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Antimicrobial Efficacy of a New Chlorhexidine-based Device Against *Staphylococcus aureus* Colonization of Venous Catheters

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ABSTRACT

Vascular catheters are a major cause of nosocomial bloodstream infections. ChloraLock (ATTWILL Medical Solutions, Inc, West Jordan, UT, and ICU Medical, Inc, San Clemente, CA) is a novel antimicrobial device containing chlorhexidine digluconate (CHG) that is fitted onto a syringe and infuses CHG into the catheter lumen during locking. The objective of this study was to evaluate the antimicrobial efficacy of ChloraLock with in vitro tests and its ability to reduce *Staphylococcus aureus* contamination of catheters in the external jugular veins of Yorkshire swine. ChloraLock significantly reduced the bacterial load in the in vitro tests by up to 6 $\log_{10}$ colony-forming units (CFU) and by 3 to 4 $\log_{10}$ CFU/lumen in vivo in a swine model with 0.9% NaCl catheter locks.

Key words: antimicrobial devices, catheter-related infection, ChloraLock, chlorhexidine digluconate, swine model

INTRODUCTION

Continuous vascular access is a common component of patient care. However, catheter-related bloodstream infections (CR-BSIs) remain a major problem in the hospital setting. In Canadian intensive care units, 5% of venous catheter insertions resulted in bacteremia.[1] In hospitals and other health care settings, more than three-quarters of cases of bacteremia and fungemia were determined to be nosocomial infections, and one-quarter of these cases were demonstrated to arise from intravenous (IV) catheter use.[2] In that study, the most commonly isolated organism in bloodstream infections was *Staphylococcus aureus* (*S aureus*).[2] This pathogen is also the main cause of infectious complications in hemodialysis patients, with the vascular access catheter being the main entry point.[3] Thorough tests must identify the catheter as the source of the bloodstream infection for classification as a CR-BSI, and in practice, the term central line-associated bloodstream infection is used for surveillance purposes where the primary bloodstream infection is preceded by a central catheter insertion within 2 days.[4] This article discusses CR-BSI and focuses on testing catheter colonization.

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Drs Kowalewska, Petrik, and Fox-Robichaud received funding for this project from the owners of ChloraLock (ATTWILL Medical Solutions, Inc, West Jordan, UT, and ICU Medical, Inc, San Clemente, CA). Mr Di Fiore is the inventor of ChloraLock and president of ATTWILL Medical Solutions, Inc. The funders had a role in designing the study, presentation of data, and reviewing the manuscript. The funders had no role in data collection and analysis. Dr Kowalewska was funded by the Ontario Centres of Excellence Talent*Edge Fellowship Program, project number 23539.*

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DOI: 10.1097/NAN.0000000000000267

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One source of CR-BSI is touch contamination[5] of stopcocks or connectors, resulting in bacterial or fungal colonization of the inner lumen of the catheter.[6] Guidelines were developed for proper handling to limit contamination of such add-on devices.[4] Most catheter hubs may be fitted with a needleless connector, and a combination of skin and needleless connector cultures was shown to have high negative predictive value for catheter tip colonization and CR-BSI.[7] Bacteria and fungi attach to a surface, form colonies, and secrete gelatinous exopolymers, forming a cell-polymer matrix referred to as a biofilm. Biofilm formation on medical devices is common and is particularly problematic as it enables bacteria to resist high levels of antibiotics and disinfectants, and it confers protection from the host's immune responses, which may result in a chronic or recurrent infection for as long as the medical device is employed.[8]

ChloraLock (ATTWILL Medical Solutions, Inc, West Jordan, UT, and ICU Medical, Inc, San Clemente, CA) is a single-use luer lock connector that contains an insert impregnated with chlorhexidine digluconate (CHG) designed for use during catheter locking (Figure 1A). The device is fitted onto a syringe with lock solution and attached to the catheter so that the solution runs through the device as it enters the catheter, allowing for CHG to be infused into the lock solution (Figure 1B). The CHG-containing indwelling solution is aspirated from the catheter and discarded during the next catheter access. The purpose of ChloraLock is to reduce catheter contamination in infusion therapy. While use of CHG as an antiseptic skin preparation is known to significantly reduce the risk of CR-BSI,[9] and promising results were obtained with catheters coated with chlorhexidine and silver sulfadiazine,[10,11] CHG has not previously been used with a lock solution such as saline to the authors' knowledge. The aim of this study was to evaluate the in vitro and in vivo efficacy of ChloraLock against *S aureus*.

MATERIALS AND METHODS

Bacterial Strain and Growth Conditions

S aureus H1559 was cultured overnight in tryptic soy broth (TSB) (EMD, Darmstadt, Germany) with 15 mcg/mL erythromycin (Sigma-Aldrich, St. Louis, MO). Subcultures were grown for 1.5 hours at 37°C to an optical density of approximately 0.3 at 600_{nm} after 100-fold dilution in fresh TSB. The subcultures were washed in sterile 1 $\times$ phosphate-buffered saline (pH 7.2, 0.14 M NaCl, 2.7 mM KCl, 0.88 mM KH_2PO_4, 6.4 mM $Na_2HPO_4 \cdot 7H_2O$) and diluted to approximately 4×10^7 colony-forming units (CFU)/mL. CFU in the inoculum and test samples were quantified after serial dilutions and plating on tryptic soy agar (TSA) supplemented with lecithin, polysorbate 80, and 10 mcg/mL erythromycin (Becton, Dickinson and Company, Sparks, MD).

