The Journal of Antibiotics (2017) 70, 775–780

www.nature.com/ja

ORIGINAL ARTICLE

The efficacy of different anti-microbial metals at preventing the formation of, and eradicating bacterial biofilms of pathogenic indicator strains

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The emergence of multidrug-resistant pathogens and the prevalence of biofilm-related infections have generated a demand for alternative anti-microbial therapies. Metals have not been explored in adequate detail for their capacity to combat infectious disease. Metal compounds can now be found in textiles, medical devices and disinfectants—yet, we know little about their efficacy against specific pathogens. To help fill this knowledge gap, we report on the anti-microbial and antibiofilm activity of seven metals: silver, copper, titanium, gallium, nickel, aluminum and zinc against three bacterial strains, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. To evaluate the capacity of metal ions to prevent the growth of, and eradicate biofilms and planktonic cells, bacterial cultures were inoculated in the Calgary Biofilm Device (minimal biofilm eradication concentration) in the presence of the metal salts. Copper, gallium and titanium were capable of preventing planktonic and biofilm growth, and eradicating established biofilms of all tested strains. Further, we observed that the efficacies of the other tested metal salts displayed variable efficacy against the tested strains. Further, contrary to the enhanced resistance anticipated from bacterial biofilms, particular metal salts were observed to be more effective against biofilm communities versus planktonic cells. In this study, we have demonstrated that the identity of the bacterial strain must be considered before treatment with a particular metal ion. Consequent to the use of metal ions as anti-microbial agents to fight multidrug-resistant and biofilm-related infections increases, we must aim for more selective deployment in a given infectious setting.
The Journal of Antibiotics (2017) **70,** 775–780; doi:10.1038/ja.2017.10; published online 15 February 2017

INTRODUCTION

The progression of bacterial resistance to antibiotics has led us to an era that urgently requires alternative anti-microbial therapies. Furthermore, recent knowledge regarding antibiotic efficacy has led to the realization that targeted anti-microbial strategies are required for use against chronic infections—such as those caused by biofilms—which are remarkably different from acute infections. Typically, more than half of infections are caused by organisms that are involved in surface-attached communities immersed in a self-produced hydrated extracellular polymeric matrix, known as a biofilm.[1] This matrix has been observed to complicate wound healing by facilitating the transition between acute and chronic infections,[2] and contaminate clinical surfaces and implanted medical devices such as catheters and endotracheal tubes.[3] The physiological changes characteristic of biofilms results in enhanced resistance to elimination by the host immune system and some antibiotics.[4] The use of modern antibiotics to treat infections caused by bacteria is now a multifactorial challenge given the threat of both multidrug-resistant bacteria and biofilm-related infections. As a consequence, the administration of metals to combat both threats has recently regained attention. Metal compounds can now be found in wound dressings,[5] liquid formulations for hand washing,[6] impregnated into textiles such as socks[7] and on medical devices like catheters.[8]

The anti-microbial properties of metals have been documented in many bodies of work[9] and continue to be the subject of investigation in an attempt to understand the mechanisms of metal toxicity and resistance.[10–14] A detailed review on the historical uses, rationale and mechanisms of action of metals can be found in Lemire *et al.*[9] Despite the wealth of literature committed to examining the anti-microbial activity of metals, less attention has been paid to determining the susceptibility of bacteria to metals within a defined set of conditions. While the minimal inhibitory concentration, minimal bactericidal concentration and minimal biofilm eradication concentrations for many metals have been determined, the lack of consistency between techniques, conditions and media has resulted in difficulties when comparing the susceptibilities of bacterial strains to metal compounds. Additionally, present data on the anti-microbial properties of metals are inadequate, which is alarming, particularly since applications have expanded into industry, agriculture and health care.[9]

Here we describe our observations from testing the anti-microbial and anti-biofilm activity of seven different metals with demonstrated anti-microbial activity and utility (silver, copper, titanium, gallium,

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Received 13 November 2016; revised 22 December 2016; accepted 3 January 2017; published online 15 February 2017

nickel, aluminum and zinc) against three indicator strains, *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Chemically simulated wound media (CSWM) was used to provide a rich environment for bacterial growth, warranting that variation in susceptibility between the three strains was not a result of nutrient limitations in the growth media. In addition, this growth media provided an environment comparable to a wound infection—a clinical challenge where metals have a realized potential for utility. Experiments were designed to reproduce an acute wound infection by assessing both the prevention and eradication of biofilms as well as the susceptibility of planktonic cultures. Using the Calgary Biofilm Device (CBD), the minimal biofilm eradication concentrations (MBECs), minimal biofilm bactericidal concentrations (MBBCs), minimal planktonic bactericidal concentrations (MPBCs) and MBECs were determined under the various metal challenges.

