5.83 \times 10^2$</td>
<td>2.20</td>
<td>$1.00 \times 10^2 \pm 1.00 \times 10^2$</td>
<td>2.00</td>
</tr>
</tbody>
</table>

**RESULTS**
DISCUSSION

The silver vascular graft and the silver-triclosan vascular graft both showed bactericidal efficacy against MRSA in this standard in vitro method. There was, however, a difference in the time when the antimicrobial efficacy reached $>3$ logRF. Although the silver-triclosan vascular graft exhibited $3.37$ logRF after 8 hours, the silver vascular graft achieved $>3$ logRF only after 24 hours.

Because both collagen-coated polyester vascular grafts are manufactured under the same standards and differ only in the presence or absence of triclosan, the observed difference in the antimicrobial efficacy is explained only by the additional antimicrobial activity of triclosan. The addition of triclosan to the already-existing silver collagen-coated polyester graft has microbiologic advantages.

The silver mode of action has already been described in details elsewhere. Although the antimicrobial efficacy of silver covers a broad microbial spectrum, its mode of action is not as fast as triclosan, as was shown in the present study. Silver needs longer contact times with possible pathogens and will exhibit its antimicrobial properties only over longer periods. Furthermore, silver is released more slowly from the graft material than triclosan.

Triclosan alone, on the other hand, would be the ideal antiseptic to be used for coating vascular grafts because it is toxicologically safe and has a rapid antimicrobial action. Triclosan, however, leaches rapidly from the graft material into the surrounding tissue and will be metabolized in the liver, principally by phase II metabolism, to glucuronide and sulfate conjugates that have an elimination half-life of 13 hours after one oral exposure. Triclosan is a

Table II. Mean log$_{10}$ reduction factors as the difference in means from three grafts—two antimicrobial and one nonantimicrobial—compared with the incubated inoculum without graft material (control)

<table>
<thead>
<tr>
<th>Variable</th>
<th>1 h</th>
<th>4 h</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGK</td>
<td>0.17</td>
<td>0.53</td>
<td>1.01</td>
<td>1.08</td>
<td>2.39</td>
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<tr>
<td>IGS</td>
<td>0.30</td>
<td>0.78</td>
<td>1.74</td>
<td>1.93</td>
<td>4.19*</td>
</tr>
<tr>
<td>IGST</td>
<td>0.37</td>
<td>2.16</td>
<td>3.37*</td>
<td>5.31*</td>
<td>6.20*</td>
</tr>
</tbody>
</table>

IGK, Non-antimicrobial InterGard vascular graft; IGS, InterGard Silver vascular graft; IGST, InterGard Silver/triclosan vascular graft.

*Bactericidal efficacy at each exposure time as defined by approved guideline Clinical and Laboratory Institute Standards M-26A, 1999.

Fig. Time-kill curve of two antimicrobial (InterGard Silver [IGS]; solid line, square; InterGard Synergy [IGST]; large-dashed line, circle) vascular grafts and one non-antimicrobial (InterGard [IGK] dotted line, triangle) vascular graft against methicillin-resistant Staphylococcus aureus (MRSA) growth without graft material during 4 to 24 hours of incubation. The mean colony-forming units/mL counts of MRSA are shown. The control curve (small-dashed line, rhombus) without any graft material shows the typical growth curve for incubated bacteria in cultivation medium.
stable, synthetic, polychlorinated, aromatic hydrocarbon with broad, antimicrobial properties. It is lipophilic and active within a broad pH range (optimum pH, 4-8) unlike other antiseptics that become inactive at high or low pH.

Triclosan passively dissipates from implanted grafts to the surrounding tissues, where it is absorbed into the bloodstream and widely distributed, but it is not confined to any particular tissue or organ system. After one exposure, triclosan is cleared from the bloodstream in ~3.8 days. Conjugated triclosan is readily water soluble and is excreted from the body by the kidneys. Combining silver acetate with triclosan compensates for its respective pharmacokinetic disadvantages in time of action and duration, and a faster and prolonged antimicrobial activity is achieved.

Triclosan is present in some medical products and in a broad range of consumer products. In health care, triclosan is used in medicated soap and skin antiseptics at concentrations of 0.2% to 0.5% and up to 3%, respectively, in ointments and impregnated/coated medical devices such as catheters and surgical sutures. Aside from its medical use, such as for decolonization of MRSA carriers or prevention of medical device contamination and implant infection, triclosan is used in a surprisingly extensive range of consumer, personal health care, and household products, which is in some cases questionable (eg, kitchenware and dishwashing liquids) and in others is frivolous and unnecessary (eg, dogs’ drinking bowls or antimicrobial underwear).

The consequences of this widespread, unregulated use of triclosan, allied with marketing that plays to consumer fears, has spurred critical comment in lay and professional media and has driven scepticism and mistrust about its use. It has also provoked calls for a wider analysis of the health and environmental issues associated with overuse of triclosan, particularly in Northern European countries.

The interest has focused on potential health concerns associated with triclosan side products released to the environment during the breakdown of triclosan, which, however, occurs in nature only in certain situations, such as presence of UV light, but never in the human body. Potentially harmful side products include chloroform gas (CHCl3), which may be produced by the reaction of triclosan and chlorine in tap water. Chloroform is classified by the EPA as a probable carcinogenic compound and has been shown to cause hepatocellular carcinoma in rats. Chloroform is also toxic at high doses (fatal oral dose, 10 mL [14.8 g]; death due to respiratory or cardiac arrest).

