The silver cation (Ag\(^+\)): antistaphylococcal activity, mode of action and resistance studies

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Objectives: To examine several poorly understood or contentious aspects of the antibacterial activity of silver (Ag\(^+\)), including its cidality, mode of action, the prevalence of resistance amongst clinical staphylococcal isolates and the propensity for \textit{Staphylococcus aureus} to develop Ag\(^+\) resistance.

Methods: The effects of Ag\(^+\) on the viability, macromolecular synthesis and membrane integrity of \textit{S. aureus} SH1000 were assessed using established methodology. Silver nitrate MICs were determined for a collection of staphylococcal isolates (\(n=1006\)) collected from hospitals across Europe and Canada between 1997 and 2010. \textit{S. aureus} biofilms were grown using the Calgary Biofilm Device. To examine the \textit{in vitro} development of staphylococcal resistance to Ag\(^+\), bacteria were subjected to continuous subculture in the presence of sub-MIC concentrations of Ag\(^+\).

Results: Silver was bactericidal against \textit{S. aureus} in buffered solution, but bacteriostatic in growth medium, and was unable to eradicate staphylococcal biofilms \textit{in vitro}. Challenge of \textit{S. aureus} with Ag\(^+\) caused rapid loss of membrane integrity and inhibition of the major macromolecular synthetic pathways. All clinical staphylococcal isolates were susceptible to \(\leq 16\) mg/L silver nitrate and prolonged exposure (42 days) to Ag\(^+\) \textit{in vitro} failed to select resistant mutants.

Conclusions: The rapid and extensive loss of membrane integrity observed upon challenge with Ag\(^+\) suggests that the antibacterial activity results directly from damage to the bacterial membrane. The universal susceptibility of staphylococci to Ag\(^+\), and failure to select for resistance to Ag\(^+\), suggest that silver compounds remain a viable option for the prevention and treatment of topical staphylococcal infections.

Keywords: antimicrobial susceptibility, \textit{Staphylococcus} spp., heavy metal resistance

Introduction

The silver cation (Ag\(^+\)) has been used as an antimicrobial agent for centuries and continues to be deployed for the prevention and treatment of bacterial infection. Silver nitrate (AgNO\(_3\)) is still used in some settings as prophylaxis against ophthalmia neonatorum (neonatal conjunctivitis) and more advanced formulations containing Ag\(^+\) are widely used to prevent and treat infection of ulcers, chronic wounds and burns.\(^1\)\(^-\)\(^3\) In 2009, the National Health Service spent \(\approx 25\) million on silver-containing dressings\(^4\) and it has been estimated that 15 metric tonnes of silver were incorporated into medical products worldwide in 2010 alone.\(^5\) Increasingly, the antimicrobial properties of Ag\(^+\) are also being exploited outside of the clinic, through incorporation into personal hygiene products, textiles and water purification devices.\(^6\)\(^-\)\(^9\)

Despite the long-standing and increasing use of Ag\(^+\), its antibacterial mode of action remains unclear. Work in \textit{Escherichia coli} has suggested that Ag\(^+\) exposure leads to uncoupling of the respiratory chain from oxidative phosphorylation and loss of the proton-motive force.\(^10\) In \textit{Staphylococcus epidermidis}, exposure to Ag\(^+\) promotes release of iron from iron–sulphur clusters and subsequent formation of the lethal hydroxyl radical, in a process believed to result from the inhibition of electron transport chain components and production of the superoxide anion.\(^11\) However, as Ag\(^+\) retains antibacterial activity under anaerobic conditions,\(^12\) it is unlikely that specific interaction with the respiratory chain and formation of reactive oxygen species is the primary mechanism by which Ag\(^+\) exerts its antibacterial effect.

Our understanding is also incomplete regarding the potential for development of bacterial resistance to Ag\(^+\) and the prevalence of silver resistance in clinical isolates. This may, in part, reflect the lack of standardized methodology for assessing bacterial susceptibility to Ag\(^+\) and that surveys of Ag\(^+\) susceptibility...
have a small sample size and differ considerably in the methodology used, making it difficult to draw firm conclusions. Disparities in methodology also occur in studies to assess the antibacterial activity of Ag⁺, including evaluations of cidal potency. Consequently, AgNO₃ has been reported to be rapidly bactericidal in some studies and bacteriostatic in others.