MATERIALS AND METHODS

Bacterial strains and culture media

Bacterial strains were stored at −70 °C in Microbank vials as described by the manufacturer (proLab Diagnostics, Richmond Hill, ON, Canada). The three bacterial strains *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were gifts from Dr Joe J Harrison (University of Calgary, Calgary, AB, Canada).

Throughout our studies—present and past—we have observed that the growth media chosen to culture bacterial cells is a significant factor that dictates the efficacy of the metal challenge. Hence, we selected a media that provides a rich environment to ensure robust bacterial growth in each strain. Chemically simulated wound media (CSWM), modified from Werthén[15] (50% bovine serum (66 g l^{-1}):50% peptone water (0.85% NaCl, 0.1 g l^{-1} peptone)) was used for metal susceptibility testing throughout this work. For the dilution of metal working solutions, a 2× peptone water (0.85% NaCl, 0.2 g l^{-1} peptone) solution was used.

Biofilm cultivation

In this work, all biofilms were cultivated using the CBD/MBEC as described in Ceri *et al.*[16] and Harrison *et al.*[17] and by the manufacturer's guidelines (Innovotech, Edmonton, AB, Canada). Following overnight growth of the preculture, colonies were suspended in CSWM and matched to a 1.0 McFarland standard. Next, the suspended cells were diluted 30 times in CSWM. To cultivate the biofilm, 150 µl of the diluted inoculum was placed into a 96-well microtiter plate (Nunclon; VWR International, Radnor, PA, USA) followed by placement of the CBD lid, which contained 96 equivalent pegs. The CBD was placed on a gyrorotary shaker operating at 150 r.p.m. in a humidified incubator at 37 °C for either 4 or 24 h.

Stock and working metal solutions. Silver nitrate ($AgNO_3$), copper (II) sulfate ($CuSO_4$), titanium (III) chloride ($TiCl_3$), gallium (III) nitrate ($Ga(NO_3)_3•H_2O$) and nickel sulfate ($NiSO_4•6H_2O$) were all obtained from Sigma-Aldrich (St Louis, MO, USA). Aluminum sulfate ($Al_2(SO_4)_3•H_2O$) was obtained from Matheson Coleman and Bell (Norwood, OH, USA) and zinc sulfate ($ZnSO_4•7H_2O$) was received from Fisher Scientific (Fair Lawn, NJ, USA). Stock solutions of $CuSO_4$, $TiCl_3$ and $Al_2(SO_4)_3•H_2O$ were made up to 1 M, $ZnSO_4•7H_2O$ was made up to 1.5 M, $NiSO_4•6H_2O$ to 2.5 M and $AgNO_3$ to 500 mM in distilled and deionized (dd)H_2O. All stock metal solutions were stored in glass vials at 21 °C for no longer than 2 weeks. No more than 30 min before experimental use, working solutions were made from stock metal solutions in equal amounts of CSWM and 2× peptone water (dilution factor of 2). In a 96-well plate (the challenge plate), serial dilutions of each metal, with a dilution factor of 2, were prepared; reservation of the first row served as a growth control (0 mM metal salt).

Prevention of planktonic growth and biofilm formation

To assess the capability of the metal salts to prevent the growth of biofilms and planktonic cells, bacterial cultures were inoculated in the CBD in the absence—to control for growth—and presence of the metal salt. The CBD was then placed in a 37 °C humidified incubator on a gyrorotary shaker at 150 r.p.m. for 4 h. This treatment provided the MPBCs and MBBCs. Overall evaluation of bacteria could establish a culture planktonically or as a biofilm in the presence of the metal salts.

Eradication of established biofilms

To evaluate the ability of the metal salts to eradicate established biofilms, a biofilm was first cultivated on the pegged lid of the CBD for 24 h. The lid was then rinsed two times with 0.9% NaCl and placed into a 96-well microtiter plate containing twofold serial dilutions of the metal salts; a column was reserved for bacterial growth in the absence of the metal salts. The plate was then incubated for 24 h in a humidified incubator at 37 °C on a gyrorotary shaker at 150 r.p.m. This treatment was used to determine the MBEC of each metal salt.

