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Antibacterial effects of pure metals on clinically important bacteria growing in planktonic cultures and biofilms

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Antibiotic resistance in pathogenic bacteria is emerging as an issue of serious concern in bio-medical research as well as food and health organizations. Metal complexes are currently being employed in medical devices for their inhibition to bacterial adherence and antibacterial activities. The primary aim of this study was to evaluate the antibacterial activities of pure metals, including selenium, germanium and lithium on planktonic cultures and biofilms of three bacterial species: *S. aureus* SH1000, *P aeruginosa* PA01 and *E. coli.* O157:H7. The antagonistic effects of selenium, germanium and lithium on these three bacterial species were examined using zone of inhibition assay. The minimum inhibitory concentrations and minimum bactericidal concentrations of antibiotics (rifampicin, mupirocin and ciprofloxacin) and metals (selenium, germanium and lithium) were measured. The minimum biofilm eradication concentrations (MBEC) of metals were determined against biofilms composed of *S. aureus* SH1000 and *P. aeruginosa* PA01. Metal susceptibility tests suggested that biofilms displayed increased resistance over their planktonic state. Differential inhibitory effects were observed for different strains of planktonic and biofilm bacteria in response to different metals and their varying concentrations. Amongst the three metals tested, selenium proved to be the most active against all three species, whereas lithium demonstrated the least inhibitory effects. Scanning electron microscope (SEM) image analysis revealed several detrimental structural changes in bacterial cells exposed to metals compared to those grown in the metal-free culture. In conclusion, the results demonstrate the antibacterial efficacy of pure metals against planktonic and biofilm bacteria paving the way for further similar investigations in search of alternative antibacterial agents.

Key words: Antibiotic resistance, antibacterial, metal, biofilm, planktonic.

INTRODUCTION

Antibiotic resistance in pathogenic bacteria is emerging as a global issue of serious concern in bio-medical research as well as food and health organizations. The ability of bacterial DNA to mutate in response to anti-biotics, oxidative stress and environmental conditions results in decreased bacterial susceptibility towards commonly administered antibacterial agents. A wide range of mechanisms are suggested to contribute to the emergence of antibiotic resistant population through previously unknown metabolic interactions and gene expression patterns (Davies and Davies, 2010; Gniadkowski, 2008).

The biofilm bacteria, defined as a structured community of cells attached to a surface and encased in a polymeric

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matrix, are phenotypically different from planktonic cells of the same species, which float as single-cells in the liquid media (Costerton et al., 1994). Bacteria in biofilms are less susceptible to antibiotics leading to various infections and diseases (Kolenbrander, 2000) than their planktonic counterparts since antibiotic resistance in biofilms appears to depend on multicellular strategies (Hoyle and Costerton, 1991; Lewis, 2005; Stewart and William, 2001; Walters et al., 2003). Biofilms are known to play a significant role in causing medical infections in clinical settings, especially in the cases of implanted therapeutic devices such as catheters and orthopaedic implants (Lindsay and von Holy, 2006; Ramsay et al., 1989; Ramsey and Whiteley, 2009; Stewart and William Costerton, 2001). Pathogenicity of microorganisms is often enhanced when growing as a biofilm, which can develop into a multi-strain, dense-layered bacterial community protected by several levels of polymeric compounds, exhibiting increased resistance against external factors and antimicrobial agents (Chambless et al., 2006; Costerton et al., 1999; Jefferson et al., 2005). The slow bacterial growth in biofilms also contributes to antibiotic resistance since antibiotics seem to function best against rapidly dividing cells (Lewis, 2005). The problem demands efforts to search for other antibacterial agents to fight against complex antibiotic resistant infections. Owing to their significant roles in science, metals have been drawing continual interest as antibacterial agents. Several studies reported encouraging results on antibacterial activities of various metal complexes (Chohan et al., 2006; Harrison et al., 2004; Sönmez et al., 2006; Sreekumari et al., 2005), but not much information is available on the antibacterial properties of pure metals. A few studies demonstrating the effects of pure metals against bacterial community show differential response of metals towards different bacterial strains(Miyano et al., 2007; Sreekumari et al., 2005; Yasuyuki et al., 2010a). Thus, a baseline data on the antibacterial efficacies of different metals is essential to develop superior antibacterial agents.

Selenium, germanium and lithium are three distinct non-related elements used in medicine, often in complex with other elements and compounds. Selenium, a component of selenoproteins, is known as an anti-cancer agent, and is used in therapies against prostate cancer (Facompre and El-Bayoumy, 2009). Tran et al. (2009) demonstrated the effectiveness of 0.2% selenium coating on cellulose dressings for wounds against *P. aeruginosa* and *S. aureus*. Yang et al. (2009) reported antagonistic effects of selenium-enriched probiotics against pathogenic *E. coli in vivo* and *in vitro*. Some of the novel organoselenium compounds are also shown to be useful against several bacterial species, including *Staphylococcus aureus*, *Staphylococcus simulans, Salmonella typhimurium, E. coli* and *Bacillus* cereus (Pietka-Ottlik et al., 2008; Radhakrishna et al., 2010).

However, the direct effects of selenium on the bacterial community are still awaited.