High-level resistance to Ag⁺ in clinically relevant Gram-negative organisms can result from the horizontal acquisition of a plasmid harbouring the Sil protein complex. Based on homology to other heavy-metal resistance mechanisms, the Sil system appears to confer resistance through a combination of Ag⁺ sequestration in the periplasm and Ag⁺ efflux. Resistance to Ag⁺ as a consequence of chromosomal mutation has also been observed in E. coli following repeated exposure to Ag⁺ in the laboratory. Proteomic analysis of the resulting resistant mutant revealed that expression of the native Cu²⁺/Ag⁺ efflux system (CusCFBA) was up-regulated when compared with the Ag⁺-susceptible parental strain. In contrast to the situation with Gram-negative bacteria, little is known about Ag⁺ resistance mechanisms in Gram-positive bacteria.

This paper describes a series of studies to gain insights into the issues described above. Using Staphylococcus aureus as a model organism, we have investigated the antibacterial activity and mode of action of Ag⁺ and examined the clinical prevalence and in vitro development of silver resistance in staphylococci.

Materials and methods

Bacterial strains and culture media

The laboratory strains used in this study are described in Table 1. Clinical staphylococcal isolates were obtained from a culture collection maintained at the University of Leeds (n=626) and from the Chelsea and Westminster Hospital, London, UK (n=54), the Freeman Hospital, Newcastle, UK (n=97), Harrogate District Hospital, Harrogate, UK (n=82), the London Health Sciences Centre, Victoria Hospital, Ontario, Canada (n=39), the Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Italy (n=29) and Smith & Nephew Ltd, York, UK (n=79). Isolates in the University of Leeds collection were collected worldwide (but primarily from the UK) between 1997 and 2007. Isolates from the Chelsea and Westminster Hospital, the Freeman Hospital and Smith & Nephew were recovered between 2008 and 2010 from patients with burns or diabetic foot ulcers in clinics where therapeutic silver was in use at the time of isolation.

Bacteria were routinely cultured using Mueller–Hinton broth and agar (MHB and MHA, respectively; Oxoid Ltd, Cambridge, UK). For studies with daptomycin, MHB was supplemented with Ca²⁺ (50 mg/L).

Antimicrobial agents, chemicals and reagents

Ciprofloxacin was from Bayer (Leverkusen, Germany), whilst daptomycin and XF-73 were received as gifts from Cubist Pharmaceuticals (Lexington, MA, USA) and Destiny Pharmaceuticals (Brighton, UK), respectively. DISC (5) and the LIVE/DEAD® BacLight™ kit were from Invitrogen Life Technologies (Paisley, UK). The following radiolabelled chemicals were from Perkin-Elmer (Cambridge, UK): [methyl-¹⁴C]-thymidine (70–95 Ci/mmol), [5,6-¹³C]-uridine (31–56 Ci/mmol), L-¹³C-gluatmine (20–50 Ci/mmol) and [¹⁴C]-glycine. All other chemicals and antimicrobial agents were from Sigma–Aldrich (Poole, UK). In all studies, AgNO₃ was used as the source of Ag⁺.

Susceptibility determinations

MICs of antibacterial agents were routinely determined by 2-fold serial dilution in MHB according to the CLSI broth microdilution method. However, susceptibility of clinical staphylococcal isolates to Ag⁺ was determined using the CLSI agar dilution method. Biofilm MICs (bMICs) and minimum biofilm eradication concentrations (MBECs) were determined in MHB using the Calgary Biofilm Device as described previously. Assessment of the cidal activity of Ag⁺ and comparator agents was performed as previously described, using exponential-phase cultures of S. aureus SH1000 grown in MHB (~10⁸ cfu/mL) or exponential-phase cultures washed and resuspended in HEPES/glucose buffer (5 mM HEPES, pH 7.2/5 mM glucose).