Preparation of Lock Solutions and Compatibility Study

The compatibility of ChloraLock was tested with the following commercially prepared lock solutions: saline (0.9% NaCl), sodium heparin (1000 USP units/mL; Sandoz Canada Inc, Boucherville, Quebec, Canada), and anticoagulant sodium citrate (4% w/v) solution (CitraFlow; MedXL Inc, Montreal, Quebec, Canada). Sodium heparin was diluted in 0.9% NaCl to 10 USP units/mL and 100 USP units/mL. The lock solutions were placed in syringes and passed through ChloraLock at 0.5, 1.0, 1.5, 2.0, and 2.5 mL to create the test solutions. The control lock solutions were not passed through ChloraLock.

A 100-μL suspension of approximately 4.1×10^6 CFU of *S aureus* was added to each test and control solution sample. The inoculated solutions were then incubated in tubes at 37°C for 20 minutes, and a separate set was incubated for 4 hours. After the appropriate contact time, samples were plated in serial dilutions on TSA and incubated overnight at 37°C. CFU were enumerated per total sample volume (lock solution volume + inoculate volume).

Time-based Efficacy Study

Saline (0.9% NaCl) was passed through ChloraLock at 0.5 mL. A 100-μL suspension of 2.9×10^6 CFU of *S aureus* was added to each tube of test and control solution, and the samples were incubated at 37°C for 1, 5, 10, 15, or 20 minutes. After the appropriate contact time, serial dilutions were immediately performed and plated on TSA. Plates were incubated overnight at 37°C, and CFU were calculated per total sample volume.

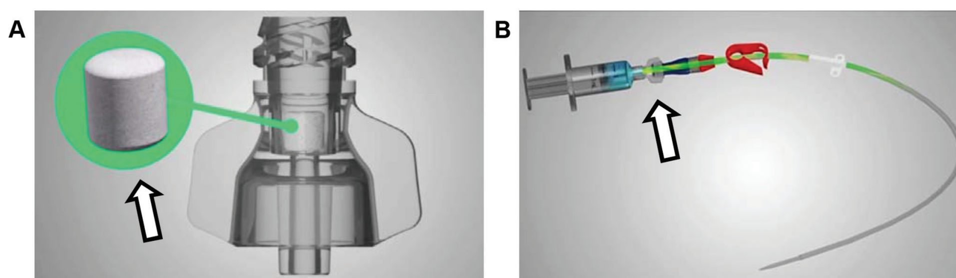

Figure 1 ChloraLock design and use. (A) The device contains a lyophilized insert (arrow) containing chlorhexidine digluconate. (B) ChloraLock (arrow) is attached to a syringe filled with lock solution and then attached to a catheter. The lock solution flows through ChloraLock, allowing for infusion of chlorhexidine digluconate into the solution as it enters the lumen of the catheter. Copyright ATTWILL Medical Solutions, Inc. Used with permission.

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Solution Preincubation Study

Before inoculation with *S aureus*, 0.5-mL samples of 0.9% NaCl and heparinized saline (10 units/mL and 100 units/mL) were passed through ChloraLock and incubated at 37°C for 168 hours. Control samples of 0.9% NaCl and heparinized saline were not exposed to ChloraLock. After the 168-hour incubation period, the test and control lock solutions were inoculated with a 100-μL suspension of approximately 3.6×10^6 CFU of *S aureus*. After 20 minutes or 4 hours of microbial incubation, serial dilutions were performed and plated on TSA. Plates were incubated overnight at 37°C, and CFU were counted per total sample volume.

Animals

Eight female Yorkshire pigs weighing between 17 and 26 kg were obtained from Arkell Research Station (University of Guelph, Puslinch, Ontario, Canada). The animal use protocol was in compliance with regulations set by the Canadian Council of Animal Care and approved by the McMaster University Animal Research Ethics Board. "Animal research: reporting in vivo experiments" guidelines were also followed.[12] Pigs were given 7 days to acclimatize to the facility and were trained to cooperate during handling using food-based rewards. Pigs were singly housed in raised pens with a 12-hour light/dark cycle and had access to drinking water as needed.