Assessment of metal efficacy

To assess the susceptibility of planktonic and biofilm populations to the metal salts, the peg lids from both treatments were first rinsed two times in 0.9% NaCl. Subsequently, the biofilms were disrupted from the pegs by sonication using a 250HT ultrasonic cleaner (VWR International) for 10 min into 200 µl of Lysogeny broth media (25 g l^{-1}) containing 0.1% Tween-20 and universal neutralizer[18] (0.5 g l^{-1} histidine (Sigma, St Louis, MO, USA), 0.5 g l^{-1} cysteine (Sigma) and 0.1 g l^{-1} reduced glutathione (Sigma) in (dd)H_2O). To establish the MBBC and MBEC of the disrupted biofilm populations, six dilutions, with a dilution factor of 10, in 0.9% NaCl were performed. The samples were spot plated on tryptic soy agar plates to determine the viable cell numbers from the biofilm, and subsequently incubated overnight at 37 °C. To determine the MPBC of the planktonic populations, eight serial dilutions, with a dilution factor of 10, were carried out into 96-well plates with 0.9% saline and universal neutralizer. Similarly, spot plating the diluted samples onto TSA plates and incubating overnight at 37 °C generated viable cell counts. The concentrations at which each metal salt gave rise to no viable microbial colonies were determined to be the MPBC, MBBC and MBEC.

RESULTS

Various metal salts can prevent planktonic growth and biofilm formation

To determine the capacity of metal salts to prevent the formation of biofilms of the selected indicator strains, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were grown for 4 h in the presence of the metal salts. This approach gave rise to the MPBC (Figure 1a), and in parallel, the MBBC (Figure 1b). In order for the biofilms to form in the presence of the metal ions, the planktonic cells would need to survive the metal concentrations long enough to permit attachment and expression of biofilm-related genes. Therefore, this experiment measures both cell attachment and biofilm proliferation in the presence of metal salts.

For all three strains the MPBCs (Figure 1a) and MBBCs (Figure 1b) of Cu, Ga and Ti were reached within the tested concentrations. A lower concentration of Cu, as opposed to Ga, was needed to prevent *P. aeruginosa* attachment and growth (Supplementary Table 1). This was not observed for *E. coli*, in which a greater concentration of Ga, in comparison with Cu, was needed to attain the MBBCs and MPBCs (Supplementary Table 2). *S. aureus* biofilms were fourfold more resistant to Ti than their planktonic counterparts indicated by the MBBCs and MPBCs (Supplementary Table 3). A fourfold higher concentration of Cu was needed to prevent planktonic growth than the formation of biofilms in *P. aeruginosa* (Supplementary Table 1).

The metals Ag and Al were successful in preventing *P. aeruginosa* and *E. coli* biofilm formation (Figure 1b). However, only Al was capable of eliminating planktonic populations in these two strains following the concurrent 4 h metal exposure and incubation period