Mode of action studies

The BacLight™ assay was used to assess membrane integrity of bacteria resuspended in water following exposure to AgNO₃ and comparator agents at 4x MIC for 10 min. The membrane potential of bacterial

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<td>S. aureus subsp. aureus Rosenbach</td>
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<td>fully genome-sequenced EMRSA-16</td>
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<td></td>
<td>MRS252</td>
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cells resuspended in HEPES/glucose buffer was monitored using the fluorescent dye DISC(S) 
(26). Leakage of K+ from cells resuspended in HEPES/glucose buffer was determined over a 1 h time course as previously described.26

To determine the inhibitory activity of Ag+ on the synthesis of cellular macromolecules, the incorporation of radiolabelled precursors into DNA, RNA, protein and peptidoglycan ([methyl-3H]thymidine, [5,6-3H]uridine, 3-(G-3H)glutamine and [1-14C]glycine, respectively) was monitored in exponential-phase cultures of SH1000 (∼105 cfu/mL) exposed to AgNO3 and comparator antibiotics.24,26 To assist in the identification of potential cellular targets of Ag+, a collection of five Bacillus subtilis antibiotic biosensors containing promoters specifically up-regulated by inhibitors of DNA, RNA, fatty acid, cell envelope and protein biosynthesis were used.28

Assessment of lysis following exposure to AgNO3 and comparator agents was carried out on exponential-phase cultures of SH1000 resuspended to an optical density at 600 nm (OD600) of 0.2 in HEPES/glucose buffer. Following the addition of AgNO3 at 4× MIC, OD600 readings were recorded at intervals over a 120 min period. To investigate possible changes in cell morphology following exposure to AgNO3, samples were removed at 5 and 120 min, prepared for scanning electron microscopy (SEM)28 and examined using a LEO 1530 FEGSEM scanning electron microscope.

Selection for silver resistance

The frequency of spontaneous mutation to Ag+ resistance was determined as previously described.30 To select Ag+ resistant mutants, bacteria were continuously exposed to AgNO3 in a manner analogous to that described by Friedman et al.26 Briefly, on day 1, broth microdilution susceptibility testing was performed using an extended range of AgNO3 concentrations instead of the standard doubling-dilution series. Following incubation of the cultures and determination of the MIC, the well that contained the highest concentration of AgNO3 permitting growth was diluted 1:100 in MHB and used to provide the inoculum for the next MIC assay; this process was repeated for up to 42 days.

Results

Methodology for Ag+ susceptibility testing

The apparent susceptibility of bacterial isolates to Ag+ varies considerably depending on the culture medium used, presumably because Ag+ binds to media components such as NaCl and proteins containing thiol groups.32,33 In addition to complicating susceptibility testing with Ag+, this effect probably also impacts studies to investigate the cidal activity of Ag+ in culture media. We therefore sought, in preliminary experiments, to establish a standard set of conditions for undertaking this work. Although originally established for the susceptibility testing of antibiotics, we found that the CLSI agar dilution protocol using MHA and MHB was appropriate for susceptibility testing with AgNO3. Thus, this method provided high reproducibility in respect of Ag+ susceptibility across multiple determinations with a variety of staphylococcal strains (SH1000, MRSA252, ATCC 29213 and ATCC 25923) (data not shown) and reliably detected silver resistance (MIC >256 mg/L) in a silver-resistant positive-control strain (E. coli NCTC 50110 harbouring silver resistance plasmid pMG101). Consequently, we adopted this method for all subsequent susceptibility testing with Ag+ and employed the same media (MHA/MHB) for our other studies to investigate the antibacterial activity of Ag+.

Susceptibility of staphylococcal isolates to Ag+

To examine the susceptibility of clinical staphylococcal isolates to Ag+, we conducted AgNO3 susceptibility testing as above for a collection of 1006 staphylococcal isolates obtained from hospitals throughout Europe and Canada between 1997 and 2010. Of these, 846 were S. aureus and 160 were coagulase-negative staphylococci. At least 230 of these isolates came from patients with burns and diabetic foot ulcers in clinics where therapeutic silver was in use at the time of isolation. All isolates tested susceptible to AgNO3 at concentrations of 8–16 mg/L, with the exception of a single isolate of Staphylococcus intermedius for which AgNO3 had an MIC of 4 mg/L.

