ORIGINAL ARTICLE

Disinfection of Needleless Connectors to Reduce *Staphylococcus aureus* Bacterial Load

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Highlights

- Compare effectiveness of chemical disinfectants in reducing *S. aureus*.
- Five disinfectants reduced the bacterial load, especially chlorhexidine solutions.
- Focus on Brazilian clinical practice of needleless connector disinfection.

Abstract

Purpose: This study aimed to gain further knowledge about the comparative effectiveness of chemical disinfectants in reducing the bacterial load of NCs inoculated with *S. aureus*.

Methods: Disinfection of needleless connectors was undertaken in vitro against Staphylococcus aureus comparing 70% isopropyl alcohol (IPA), 70% ethanol, 0.5% and 2% chlorhexidine in 70% IPA applied with gauze, and 70% IPA single-use cap (Site-Scrub®).

Results: All disinfectants reduced the bacterial load ($P < 0.001$), especially the chlorhexidine solutions. Mechanical friction should follow guidelines.

Conclusion: This study found that all tested disinfectants effectively reduced the bacterial load and more clinical studies must be developed with a focus on the Brazilian clinical practice of needleless connector disinfection.

Keywords: *Staphylococcus aureus*, catheter-related bloodstream infection, needleless connector, vascular access device, disinfection

Introduction

Needleless connectors (NCs) provide entry to vascular access devices for the administration of intravenous fluids, medications, blood products, and other intravenous therapies. However, due to connector design, environmental exposure, and manual manipulation, NCs can increase the risk of catheter-associated bloodstream infection (CABSI).[1,2] For this reason, clinical practice guidelines such as Infusion Therapy Standards of Practice[3] and Guidelines for the Prevention of Intravascular Catheter-Related Infections[4] recommend NC external surface disinfection using mechanical friction before each device manipulation. Different chemical disinfectants and NC designs have been introduced to reduce NC bacterial contamination.[5] A recent systematic review concluded that alcohol-impregnated single-use caps and alcoholic chlorhexidine gluconate (CHG) wipes were associated with significantly lower CABSI than 70% isopropyl alcohol (IPA) wipes.[1] However, this review included no randomized controlled trials or studies evaluating many of the chemical disinfectants used in lower resource settings (e.g., Brazil). In Brazil, there is a difference in available products for the disinfection of NC in comparison with the USA. In the USA, all disinfectants are available in a single-use option (a wipe or swab), while in Brazil, the disinfectants used can be bulk and applied to gauze for use. There is a lack of studies that replicate techniques used for disinfection in countries such as Brazil.

Related to the different methods of disinfection, a pilot randomized controlled study (180 patients) in Australia identified the superiority of 2% CHG in 70% IPA wipes, but this product is not widely available in low-resource settings.[5] Low-resource settings are identified as health care systems that do not meet the minimum standards set by organizations such as the World Health Organisation.

Staphylococcus aureus is the predominant Gram-positive bacteria responsible for peripheral intravenous CABSI.[6] Thus, this study aimed to gain further knowledge about the comparative effectiveness of chemical disinfectants in reducing the bacterial load of NCs inoculated with *S. aureus*.

Methods

In this *in vitro* study, we compared the antimicrobial effectiveness of 4 disinfectants applied with sterile gauze: 70% IPA liquid (Rialcool®, Rioquímica), 70% ethanol liquid (Rialcool, Rioquímica), 0.5% and 2.0% CHG in 70% IPA liquid (Riohex®, Rioquímica); to that of 70% IPA single-use cap (Site-Scrub®, Becton Dickinson). The choice of disinfectant used was based on the most common disinfection methods used in a hospital ward in Brazil in which the study was based. It is believed that there is deviation in practice related to the disinfection of NC.

Staphylococcus aureus ATCC 25923 was used as the test organism and applied onto a commonly used NC. The NC used in the study was Safeflow® (B. Braun, Rio de Janeiro, Brazil), which is a luer-activated valve developed as NC injection port in intravascular applications (Figure 1). It is an easy access for Luer Lock and Slip connections, and it provides a fluid flow rate of 360 mL/min according to the B. Braun technical information leaflet. This device is compatible with magnetic resonance imaging, lipids, and blood, and it is latex-free and Di (2-ethylhexyl) phthalate (DEHP) free.

Ninety experiments were sequentially performed by a single individual (including biological quintuplicates and 3 procedural repetitions to ensure validity results). Before testing the disinfectants, a negative control ensured absence of preexisting NC contamination, while a nontreated control certified that the bacterial load was applied.

Prestudy experiments validated the required methods including incubation and sonication times. Neutralizer was not used.