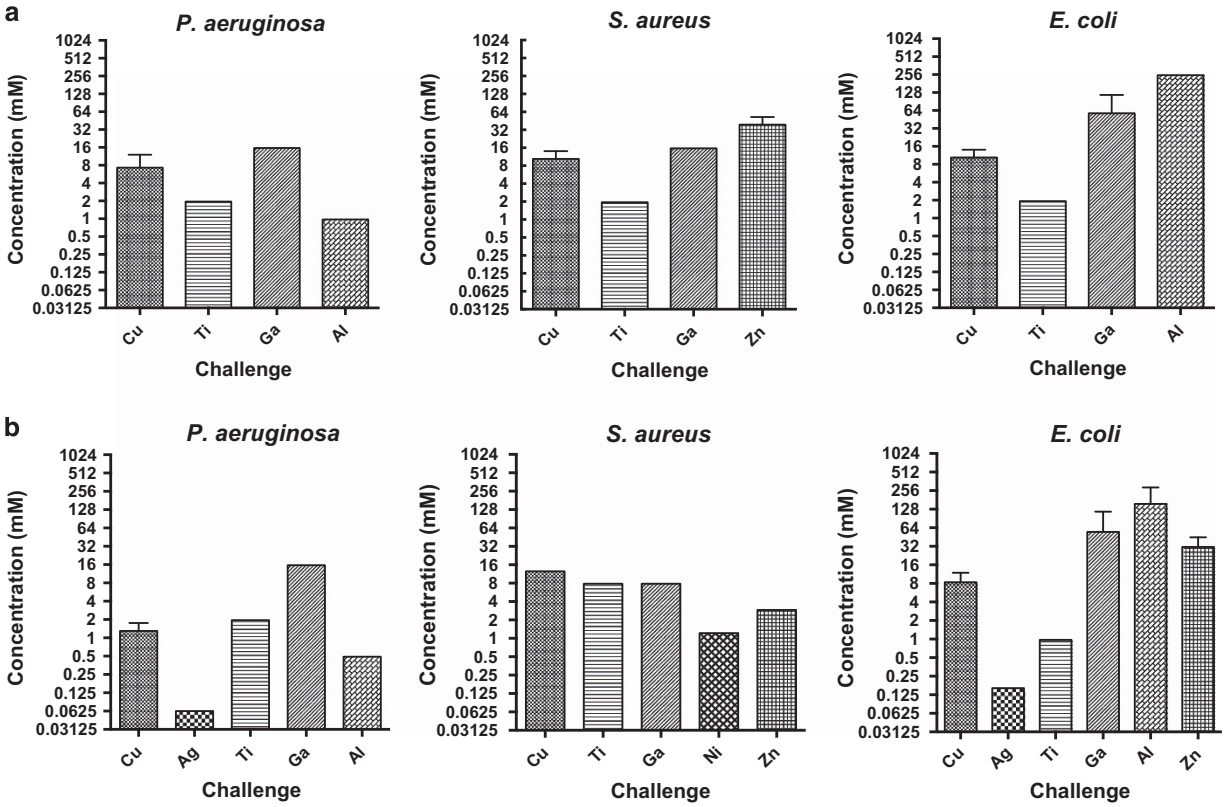

Figure 1 The efficacies of different metals for preventing the growth of planktonic and biofilm bacterial populations. (**a**) MPBCs and (**b**) MBBCs of *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) in the presence of $AgNO_3$, $CuSO_4$, $TiCl_3$, $Ga(NO_3)_3 \bullet H_2O$, $NiSO_4 \bullet 6H_2O$, $Al_2(SO_4)_3 \bullet H_2O$ or $ZnSO_4 \bullet 7H_2O$. The bacteria were grown over a concentration range defined by twofold serial dilutions of each metal; viable cells were counted to determine the MPBCs and MBBCs. Values are represented as the mean ± s.d., $n = 3$. *Note*: All metal stock solutions were prepared at equal molar equivalents of metal molecule. Hence, the concentrations found in this figure are reflective of the concentrations of metal and not the compounds themselves. Only the metal salts that were capable of preventing growth in the concentrations tested are shown.

(Figure 1a). Notably, the MBBCs for Al were found to be 250-fold lower for *P. aeruginosa* compared with *E. coli*. In addition, a greater concentration of Al was needed to reach the MPBC as opposed to the MBBC for *P. aeruginosa*. In the concentrations of Ag tested, little change in viable planktonic cells was observed for *P. aeruginosa* and *E. coli* (Figure 2a). The MPBCs and MBBCs for *S. aureus* were not reached within the concentrations of Al examined, although a 1-log decrease in biofilm formation and ~2 log decrease in planktonic cells was observed based on the reduction in viable cell numbers (Figure 2). Higher concentrations of Al were not explored because of the solubility of this metal in (dd)H_2O. Finally, the MPBCs and MBBCs of Ag for *S. aureus* were not reached within the concentrations tested. The addition of Ag at a concentration >500 mM to the CSWM led to extensive precipitation; thus, concentrations >500 mM could not be explored.

For *S. aureus*, only the MBBC was reached upon challenge with Ni (Figure 1b), whereas a twofold reduction in planktonic growth was observed (Figure 2a). Ni did not inhibit planktonic growth nor biofilm formation in *P. aeruginosa* or *E. coli* (Figure 1). Zn could not prevent the formation of biofilms and planktonic cell growth of *P. aeruginosa* (challenge with Zn or Ni resulted in a 1-log and 2-log reduction in planktonic (Figure 2a) and biofilm viable cell numbers (Figure 2b), respectively). For *S. aureus*, the attachment of biofilms and planktonic growth was prevented upon incubation with Zn, yet only biofilm attachment was prevented for *E. coli*. Last, there was no observed reduction in planktonic or biofilm viable cell numbers after exposure of *E. coli* to Ni for 4 h (Figure 2).