Killing kinetics of Ag+ against S. aureus SH1000

As for susceptibility testing with Ag+, there is currently no standardized method for assessing the killing kinetics of Ag+-containing compounds and there are conflicting data regarding the cidal activity of Ag+.11,16 In this study, we re-evaluated the cidal activity of Ag+ using a method commonly employed for assessing the killing kinetics of antibiotics in MHB.24,27 The effect of AgNO3 and comparator compounds (daptomycin and fusidic acid) at 4× MIC on the viability of early exponential-phase cultures of S. aureus SH1000 grown in MHB was determined over 6 h (Figure 1a). The silver ion demonstrated only limited killing activity compared with daptomycin, causing a <1 log drop in cell viability over 6 h. This result was comparable to that obtained for fusidic acid, a bacteriostatic antibiotic.16 After 24 h of exposure to AgNO3, a 2.2 log drop in cell viability was observed (data not shown). In contrast, Ag+ exhibited rapid bactericidal activity against staphylococcal cultures resuspended in HEPES/glucose buffer, with a >7 log drop in cell viability observed after 10 min (Figure 1b). This indicates that, as with Ag+ susceptibility testing, the choice of assay medium can have a significant effect on the antibacterial activity of Ag+.

Activity of Ag+ against biofilms

Bacteria growing as biofilms can cause persistent infections that are highly refractory to antimicrobial therapy35 and may be present in environments where silver is administered, such as in chronic wounds.36 We therefore assessed the ability of AgNO3 to inhibit the growth and/or eradicate biofilms of SH1000 and MRSA252, as determined by the bMIC and MBEC, respectively. Although AgNO3 could inhibit the growth of biofilms at a concentration equal to its planktonic MIC (bMIC 16 mg/L), it was unable to eradicate biofilms at the highest concentration tested (256 mg/L). The comparator agents, tetracycline and vancomycin, were also unable to eradicate biofilm cultures at the concentrations tested (MBEC >256 mg/L). In contrast, as previously reported,23 the compound XF-73 displayed potent activity against both SH1000 and MRSA252 biofilms, with an MBEC of 2 mg/L.

Mode of action studies with Ag+

Monitoring the ability of an antibacterial compound to inhibit the biosynthesis of cellular macromolecules can provide insights into
its mode of action.\textsuperscript{24,26,27} We therefore assessed the ability of AgNO$_3$ to inhibit the incorporation of radiolabelled precursors into DNA, RNA, protein and peptidoglycan, and compared this with the activity of antibacterial agents known to specifically inhibit these biosynthetic pathways (ciprofloxacin, rifampicin, tetracycline and vancomycin, respectively). At 4× MIC, AgNO$_3$ caused substantial and comparable inhibition of all four biosynthetic pathways after 10 min (Figure 2).

To further explore the antibacterial target of Ag$^{+}$, we employed five *B. subtilis* antibiotic biosensors, each containing a luciferase reporter gene fused to promoters induced by inhibitors of fatty acid, protein, DNA, RNA and cell envelope biosynthesis. These constructs have previously been shown to be of benefit in mode-of-action studies of antibacterial compounds.\textsuperscript{28} Exposure of the strains to AgNO$_3$ failed to elicit a significant increase in luminescence in any of the biosensors (data not shown).

The non-specific inhibition of biosynthetic processes by AgNO$_3$ may result from damage to the cell membrane and consequent loss of intracellular components.\textsuperscript{24} Thus, following exposure of *S. aureus* SH1000 to AgNO$_3$ and comparator antibiotics at 4× MIC, we assessed bacterial membrane integrity using the BacLight$^\text{TM}$ assay and membrane potential using the fluorescent dye DiSC$_3$(5). Following 10 min of exposure to Ag$^{+}$, the membrane integrity decreased by 97%. This decrease was comparable to that seen for bacteria exposed to nisin (99%), a compound known to damage bacterial membranes through pore formation.\textsuperscript{17} The membrane potential was also affected
in cells exposed to Ag⁺, with a >50% decrease observed over 1 h (Figure 3a). The kinetics of membrane damage following Ag⁺ exposure were further explored by measuring the leakage of intracellular K⁺ from S. aureus SH1000 over 1 h (Figure 3b). In <10 min, >90% of intracellular K⁺ was lost from cells exposed to Ag⁺; again, this effect was comparable to that observed for bacteria exposed to nisin.

Cells treated with nisin eventually undergo lysis as a consequence of damage to the membrane. As Ag⁺ exposure produced similar results to nisin in the BacLight and K⁺ leakage assays, we examined whether Ag⁺ also causes cell lysis. S. aureus SH1000 cells resuspended in HEPES/glucose were exposed to AgNO₃ at 4× MIC and monitored for lysis over a period of 2 h by optical density measurements. The results were compared with those of nisin-exposed cultures (Figure 4). Over 2 h, the optical density of the cultures exposed to nisin decreased by ~37%, indicating that lysis was occurring. There was no change in the optical density of cultures exposed to Ag⁺ suggesting that under these conditions silver did not cause lysis.

