



**University of Dundee**

## **Disinfection of Needless Connectors to Reduce Staphylococcus Aureus Bacterial Load**

Biazus-Dalcin, Camila; Rocha, Patricia Kuerten; Rickard, Claire M; Gales, Ana Christina; Sincero, Thaís Cristine Marques; Ray-Barruel, Gillian

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1 **DISINFECTION OF NEEDLELESS CONNECTORS TO REDUCE**  
2 ***STAPHYLOCOCCUS AUREUS* BACTERIAL LOAD**

3 **Abbreviated title: DISINFECTION OF NEEDLELESS CONNECTORS**

4 **1373 words main body**

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20 **Abstract (50 words)**

21 Disinfection of needleless connectors was undertaken in vitro against *Staphylococcus aureus*  
22 comparing 70% isopropyl alcohol (IPA), 70% ethanol, 0.5% and 2% chlorhexidine in 70%  
23 IPA applied with gauze, and 70% IPA single-use cap (Site-Scrub®). All disinfectants reduced  
24 the bacterial load ( $p < 0.001$ ), especially the chlorhexidine solutions. Mechanical friction  
25 should follow guidelines.

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

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29 **Highlights**

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

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## 46 INTRODUCTION

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

65 **Related to the different methods of disinfection,** a pilot randomized controlled study (180  
66 patients) in Australia identified the superiority of 2% CHG in 70% IPA wipes, but this  
67 product is not widely available in low-resource settings <sup>(5)</sup>. **Low-resource setting are  
68 identified as health care system that do not meet the minimum standards set by organisations  
69 such as the World Health Organisation (WHO).**

70 *Staphylococcus aureus* is the predominant Gram positive bacteria responsible for peripheral  
71 intravenous catheter associated bloodstream infection <sup>(6)</sup>. Thus, this study aimed to gain  
72 further knowledge about the comparative effectiveness of chemical disinfectants in reducing  
73 the bacterial load of NCs inoculated with *S. aureus*.

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## 75 **METHODS**

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77 In this *in vitro* study, we compared the antimicrobial effectiveness of four disinfectants  
78 applied with sterile gauze: 70% IPA liquid (Rialcool<sup>®</sup>, Rioquímica); 70% ethanol liquid  
79 (Rialcool<sup>®</sup>, Rioquímica); 0.5% and 2.0% CHG in 70% IPA liquid (Riohex<sup>®</sup>, Rioquímica); to  
80 that of 70% IPA single-use cap (Site-Scrub<sup>®</sup>, Becton Dickinson). **The choice of disinfectant**  
81 **used was based in the most common disinfection methods used in a pediatric ward from a**  
82 **Hospital in Brazil in which the study was based. It is believed that there is deviation in**  
83 **practice related to the disinfection of NC.**

84 *Staphylococcus aureus* ATCC 25923 was used as the test organism and applied onto a  
85 commonly used NC. **The NC used in the study was Safeflow<sup>®</sup>; B. Braun (Brazil), which is a**  
86 **luer activated valve developed as needleless connector injection port in intravascular**  
87 **applications [Figure 1]. It an easy access for Luer Lock and Slip connections and it provides a**  
88 **fluid flow rate of 360 ml/min according to B. Braun Brazil technical information leaflet. This**  
89 **device is compatible with MRI, lipids and blood and it is latex-free and DEHP free.**

90 Ninety experiments were sequentially performed by a single individual (including biological  
91 quintuplicates and three procedural repetitions to ensure validity results). Prior to testing the  
92 disinfectants, a negative control ensured absence of pre-existing NC contamination, while a  
93 non-treated control certified that the bacterial load was applied.

94 Pre-study experiments validated the required methods including incubation and sonication  
95 times. Neutralizer was not used.

96

## 97 **Interventions**

98 **Each** NCs were placed for 20 minutes in a tube containing 1.2 mL of  $10^6$  colony forming  
99 units (CFU)/mL bacterial suspension of *S. aureus* ATCC 25923 strains. **When inoculating the**  
100 **NC, the end was closed with a plastic device that comes with the NC for protection [Figure**  
101 **1].** Next, NCs were removed from the tubes, shaken three times in a Petri dish to remove the  
102 excess, and dried at 35 °C for 2 hours.