Certain metal ions are capable of eradicating established biofilms

The eradication of biofilms by various metal salts was assessed in a similar manner as the prevention of biofilms. However, to determine the concentration needed to eradicate an established biofilm, biofilms were established by incubating the inoculum in a CBD for 24 h. This was followed by exposure to twofold serial dilutions of the metal salts for an additional 24 h. After metal exposure, it was observed that Cu, Ag, Ga, Ti and Al had the capacity to eradicate biofilms of all three of the tested strains (Figure 3). Although Ni and Zn were found to be effective at eradicating *S. aureus* and *E. coli* biofilms after 24 h metal exposure, *P. aeruginosa* biofilms were not eliminated—rather a 50% decrease in viable cell numbers was observed (Figure 4). A higher concentration of Ag, more so than any other metal, was needed to eradicate *S. aureus*, whereas the opposite was observed for *E. coli* (Figure 3).

DISCUSSION

Numerous accounts of resistance from bacterial biofilms to conventional anti-microbials have been reported since the 1990s.[1] We are entering an era where our options to treat acute and chronic infections are limited. Consequently, alternative strategies to combat biofilm bacterial resistance and tolerance are being investigated.[19–22] Among these alternate strategies is the use of metal compounds as

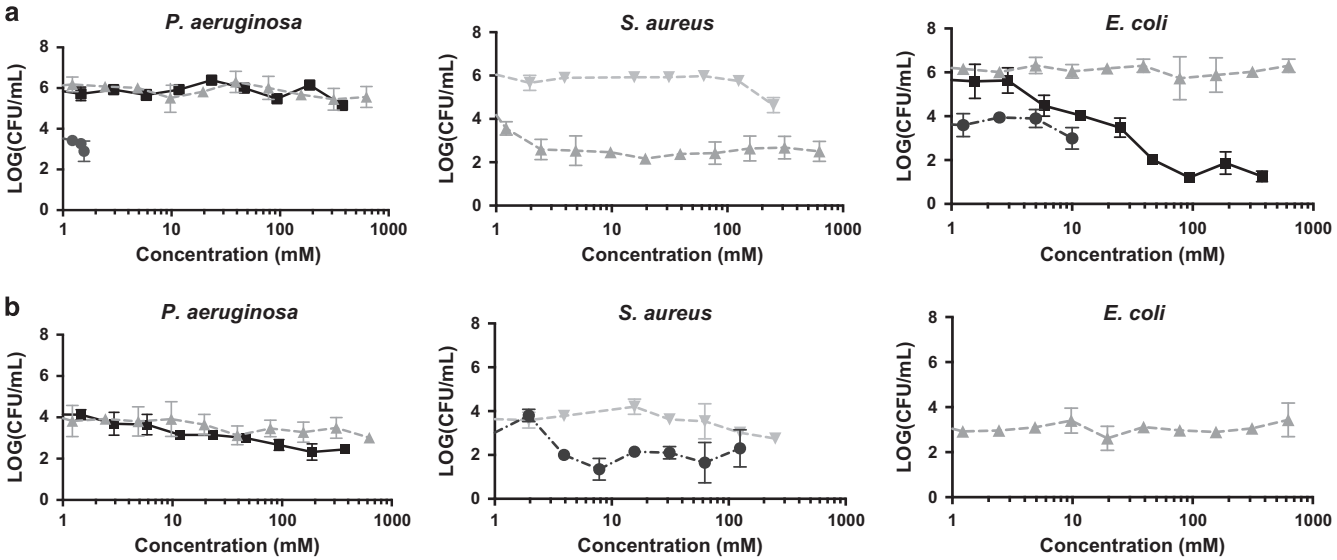

Figure 2 Growth tolerance of *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) to several metals. Within the concentrations tested, the metals that could not prevent the growth of planktonic cells are shown in (**a**), and those incapable of preventing biofilm growth are shown in (**b**). The CBD was inoculated with the bacteria in the presence of $AgNO_3$ (●), $NiSO_4 \bullet 6H_2O$ (▲), $Al_2(SO_4)_3 \bullet H_2O$ (▼) or $ZnSO_4 \bullet 7H_2O$ (■). The cells were exposed to serial dilutions (twofold) of each metal for 4 h followed by viable cell counts. Values are represented as the mean ± s.d., $n = 3$. *Note*: All metal stock solutions were prepared at equal molar equivalents of metal molecule. Hence, the concentrations found in this figure are reflective of the concentrations of metal and not the compounds themselves.