To confirm the absence of lysis and to detect any changes in the morphology of cells exposed to Ag⁺, cells were visualized using SEM following exposure to Ag⁺ or nisin for 5 and 120 min (Figure 4). Cells visualized after 120 min of exposure to nisin showed evidence of lysis, but no lysis was seen for cells treated with AgNO₃ (Figure 4).

**Selection of bacterial Ag⁺ resistance**

Spontaneous mutants of S. aureus SH1000 with reduced susceptibility to Ag⁺ could not be obtained (limit of detection <1×10⁻¹⁰) following plating of saturated cultures onto MHA containing AgNO₃ at 4× MIC. In contrast, spontaneous mutation to resistance to the comparator agents mupirocin and fusidic acid occurred at frequencies of ~5×10⁻⁶ and ~3×10⁻⁶, respectively, consistent with previously reported values. Failure to obtain spontaneous mutants exhibiting reduced susceptibility to Ag⁺ led us to adopt an alternative method to attempt to select for resistance. S. aureus SH1000 and the clinical S. aureus strain MRSA252 were continuously exposed to AgNO₃ in the form of repeated microdilution broth MICs. Attempts were also made to select Ag⁺ resistance in Pseudomonas aeruginosa PA01 and E. coli K12-BW25113, in order to determine whether the rate at which Ag⁺ resistance develops differs in Gram-negative organisms. Following 6 days of exposure, the AgNO₃ MIC for E. coli increased from 4 to >256 mg/L, while AgNO₃ MICs for S. aureus and P. aeruginosa cultures remained at 16 and 4 mg/L, respectively, for the duration of the experiment (42 days). In contrast, S. aureus cultures exposed to daptomycin developed clinically significant levels of daptomycin resistance (>2 mg/L) following 3 days of exposure (data not shown).

**Discussion**

In this study, the CLSI agar dilution susceptibility testing method was used to determine Ag⁺ MICs for 1006 staphylococcal isolates, using AgNO₃ as the source of Ag⁺. Since there are no published breakpoints for Ag⁺, the Ag⁺ MIC that corresponds to clinically significant Ag⁺ resistance is unknown. However, as the MICs of Ag⁺ for the isolates in this study were comparable to those for S. aureus SH1000 and other laboratory strains, no...
evidence of reduced susceptibility to silver in clinical isolates of staphylococci was found. The universal susceptibility of staphylococci to Ag\(^+\) suggests that silver therapy could be considered as an alternative for the treatment of topical staphylococcal skin infections that are resistant to commonly used topical antibiotics. Furthermore, as this method is based on CLSI guidelines commonly used for antibiotic susceptibility testing, it could easily be established in clinical laboratories to allow for continued monitoring of Ag\(^+\) susceptibility.

Studies in the recent scientific literature have employed various methodologies to assess the killing kinetics of Ag-containing compounds. Differences include the choice of culture medium, the starting inoculum size and the concentration of the Ag-containing compound used.\(^{11,16}\) This has led to conflicting results regarding the cidality of Ag\(^+\). Using a method frequently employed for assessing the bactericidal activity of antibiotics in susceptibility-testing media (MHB), Ag\(^+\) was found to exhibit bacteriostatic activity against \(S.\) aureus SH1000, causing only a 2.2 log\(_{10}\) drop in cell viability over 24 h. However, in buffer, Ag\(^+\) was rapidly bactericidal, causing a \(>7\) log\(_{10}\) drop in cell viability in 10 min. The considerably increased cidal potency of Ag\(^+\) in buffer is presumably primarily due to the lack of NaCl and thiol-containing proteinaceous components that are present in culture media, which have been reported to bind and inactivate Ag\(^+\).\(^{32,33}\) These results emphasize the importance of adopting standardized culture media (such as MHB) when assessing the antibacterial activity of Ag\(^+\)-containing compounds.