Catheter Insertion

After fasting animals for at least 8 hours, general anesthesia was induced with an intramuscular (IM) injection of ketamine (33 mg/kg, Vetalar; Bioniche, Belleville, Ontario, Canada) and acepromazine (1.1 mg/kg, Acevet; Vetoquinol, Lavaltrie, Quebec, Canada) and maintained with isoflurane (2%-3%) after endotracheal intubation. Atropine (0.05 mg/kg, ATRO-SA; Rafter, Calgary, Alberta, Canada), butorphanol (0.4 mg/kg, Torbugesic; Zoetis, Kirkland, Quebec, Canada), and meloxicam (0.2 mg/kg, Metacam; Boehringer Ingelheim, Burlington, Ontario, Canada) were administered IM after induction. A single dose of cefazolin (20 mg/kg; Sandoz Canada Inc, Boucherville, Quebec, Canada) was infused through the cannulated auricular vein preoperatively, and 0.9% NaCl was infused at 10 mL/kg/h throughout the surgical procedure. A Bair Hugger warming blanket (3M, Maplewood, MN) was used to maintain temperature intraoperatively, and vital signs were monitored at least every 15 minutes throughout the protocol.

The surgical protocol was performed by trained personnel (doctor of veterinary medicine and doctor of medicine). Incisions were made in the right and left jugular furrows, and the external jugular veins were exposed through blunt dissection and occluded with silicone vessel loops (Sterion, Caledonia, MI). Dual-lumen infusion catheters (7-Fr, 20 cm, 0.5-mL proximal priming volume, 0.6-mL distal priming volume; *med*COMP, Harleysville, PA) were capped with needleless connectors (MicroClave Neutral Connector; ICU Medical, Inc, San Clemente, CA) and primed with 0.9% NaCl. Following a small incision, the catheter was inserted into the external jugular vein and tunneled subcutaneously to the dorsal interscapular region. The catheter was secured to the jugular vein using circumferential ligatures (synthetic absorbable 3-0 PDS; Ethicon, Inc, Juarez, Mexico). Skin incisions were closed with subcuticular sutures (3-0 PDS), and cyanoacrylate glue incisional overlay was applied (3M Vetbond; 3M Animal Care Products, St. Paul, MN). The catheters were secured at the exit sites in the interscapular region with sutures (3-0 PDS), and the external ends of the catheters were secured to the skin with Tegaderm dressings (3M, Neuss, Germany). The catheters were flushed with 0.9% NaCl and further secured using pig jackets (juvenile Yorkshire swine jacket three-quarter-length medium; Lomir Biomedical Inc, Notre-Dame-de-I'lle-Perrot, Quebec, Canada).

Catheter Care and Study Design

Each catheter lumen was flushed with 5 mL of 0.9% NaCl once the animals were able to stand. The catheters were accessed after cleaning connectors with a 70% isopropyl alcohol wipe using an aggressive circular motion for 3 seconds and allowing them to air-dry. Following surgical recovery, the pigs were treated with oral meloxicam (0.2 mg/kg; Metacam, Boehringer Ingelheim, Burlington, Ontario, Canada) once daily for 3 days and butorphanol (0.2 mg/kg) subcutaneously for pain. The animals did not receive antibiotics or anticoagulants during the postoperative period.

Samples of catheter lock solution and a blood sample were taken 24 hours after surgery before daily flushes. Samples were plated on 5% sheep's blood agar plates with 3 mcg/mL of penicillin (Teknova, Hollister, CA). The 4 catheter lumens per pig were randomly assigned to the test group (ChloraLock) or control group (0.9% NaCl lock). Catheters were kept patent with 5-mL 0.9% NaCl flushes of each lumen twice a day during the catheter acclimatization period. Following the second flush, the lumens were locked according to their assigned group. The dressings were changed as needed, and the needleless connectors were changed 3 days after surgery. Twenty-four hours before bacterial inoculation of catheters, blood and samples of catheter lock fluid were obtained and plated on sheep's blood agar to check for contamination. After the 5-day catheter acclimatization period, the animals were moved into a quarantine room in preparation for catheter contamination with *S aureus*.

Catheter Contamination and Fluid Sampling

Each catheter lumen was flushed with 10 mL of 0.9% NaCl, and then 1 of the lumens on each dual-lumen catheter was inoculated with a 300-μL suspension (half of lumen volume) of approximately 3.9×10^6 CFU of *S aureus*. One of the 2 inoculated lumens on each pig was immediately locked with 300 μL of 0.9% NaCl (control lumen) and the other with 300 μL of 0.9% NaCl with ChloraLock (test lumen). The other 2 lumens on each pig were not inoculated and

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were instead locked with either 0.9% NaCl or 0.9% NaCl with ChloraLock (entire lumen volume) according to the assigned group. After inoculation, lock solutions were sampled from each lumen at 4, 12, 24, 36, and 48 hours. After every aspiration, the lumens were locked with 0.9% NaCl with or without ChloraLock as described above. CFU of *S aureus* were enumerated from the aspirated lumen fluid after plating of serial dilutions on TSA. Throughout the entire *S aureus* H1559 contamination period, the pigs were monitored for signs of pain and infection. At the study end point, the pigs were sedated using IM ketamine (33 mg/kg) and IM acepromazine (1.1 mg/kg), and an angiocatheter (22 GA BD Angiocath; Becton Dickinson, Sandy, UT) was inserted into the marginal auricular vein for blood to be sampled and plated to detect peripheral bacteremia. The animals were euthanized using IV sodium pentobarbital (120 mg/kg, Euthanyl; Bimeda-MTC, Cambridge, Ontario, Canada) through the auricular angiocatheter.