103 For disinfection, 1 mL of each disinfectant solution was applied to sterile gauze, then applied  
104 to the external NC surface to simulate clinical practice. **The method of disinfection and the**  
105 **amount of disinfectant used aimed to reproduce the practice of disinfection in a specific**  
106 **hospital ward.** The NCs were scrubbed with movements of 180° (15 times) for 15 seconds (1  
107 time/second) and left to dry for 15 seconds, as per guideline recommendations<sup>(3)</sup>. **This**  
108 **studied was submitted and conducted following the Infusion therapy standards of practice from**  
109 **2016<sup>(3)</sup>.** The NC with the 70% IPA single-use cap disinfection met the manufacturer's  
110 recommendations **and are not designed to be left on the NC.**

111 Each connector was then placed inside a tube containing 2 mL of Ringer's Lactate®  
112 (BBraun), then immediately vortexed for 5 minutes and sonicated (40 Hz) for 10 minutes.  
113 The connectors were removed and 100 µL of the suspension was plated on mannitol salt agar.  
114 The plates were incubated for 24 hours at  $35 \pm 2$  °C, and the CFU were counted for each  
115 treated NC and the non-treated control.

116

117 **Statistical analysis**

118 Statistical analysis was carried out using MedCalc® version 19.1.7 (Belgium). Differences  
119  $p \leq 0.001$  were considered statistically significant. Evaluation of effectiveness, using Kruskal  
120 Wallis test and Conover as post hoc analysis, was performed by comparing bacterial loads  
121 recovered after each NC treatment and with non-disinfected controls.

122

123 **RESULTS**

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125 The final analysis included 83 experiments (outliers were excluded). The median bacterial  
126 load of the non-treated control was 223.5 CFU. Following the disinfection procedure as per  
127 international guidelines outlined above, all disinfectants were found to be effective in  
128 reducing the bacterial load, with rates varying from 93.3% (70% IPA single-use cap) to 100%  
129 (0.5% and 2% CHG in 70% IPA) ( $p < 0.001$ ) [Table 1].

130 No differences in effectiveness were identified between 0.5% and 2% CHG concentrations.

131 No differences in effectiveness were identified between the types of alcohol (IPA or  
132 Ethanol). However, CHG solutions were significantly more effective than alcohol-based  
133 disinfectants. We found significant but lower reduction in bacterial load with the 70% IPA  
134 single-use cap [Figure 2].

135

136 **DISCUSSION**

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138 This study compared the impact of five disinfectants frequently used to reduce bacterial load  
139 on NCs. *S. aureus* was selected as the test organism because this pathogen is one of the most  
140 common causes of bloodstream infections globally <sup>(7)</sup>. In fact, *S. aureus* imposes an important



141 healthcare burden, particularly in low-resource countries <sup>(6, 7)</sup>. Despite the adoption of 70%  
142 IPA single-use cap by some Brazilian institutions, liquid disinfectants are still used in most  
143 public hospitals to scrub NCs due to their low cost.

144 Four tested disinfectants (70% IPA, 70% Ethanol, and 0.5% and 2% CHG in 70% IPA) were  
145 the most effective at removing bacteria from NCs. A recent factorial randomized trial found  
146 70% IPA and 2% CHG in 70% IPA to be respectively 97% and 99% effective at NC  
147 decontamination in adults <sup>(8)</sup>.

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

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

160 Strengths of our study are: (1) the standardization of disinfection technique: the time to scrub  
161 and dry the disinfectant on the NC surface was the same (15 seconds) and performed by a  
162 single person; (2) the use of non-treated and negative controls; and (3) experiments were  
163 performed in triplicate and with biological quintuplicates. Although the use of a single NC  
164 type could be considered a limitation of this study, the NC evaluated was the type most

165 commonly used in Brazilian clinical practice.

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220

221 Figure 1. Multiple comparison graph of bacterial load on NCs between control and  
222 disinfection treatments  
223  
224

225 Legend: NTC: Non-treated Control; IPA: Isopropyl Alcohol; CHG: Chlorhexidine. The white  
226 circles represent each individual experiment. The central box represents the values from the  
227 lower to upper quartile (25 to 75 percentile). The middle line represents the median. A line  
228 extends from the minimum to the maximum value.

229

230 Table 1. Comparative effectiveness of each disinfectant to reduce the bacterial load on  
 231 needleless connector surfaces

Treatments	n	CFU median	% reduction in bacterial load	Different (P<0,001) from treatments n <sup>o</sup>
(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)

232 Legend: NTC: non-treated control; IPA: isopropyl alcohol; CHG: chlorhexidine; CFU:  
 233 colony forming units. Differences calculated using Kruskal Wallis test and Conover as post  
 234 hoc analysis.

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