In the presence of hydrogen chloride, triclosan can also form the dioxin 2,4-dichlorophenol (a chlorinated derivative of phenol). However, this reaction occurs only in presence of UV radiation. The mere mention of dioxins alarms many people, but of 210 known dioxins, only 17 are harmful. The most toxic dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is not formed with triclosan.

Calls for the restriction of triclosan use are justified in context with the increase of triclosan in a wide range of consumer products. However, if the use of triclosan is associated with demonstrable health benefits, such as in medical applications for the prevention or treatment of infection, its use should be considered and shall be distinguished from indications with no plausible or proven benefit.

Although beneficial, the use of vascular prosthetic grafts is correlated with an increased risk of infection, morbidity, and death. This, however, is not only restricted to vascular grafts but also applies to all implanted prosthetic material. The risk for infection varies by medical specialization and medical device. Reported infection rates range from 1% to 6% after cardiac valve implantation, 0.5% to 6% for vascular grafts, 10% to 30% for urinary catheters, 25% to 50% for aortic balloon pumps, 5% to 41% for neurosurgical liquor shunts, and 1% to 20% for hernia mesh implants.

The morbidity associated with prosthetic graft infection is high. For peripheral vascular prostheses, morbidity of up to 41% has been reported, mostly resulting in amputation, together with an attributable mortality rate of 17%. A mortality rate of 24% to 75% was observed in patients with aortic vascular grafts, with a 5-year survival rate <50%.

Prosthetic graft infection starts with contamination of the graft and formation of biofilms. Biofilms result from microbial adhesion to surfaces in moist environments and can be found in virtually every environment with sufficient provision of water and nutrients. Formation of a biofilm begins with the attachment of planktonic microorganisms to a surface. The microbial attachment is initially supported by reversible van der Waals forces and electrostatic bonds, dipole interactions, hydrogen bonds, and covalent bonds. If the attached organisms are not immediately separated from the surface or inactivated, they will start colonizing the surface permanently, supported by cell adhesion structures and active, progressive movement. Within the formed biofilm, bacteria may communicate by the release of signal molecules ("quorum sensing") that coordinate the formation of structured and stable biofilms. Once colonization begins, the biofilm will grow by a combination of cell division and by genesis of a matrix of excreted extracellular polymeric substance. This matrix protects the microorganisms embedded within the biofilm. From the microorganism’s perspective, one major benefit of this matrix is the increased protection against the immune system and considerably decreased susceptibility to antiseptics and antibiotics. It is therefore desirable to minimize the formation of biofilms on medical implants or to prevent their formation completely.

Antimicrobial prosthetic grafts might limit colonization by microorganisms, and this study provides quantitative and objective results of the in vitro efficacy of two antimicrobial vascular grafts. However, we recognize that our study has some limitations. The study was designed to assess the antimicrobial efficacy within the immediate postoperative period, defined as 24 hours; however, depending on the product type, this time frame can be discussed. This choice was made considering that it is generally believed that most vascular graft infections have their origins in the immediate postoperative period. With this in mind, the silver collagen and silver triclosan grafts were designed for inhibiting microbial colonization of the graft during the
early postoperative period only by providing a release of antimicrobial agents up to 30 days. Whether this is sufficient in current clinical practice to prevent vascular graft infections is unknown.

Furthermore, the in vitro tests applied here were performed on planktonic bacterial suspensions and not on established biofilms. Considering that this experimental work aimed to assess antimicrobial efficacy during the immediate postoperative period, the use of planktonic culture is relevant because biofilms require a longer time to develop. However, on a longer time scale, some bacterial species may initiate biofilm formation for protection against the environment. Biofilms are less susceptible to antimicrobial agents because the bacteria are protected by the extra-cellular polymeric substance.\textsuperscript{44,45} This aspect should also be addressed when assessing long-term antimicrobial efficacy in future experiments.

Finally, this study tested the antimicrobial efficacy against one MRSA strain only. Therefore, our conclusions are valid only for Gram-positive cocci, but no statement can be made about Gram-negative and -positive bacteria. How-ever, because \textit{S. aureus} is the most common pathogen causative for vascular graft infection\textsuperscript{25-29} and methicillin resistance is rapidly increasing worldwide,\textsuperscript{46} preventing \textit{S. aureus} vascular graft infections is of particular relevance.

CONCLUSIONS

The two antimicrobial collagen-coated polymer vascular grafts showed bactericidal activity against MRSA in vitro. The silver-triclosan vascular graft showed faster antimicrobial efficacy, and the silver graft exhibited its antimicrobial properties after 24 hours. It remains unknown if this concept will protect an implanted vascular prosthesis better from bacterial contamination and infection. This aspect needs to be investigated further in in vivo animal and clinical studies.

AUTHOR CONTRIBUTIONS

Conception and design: JR, OA, FS, AA
Analysis and interpretation: JR, OA, AA
Data collection: JR, OA
Writing the article: OA, JR, AA, FS
Critical revision of the article: JR, AA, FS, OA
Final approval of the article: JR, AA; FS, OA
Statistical analysis: OA
Obtained funding: Not applicable
Overall responsibility: JR
JR and OA contributed equally to this work.

REFERENCES