Scanning Electron Microscopy

After euthanasia, the catheters were extracted and a 1-cm sample of each catheter was cut from the midsection, fixed in 2% glutaraldehyde, and prepared for scanning electron microscopy as previously described.[13] The samples were imaged with a Tescan Vega II LSU scanning electron microscope (Tescan USA, Cranberry Township, PA) operating at 20 kV. Images were acquired with Tescan VegaTC operating software (Tescan USA, Cranberry Township, PA).

Statistical Analysis

Data were transformed to $\log_{10}$ CFU, and $\log_{10}$ reduction in CFU/sample was calculated by subtracting $\log_{10}$ CFU in ChloraLock-treated samples from $\log_{10}$ CFU in control samples. Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was set at $P < .05$ and calculated using the Kruskal-Wallis test or Student *t* test for parametric data using the computer software package KaleidaGraph 4.5.2 (Synergy Software, Reading, PA).

RESULTS

In Vitro Efficacy and Compatibility of ChloraLock With Commercially Prepared Lock Solutions

The efficacy of ChloraLock was tested with increasing volumes of lock solutions that were passed through the device with consequently decreasing CHG concentrations (Table 1). It was noted that a precipitate formed in the 100 units/mL heparin solution immediately after passage through the device (data not shown). ChloraLock reduced CFU counts in the 0.9% NaCl samples by $> 6 \log_{10}$ after 20 minutes of incubation with *S aureus*. No colonies were cultured from these samples after treatment with ChloraLock. With 10 units/mL of heparin, 0.5 mL of ChloraLock solution also sterilized the *S aureus* suspensions, but its efficacy decreased with higher solution volumes, from a $>$ 6-$\log_{10}$ reduction at 0.5 mL to a factor of less than 10 at 2.5-mL solution volume after 20 minutes of incubation with *S aureus*. Use of ChloraLock with heparinized saline (100 units/mL) or sodium citrate 4% solution did not significantly reduce CFU compared with control samples after 20 minutes of microbial incubation.

After 4 hours of microbial incubation (Table 2), ChloraLock used with 0.9% NaCl resulted in $>$ 5-$\log_{10}$ reduction in CFU compared with control. No colonies were grown from ChloraLock-treated samples of 10 units/mL of heparin with $>$ 4-$\log_{10}$ reduction in CFU compared with control. Heparin (100 units/mL) and sodium citrate resulted in approximately

TABLE 1

$\log_{10}$ Reduction[a] in CFU of *Staphylococcus aureus* After Exposure to ChloraLock With 20 Minutes of Microbial Incubation

Solution Volume	0.9% NaCl[b]	Heparin (10 units/mL)[c]	Heparin (100 units/mL)[d]	Sodium Citrate (4%)[e]
0.5 mL	6.612 ± 0.037[f]	6.519 ± 0.062[f]	0.091 ± 0.074	0.023 ± 0.071
1.0 mL	6.621 ± 0.036[f]	4.535 ± 0.957[f]	0.066 ± 0.025	0.096 ± 0.104
1.5 mL	6.692 ± 0.014[f]	1.818 ± 0.568[f]	−0.093 ± 0.063	0.133 ± 0.093
2.0 mL	6.684 ± 0.036[f]	1.294 ± 0.607[f]	−0.095 ± 0.140	0.267 ± 0.128
2.5 mL	6.592 ± 0.056[f]	0.602 ± 0.256	−0.031 ± 0.135	0.075 ± 0.124

Abbreviation: CFU, colony-forming units.
[a]$\log_{10}$ reduction = ($\log_{10}$ control samples) − ($\log_{10}$ ChloraLock-treated samples)
Bacterial inoculum:
[b]~6.670 $\log_{10}$ CFU
[c]~6.531 $\log_{10}$ CFU
[d]~6.620 $\log_{10}$ CFU
[e]~6.621 $\log_{10}$ CFU
[f]$P < .05$, ChloraLock-treated samples compared with controls, analyzed using Kruskal-Wallis test.

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TABLE 2

Log_{10} Reduction[a] in CFU of *Staphylococcus aureus* After Exposure to ChloraLock With 4 Hours of Microbial Incubation

Solution Volume	0.9% NaCl[b]	Heparin(10 units/mL)[c]	Heparin(100 units/mL)[d]	Sodium Citrate(4%)[e]
0.5 mL	5.611 ± 0.182[f]	5.244 ± 0.161[f]	4.632 ± 0.380[f]	4.754 ± 0.534[f]
1.0 mL	5.418 ± 0.179[f]	4.852 ± 0.207[f]	4.698 ± 0.682[f]	5.561 ± 0.144[f]
1.5 mL	5.397 ± 0.333[f]	4.809 ± 0.166[f]	5.629 ± 0.304[f]	4.523 ± 0.436[f]
2.0 mL	5.112 ± 0.454[f]	4.739 ± 0.346[f]	4.422 ± 0.646[f]	5.052 ± 0.528[f]
2.5 mL	5.178 ± 0.344[f]	4.845 ± 0.346[f]	3.948 ± 0.591[f]	4.857 ± 0.516[f]

Abbreviation: CFU, colony-forming units.
[a]Log_{10} reduction = (log_{10} control samples) − (log_{10} ChloraLock-treated samples)
Bacterial inoculum:
[b]~6.670 log_{10} CFU
[c]~6.531 log_{10} CFU
[d]~6.620 log_{10} CFU
[e]~6.621 log_{10} CFU
[f]$P < .05$, ChloraLock-treated samples compared with controls, analyzed using Kruskal-Wallis test.