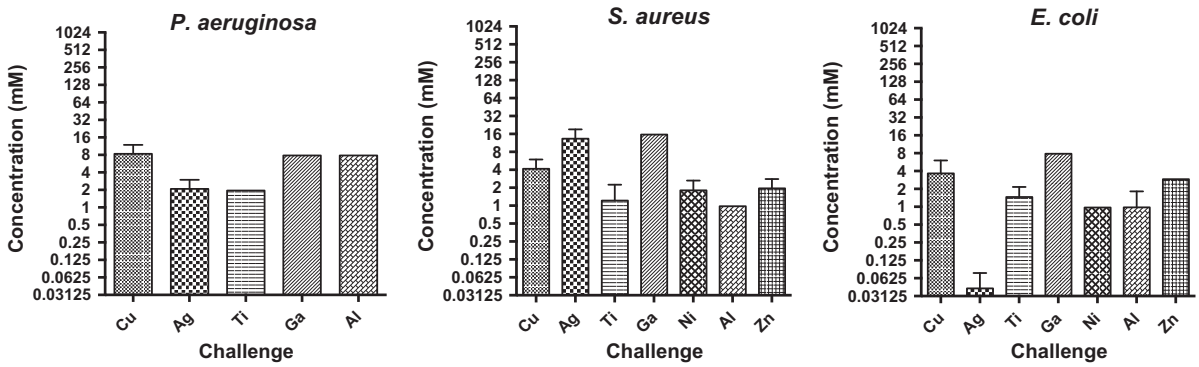

Figure 3 Ability of the metals to eradicate established biofilms. The MBECs of *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) in the presence of $AgNO_3$, $CuSO_4$, $TiCl_3$, $Ga(NO_3)_3 \bullet H_2O$, $NiSO_4 \bullet 6H_2O$, $Al_2(SO_4)_3 \bullet H_2O$ or $ZnSO_4 \bullet 7H_2O$. The CBD was inoculated in the absence of the metals salts and grown for 24 h. The established biofilms where then exposed to twofold serial dilutions of each metal; viable cells were counted to determine the MBEC. Values are represented as the mean ± the s.d., $n = 3$. *Note*: All metal stock solutions were prepared at equal molar equivalents of metal molecule. Hence, the concentrations found in this figure are reflective of the concentrations of metal and not the compounds themselves. Only the metals that were capable of eradicating established biofilms in the concentrations tested are shown.

anti-microbial agents that are capable of disrupting growth and/or eradicating biofilms.[9] Despite their re-emerging use, little effort has been directed toward comparing the susceptibility of planktonic cells and biofilm communities to metals under a defined set of conditions. Here we demonstrate how a reproducible screening method was used to compare the susceptibility of bacterial strains to several metal salts. Chemically simulated wound media were used to provide a rich environment containing proteins, lipids and a large variety of ions for promoting bacterial growth. The aim of this study was to provide a robust comparison of the efficacy of various metals against three defined indicator strains, namely *P. aeruginosa*, *S. aureus* and *E. coli.*

Ag has been studied for its efficacy at disrupting and/or eliminating biofilms.[23] Contrary to such studies, the MPBCs and MBBCs for *S. aureus* were not reached in the concentrations tested in this work (Figure 1). Decreased anti-microbial susceptibility may be regarded as the most consequential phenotype of bacterial biofilms, and for many anti-microbial agents this concept holds true.[24] Despite this, data have suggested that under selected growth conditions residence within a biofilm does not always provide enhanced resistance against anti-microbials,[25–27] and several of our observations support this. In fact, Ag was successful at preventing the formation of *P. aeruginosa* and *E. coli* biofilms (Figure 1b); however, this metal was incapable of inhibiting planktonic growth within these two strains (Figure 1a).