Exposure of \(S.\) aureus to Ag\(^+\) caused rapid loss of membrane integrity. Since assays to measure bacterial membrane integrity require that bacterial cells are resuspended in buffer (or water), in conditions under which Ag\(^+\) is potently bactericidal, care must be taken when relating these findings to the antibacterial mode of action of Ag\(^+\) in growth media. However, when bacterial cells were exposed to Ag\(^+\) for 10 min in MHB, simultaneous inhibition of four major macromolecular biosynthesis pathways was observed. Such non-specific inhibition has previously been observed for compounds that cause membrane damage and is likely a result of leakage of cytoplasmic constituents from the cell and subsequent de-energization of biosynthetic pathways.\(^{24,26}\) As this process occurs rapidly upon addition of Ag\(^+\) to cultures and correlates with the results of membrane damage assays in buffer, it seems likely that the cell membrane is indeed the primary target of Ag\(^+\).

The mechanism by which Ag\(^+\) damages the bacterial membrane is unclear. Unlike cells exposed to nisin, no lysis or changes in morphology were observed for cells exposed to Ag\(^+\). The direct interaction of Ag\(^+\) with thiol-containing macromolecules in the membrane, or hydroxyl radical production as a consequence of Ag\(^+\) inhibiting electron transport, may contribute to the observed membrane damage.\(^{11}\) However, since Ag\(^+\) maintains activity (albeit at a reduced level) in anaerobic conditions,\(^{12}\) hydroxyl radical production cannot be the sole cause of membrane damage. Future studies will focus on understanding how Ag\(^+\) acts to compromise the integrity of the bacterial membrane.

It has been suggested that antibacterial agents that target the bacterial membrane may be beneficial for the treatment of persistent infections, such as those caused by non-growing bacteria or those resident in biofilms,\(^{41,42}\) since such membrane-active agents typically retain antibacterial activity against metabolically inert bacteria.\(^{23,41}\) We therefore sought to determine the antibacterial activity of Ag\(^+\) against biofilm cultures of SH1000 and

![Figure 4. Effects of Ag\(^+\) and nisin on the integrity of \(S.\) aureus SH1000 resuspended in HEPES/glucose buffer. Values shown are the means of three replicates from three independent experiments. Broken lines point to scanning electron micrographs of cells taken from cultures at indicated timepoints. Error bars represent standard deviations from the mean.](image-url)
MRSA252. At a concentration of 16 mg/L, AgNO₃ could prevent shedding of planktonic cells from established biofilms in vitro. This may indicate that, in vivo, Ag⁺ could prevent further biofilm growth and dissemination to other sites. However, as this bMIC is equivalent to the MIC of Ag⁺, it seems likely that Ag⁺ inhibits the growth of planktonic cells following their release from the biofilm and has no direct effect on the viability of the biofilm itself. Indeed, we determined that Ag⁺ was unable to eradicate established biofilms (MBEC >256 mg/L). This may be a consequence of Ag⁺ binding to negatively charged extracellular polymeric substances within the biofilm, thereby preventing Ag⁺ from reaching cells in a sufficient concentration to exert an antibacterial effect.

The continuous exposure of cells to AgNO₃ for 42 days failed to select for Ag⁺ resistance in S. aureus or P. aeruginosa. The inability to select endogenous bacterial Ag⁺ resistance highlights another potential advantage of using Ag⁺ in place of antibiotics for the treatment of topical staphylococcal infections. Although we could not generate Ag⁺-resistant S. aureus or P. aeruginosa in this study, we were able to select E. coli resistant to >256 mg/L AgNO₃ following a short period of continuous subculture. The generation of Ag⁺-resistant E. coli in the laboratory has previously been reported and appears to result from the increased expression of the CusCFBA efflux transporter. However, as E. coli is rarely isolated from wounds where therapeutic Ag⁺ is used, it remains unclear if Ag⁺-resistant E. coli would arise or persist in the clinical setting. It is worth noting that neither S. aureus nor P. aeruginosa harbour homologues of the CusCFBA system, which may provide an explanation for the inability to select for Ag⁺ resistance in these species.

In summary, our work suggests that standardization of culture media and experimental methodology are critical when evaluating the antibacterial activity of Ag⁺. Although no overt lysis can be observed following exposure to Ag⁺, the rapid and extensive loss of membrane integrity strongly suggests that the antibacterial activity of Ag⁺ results from direct damage to the cell membrane. The universal susceptibility of staphylococcal isolates to Ag⁺, coupled with the inability to select for Ag⁺ resistance following repeated exposure, suggest potential advantages of silver-based therapy over antibiotics for the treatment and prevention of superficial staphylococcal infections.

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