Figure 1. **Needleless connectors Safeflow®; B. Braun, Brazil.**

Interventions

Each NC was placed for 20 minutes in a tube containing 1.2 mL of 10^6 colony forming units (CFUs)/mL bacterial suspension of *S. aureus* ATCC 25923 strains. When inoculating the NC, the end was closed with a plastic device that comes with the NC for protection (Figure 1). Next, NCs were removed from the tubes, shaken 3 times in a Petri dish to remove the excess, and dried at 35°C for 2 hours.

For disinfection, 1 mL of each disinfectant solution was applied to sterile gauze, then applied to the external NC surface to simulate clinical practice. The method of disinfection and the amount of disinfectant used aimed to reproduce the practice of disinfection in a specific Brazilian hospital. The NCs were scrubbed with movements of 180° (15 times) for 15 seconds (1 time/second) and left to dry for 15 seconds, as per guideline recommendations.[3] This study was submitted and conducted following the 2016 Infusion Therapy Standards of Practice.[3] The NC with the 70% IPA single-use cap disinfection met the manufacturer's recommendations and is not designed to be left on the NC.

Each connector was then placed inside a tube containing 2 mL of Ringer's Lactate® (B. Braun), then immediately vortexed for 5 minutes and sonicated (40 Hz) for 10 minutes. The connectors were removed, and 100 µL of the suspension was plated on mannitol salt agar. The plates were incubated for 24 hours at 35 ± 2°C, and the CFU were counted for each treated NC and the nontreated control.

Statistical analysis

Statistical analysis was carried out using MedCalc® version 19.1.7 (Belgium). Differences $P \leq 0.001$ were considered statistically significant. Evaluation of effectiveness, using the Kruskal–Wallis test and the Conover test for post hoc analysis, was performed by comparing bacterial loads recovered after each NC treatment and with nondisinfected controls.

Results

The final analysis included 83 experiments (outliers were excluded). The median bacterial load of the nontreated control was 223.5 CFU. Following the disinfection procedure as per international guidelines outlined above, all disinfectants were found to be effective in reducing the bacterial load, with rates varying from 93.3% (70% IPA single-use cap) to 100% (0.5% and 2% CHG in 70% IPA; $P < 0.001$; Table).

No differences in effectiveness were identified between 0.5% and 2% CHG concentrations. No differences in effectiveness were identified between the types of alcohol (IPA or ethanol). However, CHG solutions were significantly more effective than alcohol-based disinfectants. We found significant but lower reduction in bacterial load with the 70% IPA single-use cap (Figure 2).

Discussion

This study compared the impact of 5 disinfectants frequently used to reduce bacterial load on NCs. *S. aureus* was selected as the test organism because this pathogen is one of the most common causes of bloodstream infections globally.[7] In fact, *S. aureus* imposes an important health care burden, particularly in low-resource countries.[6,7] Despite the adoption of 70% IPA single-use cap by some Brazilian institutions, liquid disinfectants are still used in most public hospitals to scrub NCs due to their low cost.

Table. **Comparative Effectiveness of Each Disinfectant to Reduce the Bacterial Load on Needleless Connector Surfaces**

Treatments	n	CFU median	% reduction in bacterial load	Difference ($P < 0.001$) between treatments[a]
(1) 0.5% CHG in 70% IPA	15	0	100	(3)(4)(5)(6)
(2) 2% CHG in 70% IPA	15	0	100	(3)(4)(5)(6)
(3) 70% Ethanol	13	4	98.2	(1)(2)(5)(6)
(4) 70% IPA	15	2	99.1	(1)(2)(5)(6)
(5) 70% IPA single-use cap	11	15	93.3	(1)(2)(3)(4)(6)
(6) NTC	14	223.5	-	(1)(2)(3)(4)(5)

CFU = colony forming units; CHG = chlorhexidine; IPA = isopropyl alcohol; NTC = nontreated control.
[a] Treatments indicated by the numbers in the first column. Differences calculated using the Kruskal–Wallis test and the Conover test for post hoc analysis.