4- to 5-log_{10} reduction in CFU after 4 hours of contact time, representing a 99.99% reduction in CFU. Taken together, these results indicate that longer contact times are required for ChloraLock to significantly reduce CFU of *S aureus* with heparin and sodium citrate compared with 0.9% NaCl.

Time-based Antimicrobial Efficacy

The microbial kill time of ChloraLock was examined at 1 to 20 minutes. ChloraLock significantly reduced CFU counts in 0.5-mL samples of 0.9% NaCl after 1 minute of contact time, with > 5-log_{10} reduction in CFU compared with control (Table 3).

TABLE 3

Log_{10} Reduction[a] in CFU of *Staphylococcus aureus* in 0.9% NaCl After 1 to 20 Minutes of Exposure to Chlorhexidine Digluconate in ChloraLock

Incubation Time	Log_{10} Reduction in CFU[b]
1 minute	5.799 ± 0.396[c]
5 minutes	6.308 ± 0.044[c]
10 minutes	6.498 ± 0.044[c]
15 minutes	6.445 ± 0.055[c]
20 minutes	6.428 ± 0.051[c]

Abbreviation: CFU, colony-forming units.
[a]Log_{10} reduction = (log_{10} control samples) − (log_{10} ChloraLock-treated samples)
[b]Bacterial inoculum was approximately 6.464 log_{10} CFU.
[c]$P < .05$, ChloraLock-treated samples compared with controls, analyzed using Kruskal-Wallis test.

After 5 minutes, no viable bacterial cells were found in the treated samples with > 6-log_{10} reduction in CFU.

Antimicrobial Efficacy of ChloraLock After 168 Hours of Solution Preincubation

To imitate lock solutions in catheters with a dwell time of more than a week between accesses, 0.5-mL samples of 0.9% NaCl that were passed through ChloraLock were incubated for 168 hours at 37°C before inoculation with *S aureus*. This preincubation decreased the antimicrobial activity of ChloraLock, resulting in a very small decrease in *S aureus* CFU counts after 20 minutes of microbial contact time (Table 4). Similarly, preincubated heparinized saline (10 units/mL and 100 units/mL) that was exposed to ChloraLock resulted in counts comparable to the control levels after 20 minutes of exposure to *S aureus*. Notably, precipitate appeared in the 0.9% NaCl and 10 units/mL heparin solution after 1 day of incubation at 37°C. After 4 hours of contact time, all 3 lock solutions significantly reduced CFU counts compared with the control (Table 4). This corresponded to a > 4-log_{10} reduction in CFU. With the heparinized saline samples, no colonies were grown after 4 hours of microbial incubation, and with 0.9% NaCl, only 1 sample out of 4 contained viable bacterial cells. Thus, solutions with ChloraLock that are used with longer dwell times require increased contact time for microbial killing.

Animal Characteristics

The animals recovered from general anesthesia smoothly, and surgical sites healed with no signs of infection or other complications. Neck swelling was apparent for approximately 2 days postoperatively, with full resolution following this time. Samples of catheter lock solution from each lumen and a blood sample were taken from each animal 24 hours after surgery and, again, 24 hours before catheter

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TABLE 4

Antimicrobial Efficacy of ChloraLock After 168 Hours of Solution Preincubation at 37°C[a]

Solution	20 minutes	4 hours
0.9% NaCl	0.226 ± 0.069[b]	4.791 ± 0.504[b]
Heparinized saline (10 units/mL)	−0.070 ± 0.062	5.422 ± 0.060[b]
Heparinized saline (100 units/mL)	0.059 ± 0.095	5.444 ± 0.040[b]

Abbreviation: CFU, colony-forming units.

[a]After passage through ChloraLock, the solutions were incubated for 168 hours at 37°C before bacterial inoculation with approximately 6.612 log_{10} CFU. The table reports values for log_{10} reduction in CFU of *Staphylococcus aureus* after exposure to ChloraLock in 0.5 mL of 0.9% NaCl or heparinized saline with 20 minutes or 4 hours of microbial incubation. Log_{10} reduction was calculated by subtracting log_{10} ChloraLock-treated samples from log_{10} control samples.