Cu(II) is known to increase intracellular levels of reactive oxidative species,[28–30] catalyze hydroxyl radical formation[31] and target enzymes in the iron-sulfur dehydratase family.[12] Both Cu(II) and Ag(I) are thiophilic metals and share similar selectivity for biological donor ligands in the bacterial cell.[9] Yet, one key difference between the two metals is their biological function. Cu(II) is an essential metal for many cellular redox enzymes, whereas Ag(I) is a non-essential metal in

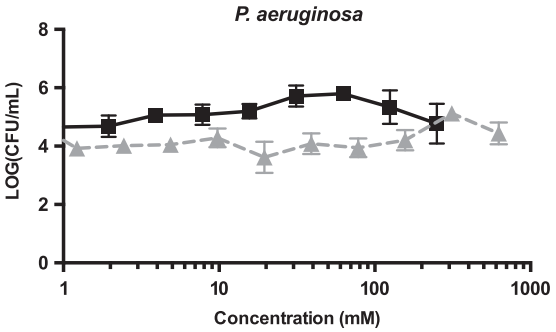

Figure 4 Biofilm eradication tolerance. Efficacy of $NiSO_4 \bullet 6H_2O$ (▲) and $ZnSO_4 \bullet 7H_2O$ (■) against *P. aeruginosa* ATCC 27853. The CBD was inoculated and incubated for 24 h in the absence of the metal challenges. The established biofilm was then treated with serial dilutions (twofold) of the metal salts. Values are represented as the mean ± s.d., $n = 3$. *Note*: All metal stock solutions were prepared at equal molar equivalents of metal molecule. Hence, the concentrations found in this figure are reflective of the concentrations of metal and not the compounds themselves.

which the precise manner of toxicity within all cell types still remains unclear. In this work, we found Cu to be effective for preventing biofilm attachment (Figure 1b) and eradicating established biofilms (Figure 3). In addition, this metal was capable of preventing the growth of planktonic cells (Figure 1a), different from what was observed with Ag. In general, we determined that the tendency of Ag to precipitate in CSWM proved its efficacy as an anti-microbial agent against cells in either cellular state to be secondary to Cu. Nonetheless, the efficacy of Ag as an anti-microbial agent continues to be observed,[32] and a substantial amount of effort has gone into developing silver-based materials.[33]

Certain transition metals have a documented capacity to disrupt cellular donor ligands that coordinate the essential ion Fe(III).[9] Destruction of [Fe-S] clusters may release additional Fenton-active Fe into the cytoplasm increasing intracellular reactive oxidative species formation.[11,14,34] Ga(III) has been found to target solvent-exposed [Fe-S] clusters as many biological systems are unable to distinguish between Ga(III) and Fe(III).[35] In fact, we observed that this metal was effective at inhibiting biofilm and planktonic cell growth in all three strains (Figures 1 and 3). The use of Ga as an anti-microbial agent is not novel, and in parallel with our data, the anti-microbial properties of this metal have been demonstrated both *in vitro* and *in vivo* against numerous organisms.[36] It should be noted, however, that upon comparison with other bodies of work, we observed that higher concentrations of Ga were needed to eliminate all three strains.[10,37] This observation provides insight into the influence of experimental conditions on biofilm and planktonic anti-microbial susceptibility. In fact, we have repeatedly observed that different media formulations give rise to exceedingly different tolerance levels (unpublished data).

Al(III), like Ag(I), is also a non-essential metal in which the precise mechanism of cellular uptake has yet to be determined. This metal was found to be effective at preventing the formation of biofilms and planktonic cells in *P. aeruginosa* and *E. coli* (Figure 1). Contrary to this, Al was not effective at preventing biofilm formation and planktonic cell growth in *S. aureus* in the concentrations tested; however, a single-fold reduction in viable cell numbers was observed during a 4 h metal exposure (Figure 2b). As the MBEC was reached for *S. aureus* in the presence of Al during the 24 h incubation, we speculate that the mechanism of Al toxicity is subject to longer metal exposure. *E. coli* was found to comply to the same trend based on the concentrations needed to reach the MBBC and MBEC, again reflecting the requirement of prolonged metal exposure for the efficacy of some metals.[25]

Contrary to what was observed for Ag and Al, the biofilms of each indicator strain were found to be less susceptible to Ti when compared with the planktonic cells (Figure 1). This was particularly evident for *S. aureus*, in which there was a fourfold increase in the concentration of Ti needed to prevent the formation of a biofilm when compared with the concentration needed to eliminate the planktonic cells.