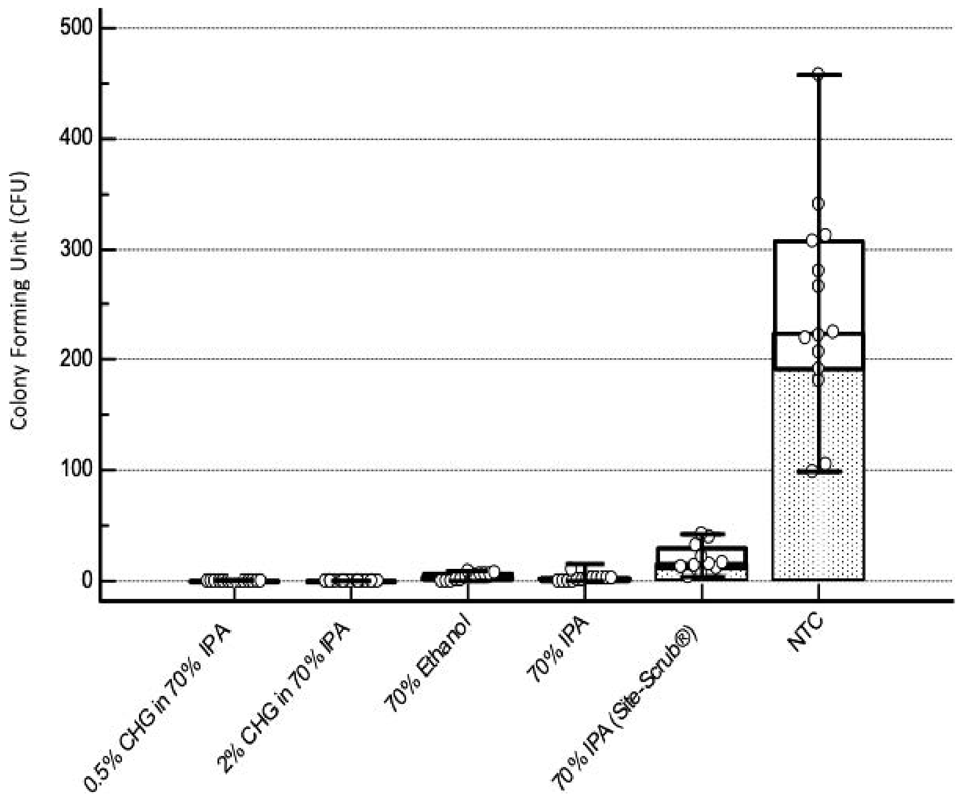

Figure 2. **Multiple comparison graph of bacterial load on needleless connectors between control and disinfection treatments. CHG = chlorhexidine; IPA = isopropyl alcohol; NTC = nontreated control. The white circles represent each individual experiment. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. A line extends from the minimum to the maximum value.**

Four tested disinfectants (70% IPA, 70% ethanol, and 0.5% and 2% CHG in 70% IPA) were the most effective at removing bacteria from NCs. A recent factorial randomized trial found 70% IPA and 2% CHG in 70% IPA to be, respectively, 97% and 99% effective at NC decontamination in adults.[8]

Although 70% IPA based, the single-use cap was not as effective as other disinfectants, likely due to the differences of mechanical friction (possibly higher using gauze) and the cap size (smaller than the NC surface area). This finding is in accordance with previous *in vitro* studies comparing different CHG and alcohol disinfectant solutions with 70% IPA caps.[9] Another *in vitro* study reported a favorable reduction in *S. aureus* bacterial contamination with IPA single-use caps, but in that study, the caps remained connected to the NCs for 1, 3, or 7 days[10]; therefore, the passive disinfection time was not comparable with our study.

All disinfectants reduced the bacterial load of *S. aureus*, with CHG in IPA formulations being most effective. More studies are necessary to confirm our observation, especially in the clinical setting where compliance to guidelines is often lacking. The clinical implications of this study are to test NC disinfection methods used in countries such as Brazil and the possibility of developing a follow-up study in a clinical situation based on these findings.

Strengths of our study are (1) the standardization of disinfection technique: the time to scrub and dry the disinfectant on the NC surface was the same (15 seconds) and performed by a single person, (2) the use of nontreated and negative controls, and (3) experiments were performed in triplicate and with biological quintuplicates. Although the use of a single NC type could be considered a limitation of this study, the NC evaluated was the type most used in Brazilian clinical practice.

Disclosures

CMR's previous employer, Griffith University, has received unrestricted investigator-initiated research grants on her behalf from BD-Bard and Cardinal Health, and consultancy payments on her behalf from manufacturers (3M, BD-Bard; unrelated to current project).

GRB's employer, Griffith University, has received payments for educational lectures on her behalf from 3M, Becton Dickinson (BD)-Bard, and Medline, unrelated to this project.

AJU's employer, Griffith University, has received unrestricted research grants and payments for educational lectures from 3M, Becton Dickinson (BD)-Bard, B. Braun, and Cardinal Health on her behalf (unrelated to current project).

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Conception and study design: PKR, TCMS, CBD. Data collection: PKR. Data analysis and interpretation of data for the work: PKR, CMR, ACG, TCMS, GRB, AJU, CBD, MLGP. Drafting the work or revising it critically for important intel-

lectual content: PKR, CMR, ACG, TCMS, GRB, AJU, CBD, MLGP. Final approval of the version to be published: PKR, CMR, ACG, TCMS, GRB, AJU, CBD, MLGP. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: PKR, CMR, ACG, TCMS, GRB, AJU, CBD, MLGP.

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