[b]$P < .05$, ChloraLock-treated samples compared with controls, analyzed using Kruskal-Wallis test.

inoculation to check for catheter contamination secondary to catheter handling during surgery or during the catheter acclimatization period. There were no animals with positive blood cultures identified postoperatively or prior to the experimental contamination with *S aureus*. However, 1 out of 16 lumens was culture-positive after surgery, which was cleared with ChloraLock treatment. Also, 3 out of 16 lumens were culture-positive with unidentified organisms before purposeful infection (1 contaminated lumen in the control group and 2 contaminated lumens in the ChloraLock group; not statistically significant). Following *S aureus* inoculation of the catheters, all of the animals continued to exhibit normal eating and drinking patterns, and feces appeared normal. There were no abnormal observations in behavior; the animals were active and cooperative during handling. The skin temperature, obtained using an infrared dermal thermometer, was slightly elevated from an average temperature of 36.5°C ± 0.1°C during the catheter acclimatization period to 37.2°C ± 0.2°C during the infection period.

In Vivo Efficacy of ChloraLock Against Purposeful Contamination With *S aureus*

Catheter lock fluid was not retrievable from 21% of the lumens on average at any given sampling time point, likely because of clot formation in the lumen. The number of CFU of *S aureus* in lumens treated with ChloraLock decreased rapidly over 48 hours, corresponding to a 6-log_{10} reduction in CFU, and a steady decline in CFU was also noted in the contaminated control lumens, corresponding to a > 2-log_{10} reduction in CFU (Figure 2). There was no significant contamination of the lumens that were not inoculated with *S aureus*. At each sampling time, use of ChloraLock reduced the CFU/lumen by approximately 3 to 4 log_{10} compared with the contaminated controls (Figure 2). At 24 to 48 hours after contamination, most of the ChloraLock-treated lumens were devoid of *S aureus* CFU, while 3.8 log_{10} CFU/lumen persisted in the control samples at 48 hours, $P < .01$. Specifically, 48 hours after inoculation, bacterial colonies were grown from 7 out of 8 lumens from the inoculated control group and 1 out of 8 lumens from the inoculated ChloraLock-treated group, while fluid samples from the uninoculated lumens were devoid of CFU at 48 hours in both the control and ChloraLock groups. These results show that ChloraLock is effective against *S aureus* contamination of intravascular catheters. At the end of the study, blood was collected from an auricular vein of each pig and plated on selective media to determine whether *S aureus* spread from the catheters into the bloodstream. *S aureus* H1559 was grown from blood samples of 2 out of 8 pigs despite these animals not showing signs of illness.

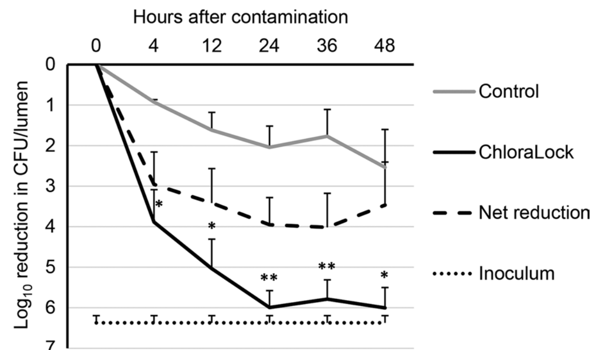

Figure 2 ChloraLock significantly decreased bacterial loads in catheter lumens. Eight Yorkshire pigs had 2 dual-lumen, 7-Fr catheters implanted bilaterally into the external jugular veins. During the 5-day catheter acclimatization period, half of the lumens were locked daily with 0.9% NaCl using ChloraLock; the other half of the lumens served as controls and were locked with 0.9% NaCl without the device. On the sixth day after surgery, half of the lumens in each group were contaminated with *S aureus* by injection of approximately 3.9×10^6 CFU in 300 μL. Fluid was sampled from the lumens at 4, 12, 24, 36, and 48 hours after contamination. After each sampling, the lumens were locked according to their assignment to ChloraLock or control group. CFU were quantified per lumen. After log_{10} transformation, 5 to 7 counts were averaged per sample, and data were recorded as mean ± SEM and analyzed with Student *t* test, $*P < .01$ compared with control, $**P < .001$ compared with control. Abbreviations: CFU, colony-forming units; SEM, standard error of the mean.

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Bacterial Attachment and Host Material Deposition in the Catheter

The clots observed on the intraluminal surfaces did not differ between the control and ChloraLock-treated lumens or between inoculated and uninoculated lumens (Figure 3). Inoculated lumens had leukocytes bound to the catheter surface within a fibrin network with what appeared to be interspersed *S aureus* cells. In many areas, the fibrin network appeared to be in a thrombolytic state (Figure 3B-E). There was extensive host material deposition, and no significant biofilm formation was noted, likely because the device was in place for a brief time. However, structures resembling *S aureus* microcolonies were observed (Figure 3B). A limitation of this study is that only small samples (1-cm-long segments) of the luminal surface were examined from the midsection of the catheter.

DISCUSSION

In recent years, plasmid-mediated resistance has emerged against the antibiotics used as a last resort,[14] and we are entering a postantibiotic era. This highlights the urgent need for development of other strategies to prevent and treat infections. Use of novel antimicrobial devices is particularly needed for high-risk patients and those with recurrent catheter-related infections. The current study reports on in vitro and preclinical animal tests of the antimicrobial efficacy of ChloraLock. Use of ChloraLock resulted in significant reduction in CFU of *S aureus* in 0.9% NaCl in vitro. However, use of sodium heparin or sodium citrate increased the required time for its antimicrobial effectivity. In our swine model of venous catheter contamination, ChloraLock reduced the bacterial burden by 3 to 4 $\log_{10}$ compared with the contaminated control lumens. These results show promise of this device in preventing vascular catheter contamination during access, which may eventually add to infection prevention strategies in infusion therapy.