The MBBC was reached upon the addition of Zn in *E. coli* and *S. aureus* in the concentrations tested (Figure 1). For both strains, the MBBCs were found to be comparable to work completed in other studies, in which biofilm growth was found to decrease by at least 50% upon exposure to $ZnSO_4$.[38] *P. aeruginosa* was found to be tolerant to this metal salt within the concentrations tested as no change in the growth of planktonic cells and biofilms were observed after 4 and 24 h treatments (Figures 1 and 3). Upon longer metal exposure, *E. coli* and *S. aureus* biofilms were eradicated, again giving insight into the time dependence of metal toxicity (Figure 3).

Ni, similar to Zn, was also observed to be less effective against all three strains. In *P. aeruginosa* and *E. coli*, no change in viable cell numbers were found upon Ni exposure. This metal was only capable of preventing the assembly of a biofilm in *S. aureus* (Figure 1b). The results suggest that a concentration well above 650 mM may be needed to reach the MPBC for all three strains, the MBBC for *P. aeruginosa* and *E. coli* and the MBEC for *P. aeruginosa* in the conditions tested. Still, this would be problematic as at these concentrations the metal salts precipitate. Nonetheless, this does not preclude the use of Ni and Zn as surface contact anti-microbials for certain infectious settings.[9]

The literature suggests a variety of mechanisms responsible for metal toxicity, and it is likely that each metal has different cellular targets and resultant toxicological effects.[9] Here we observed that a comparison between the seven metals gave rise to remarkably different efficacies versus three bacterial species. Additionally, comparing the susceptibilities of the three strains to even a single metal revealed pronounced differences. Upon further analysis, we revealed that the planktonic and biofilm cells of *P. aeruginosa* appeared to behave similarly with a 4 h metal exposure (Figure 3a). This trend was not observed for *E. coli* and *S. aureus*, in which the concentrations capable of inhibiting growth were different between planktonic cells or those residing within a biofilm. The planktonic cells of the Gram-negative strains demonstrated similar MPBCs for Ti, Ag and Ni; however, the biofilms did not share these similarities (Supplementary Figure 1a). Furthermore, differences were found in biofilm susceptibility of *S. aureus* and *E. coli*, revealing the greatest degree of dissimilarity between the MBBCs within the experimental conditions used in this study. Finally, upon biofilm establishment followed by 24 h metal exposure, the biofilms of *S. aureus* and *E. coli* had similar MBECS, particularly following Al, Cu, Zn and Ni addition (Supplementary Figure 1b).

CONCLUSIONS

Based on the MPBC, MBBC and MBEC data generated in this study, Cu, Ti and Al were the most effective metals for preventing the formation of, and eradication of *P. aeruginosa* biofilms. Meanwhile, Cu, Ti and Ga were the most efficacious metals against *S. aureus* and *E. coli* biofilms. From our observations in this study, Cu, Ti and Ga were found to have extended activity against planktonic cell growth, the attachment of biofilms and biofilm proliferation. This leads us to conclude that Cu and Ti are the only metals that have reasonable broad-spectrum efficacy against the strains used in this study.

However, an overarching theme of this study is that no metal should be considered a 'silver bullet'. The study of metal resistance genes during the 1990s has revealed that specific resistance mechanisms exist for almost all metals studied to date.[39] Nonetheless, reports have demonstrated that certain metals can enhance anti-microbial activity[40] and broaden the anti-bacterial spectrum of antibiotics.[41] Therefore, as a follow-up to this study, future directions include examining the ability of metals to increase bacterial susceptibility to antibiotics, and antibiotic activity against bacterial biofilms.

With the ever-increasing use of metal ion formulations and nanoparticles as anti-microbials, we must heed to the history of the evolution of antibiotic resistance and aim for more responsible use of anti-microbial metals—a situational approach of the appropriate metal, at the appropriate concentration for a given infectious setting.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This project was funded by the Discovery grant from the Natural Sciences and Engineering Research Council of Canada (grant number RJPIN/216887). JAL was supported by the Natural Sciences and Engineering Council of Canada—Banting Postdoctoral Fellowship Program and Alberta Innovates Health Solutions. NG was supported by the Eyes High Graduate Scholarship from the University of Calgary.

Author contributions: NG designed experimental methodology, conducted experiments, analyzed the data and wrote the manuscript. JAL designed experimental methodology, analyzed data and contributed in writing the manuscript. RJT, the corresponding author, contributed in writing the manuscript and provided additional research funding. All authors have read and approve the manuscript.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)