The standard practice for disinfecting catheter components, such as stopcocks and needleless connectors, is to use 70% isopropyl alcohol wipes. Isopropyl alcohol wipes were shown to be superior in sanitizing conventional open-lumen stopcocks compared with Site-Scrub (Bard Access Systems, Salt Lake City, UT) isopropyl alcohol devices when the outside rim of the stopcock was contaminated with *Staphylococcus epidermidis* or *Pseudomonas aeruginosa*.[6] However, when the inner surface of the stopcock was contaminated, neither technique was effective in reducing bacterial burden in the study. Thus, ChloraLock was designed to act on the internal surfaces of the infusion set components and is intended to be used with a 70% isopropyl alcohol wipe and not as a replacement.

Lock solutions used to prevent catheter complications include various anticoagulant solutions, antimicrobial solutions, antibiotics, and antithrombotic agents. Heparin sulfate is widely used as an anticoagulant in the United States; however, it may induce thrombocytopenia[15] and was reported to enhance biofilm formation with *S aureus*.[16] Sodium citrate (4%) was shown to be as effective as heparin sulfate in keeping catheters patent[17] and appeared to inhibit *S aureus* biofilm formation in vitro.[16] A systematic review and meta-analysis of randomized controlled trials showed that citrate

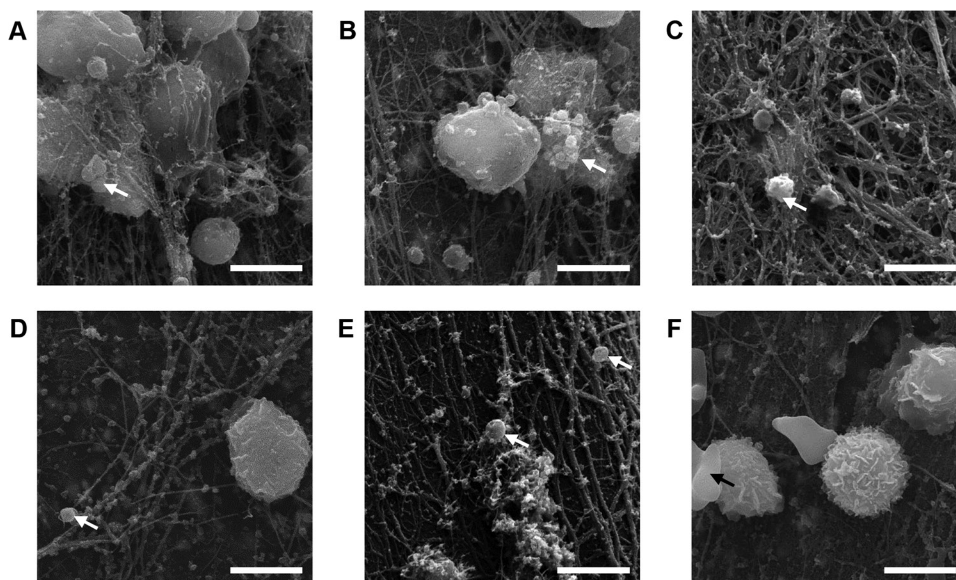

Figure 3 Scanning electron micrographs of bacterial attachment and host reaction to the catheter material at the luminal surface. After 5 days of catheter acclimatization and 2 days of catheter contamination with *S aureus*, the catheters were removed from the jugular veins of the pigs, and samples from the middle section of the catheters were prepared for scanning electron microscopy. Surfaces from inoculated control lumens (A and B) and from inoculated ChloraLock-treated lumens (D and E) contained leukocytes bound to the catheter surface on a fibrin scaffold with structures resembling interspersed *S aureus* cells and microcolonies (white arrows). Clots were also observed in the uninoculated samples from the control group (C) and ChloraLock-treated group (F) with platelets (white arrow) and crenated erythrocytes (black arrow) along with leukocytes on a fibrin network. Scale bar = 5 μm.

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(1%-7%) locks containing antimicrobial agents are superior to heparin in preventing CR-BSI in hemodialysis patients with central venous catheters.[18] In Canadian hospitals, sodium citrate (4%) is the preferred lock solution for dialysis catheters to avoid systemic heparinization in uremic patients who already have an increased risk of bleeding complications.[17] Use of 70% ethanol locks was shown to be associated with lowered occurrence of catheter-related infection compared with heparin in oncology patients,[19] but it was also found to be associated with increased catheter occlusion, decreased catheter integrity, and adverse events in patients.[20-22] The in vitro work in this study showed that use of the anticoagulants, heparin (10 USP units/mL and 100 USP units/mL) and sodium citrate 4% solution, increased the contact time needed to achieve $> 4\text{-}\log_{10}$ reduction in CFU with ChloraLock. However, whether other contact times between 20 minutes and 4 hours are sufficient to achieve $4\text{-}\log_{10}$ reduction with these solutions was not examined in this study. Thus, the time-dependent effectivity of ChloraLock should be taken into consideration when used with salt-containing lock solutions, such as heparin and sodium citrate, with short periods of time between catheter accesses.

Numerous antibiotics, alone or in combination, were shown to prevent catheter-related infections.[23] A meta-analysis of catheter-related infections in hemodialysis patients showed that antibiotic lock solutions reduce infections without significant side effects.[24] A few trials that assessed the effectiveness of antibiotic lock solutions in neonates reported decreased infection rates in the antibiotic treatment groups with central venous catheters, but more studies need to be done to draw conclusions on the safety and effectiveness of antibiotic lock therapy in the neonatal population.[25] Early treatment of bacteremia with an antibiotic lock is more effective than late treatment aimed to salvage a catheter.[26] However, some studies report a moderate to no benefit of antibiotic locks over standard treatment. For example, a review of trials on antibiotic locks in pediatric oncology patients with CR-BSI showed that antibiotic lock treatment does not add benefit to systemic antibiotic treatment.[27] In the case of peripherally inserted central catheters, there is a lack of studies that address the use of antibiotic locks.[28] Thus, more research is needed to reach an agreement for clinical practice regarding antibiotic locks.

Polymicrobial CR-BSIs are more difficult to treat with antibiotic locks.[26] Also, considerations of toxicity and bacterial resistance discourage their use. Thus, development of antimicrobial alternatives to antibiotic locks may add the benefit of prophylactic use without risking antimicrobial resistance. Since ChloraLock treatment reduced bacterial burden in the catheters in this study, it may be an effective alternative to antibiotic locks. However, it is important to note that this product is still investigational, and a drawback of this study is that the catheters were in place for only 7 days, limiting the conclusions to short-term catheters.

Pigs colonized with methicillin-resistant *S aureus* did not show clinical signs of infection.[29,30] However, IV injection of 10^8 CFU of *S aureus*/kg in Yorkshire-Landrace swine resulted in severe signs of sepsis.[31] In the current study, the authors attempted to maintain the bacterial suspension of 3.9×10^6 CFU in the catheter lumen during sampling and locking. None of the animals showed clinical signs of illness over the 2-day infection period, and only 2 out of 8 animals had *S aureus* H1559 growth from peripheral blood samples taken from an auricular vein. Nevertheless, the experimental strain colonized the lumens and persisted in most of the control lumens up to the study end point. Comparatively, no CFU were grown from all but 1 ChloraLock-treated lumen at the study end point.

The other serious complication associated with vascular catheters is thrombosis, and infections and thrombosis appear to enhance each other. Presence of a vascular catheter is a known risk factor for deep vein thrombosis,[32] and patients with a single episode of catheter-related infection had an increased risk of catheter-related thrombosis.[33] Conversely, thrombosis promoted catheter colonization and septicemia in patients.[34] A fibrin sheath develops around a catheter soon after insertion into a vessel, and fibrin sheath formation was shown to promote colonization and bacteremia in an animal study as well.[35] While the intraluminal catheter clot does not generally cause circulatory problems, it serves as a nidus for colonization by bacteria and fungi. In fact, *S aureus* manipulates host defenses by generating staphylothrombin, which produces fibrin fibers without activating clotting and inflammatory factors, thereby avoiding recruitment and detection by leukocytes while it embeds itself into the fibrin network.[36]

Compared with other species, pigs appear to be hypercoagulable.[37] In the present study, 0.9% NaCl flushes were performed twice daily during the catheter acclimatization period to discourage clot formation in the catheters. However, following intraluminal inoculation with *S aureus*, to retain the pathogen in the catheter, flushes were not performed. Thus, intraluminal clot formation was likely the cause of inadequate fluid withdrawal from some lumens. Scanning electron microscopy revealed that ChloraLock-treated lumens had similar amounts of thrombotic occlusions on the intravascular surface as the control lumens, and lumen occlusion was similar between the 2 groups. Thus, despite its efficacy in decreasing bacterial contamination, the device did not appear to diminish thrombotic occlusions in the catheter.

This study describes the successful application of a swine model to test antimicrobial vascular devices. It has shown that ChloraLock significantly reduces *S aureus* colonization of catheters in vitro and in vivo with 0.9% NaCl. However, the use of heparin and sodium citrate increased the contact time needed to kill bacteria. Thus, the dwell time and the type of lock solution used with ChloraLock are important considerations. Furthermore, studies are now required to determine the safety and feasibility of using ChloraLock in a patient population.

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ACKNOWLEDGMENTS

The authors thank Peng Liao, MD, of the Thrombosis and Atherosclerosis Research Institute, Comparative Medicine at McMaster University for help with surgical procedures. The authors also thank Jeffrey Weitz, MD, FRCPC, of the Thrombosis and Atherosclerosis Research Institute at McMaster University for the use of his spectrophotometer.

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