Germanium is used in small portions in pharmaceuticals and nutritional supplement formulations (Rosenberg, 2009). Organogermanium compounds are shown to possess antioxidant-like properties (Yang and Kim, 1999), antitumor activities (Shangguan et al., 2005; Zhang et al., 2009) and several other biological roles, including neurotropic, anticoagulant, vasodilating and cardioprotective functions (Lukevics and Ignatovich, 2003). Compounds like germanium oxide have been shown as potent antimutagens (Kada et al., 1984), although different microorganisms show differential sensitivities to these compounds (Dyke et al., 1989). The potential of organic germanium compounds as effective antibacterial agents has been successfully proved against some of the human pathogenic bacteria (Sellapa and Jeyaraman, 2011).

Lithium, used to treat depression and bipolar disorder, is suggested to stimulate immune function by inhibiting the production of prostaglandin E2, and in turn fighting against microorganisms (Lieb, 2004 ; Lieb, 2007). Recently, the combined inhibitory effects of lithium fluoride (LiF) with commonly used antimicrobials was examined on the growth of several bacterial species (Syed and Ravaoarinoro, 2012). The results show synergistic effects of LiF with the antibiotics used and suggested its potential as an adjuvant in antibacterial treatment. The suggested roles of lithium, if investigated and exploited well, can help in preventing critical infections and surgeries.

Due to such scientific support on the antibacterial effects of these metal complexes, it becomes logical to investigate their direct effects on bacterial species. Thus, the primary aim of this study was to evaluate and compare the antibacterial activities of pure metals, including selenium, germanium and lithium on planktonic cultures and biofilms. The study tested the effects of these metals on three bacterial species: *S. aureus SH1000*, *P. aeruginosa PA01* and *E. coli. O157:H7* by measuring their bacterial growth, cell viability and visualizing the ultrastructural cell damage using SEM. Response to planktonic bacteria was measured by determining the minimum inhibitory concentration (MIC) at which the bacterial growth is inhibited, and minimum bactericidal concentration (MBC) of antibiotics and metals against the planktonic bacterial cultures that effectively kills the test bacteria. The metal activity against biofilms was estimated by determining the minimum biofilm eradication concentration (MBEC) using the commercially available MBEC™ High-Throughput (HTP) assay (Innovotech, USA).

MATERIALS AND METHODS

Zone of inhibition assay

The primary antimicrobial activity of the pure metals selenium (Se),

germanium (Ge) and lithium (Li) was determined using the zone inhibition assay as described by Negi et al. (2011). Planktonic bacterial cultures of *S. aureus* SH1000, *P. aeruginosa* PA01 and *E. coli* were obtained from pure stocks of the Department of Molecular Biology and Biotechnology (University of Sheffield, UK). Each was inoculated into 5 ml of Mueller-Hinton Broth (MHB) (Sigma-Aldrich,UK), and grown overnight for 18 h at 37°C with shaking at 120 rpm. 500 µl of the overnight grown cultures were inoculated into 50 ml MHB and incubated for 4 h at 37°C with shaking at 120 rpm. 1 ml each of the three cultures was added to 10 ml of lukewarm broth and poured and carefully distributed over the surface of solidified nutrient agar plates. Cylindrical wells of 1 cm diameter were then bored into the agar using a sterilized cork borer. For all three species, varying amounts of the three pure metal sources (1.25, 6.25, 12.5, 25, 50 and 100 mg/ml) were added to the wells and allowed to diffuse for 12-16 h at 37°C. The circular and clear inhibition zones around each hole were then measured in millimetres (mm.) (Negi et al., 2011).

Cell viability test

1 ml each of the overnight grown planktonic cultures of *S. aureus* SH1000, *P. aeruginosa* PA01 and *E. coli* were added to 10 ml of MHB in 50 ml flasks. Varying amounts of pure metal source (1.25, 6.25, 12.5, 25, 50 and 100 mg) of Se, Ge and Li was added to each flask and incubated for 16 h at 37°C with shaking at 120 rpm. Aliquots of samples were taken and imaged under a scanning electron microscope (SEM), however only the cultures grown in 50 mg metals showed clear differences.

The number of viable *S. aureus*, *P. aeruginosa* and *E. coli* cells remaining in the suspension was measured by making a series of dilutions. 1 ml aliquot of each dilution was spot plated on solid nutrient agar plates followed by an overnight incubation at 37°C. The colonies formed in the agar gel were counted and the cell counts were multiplied by the dilution factor and expressed as colony forming units (CFU) per ml.

Determination of minimum inhibitory (MIC) and minimum bactericidal concentration (MBC)

Colonies for *S. aureus* and *P. aeruginosa* were formed by streaking bacterial inoculums on sterile Mueller-Hinton Agar (MHA) (Sigma-Aldrich UK) plates followed by incubation for 24 h at 37°C. Single colonies were picked, inoculated into tubes with MHB and incubated for 18 h at 37°C with shaking. The resulting cultures were then diluted (1:100) by adding 0.1 ml of culture to 9.9 ml of MH broth for determining MICs and MBCs. To determine the MIC of mupirocin (MUP) (Sigma-Aldrich UK), the starting MUP concentration was 16 µg/ml. Serial dilutions were made with sterile saline to further achieve the following concentrations: 8, 4, 2, 1, 0.5, 0.25, 0.0625 and 0.03125 µg/ml. Similarly, the starting concentration for rifampicin (RIF) (Sigma-Aldrich UK) and ciprofloxacin (CIP) (Sigma-Aldrich UK) was 2 µg/ml and double dilutions were made to obtain the following concentrations for their MIC determination: 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.01563, 0.0078 and 0.0039 µg/ml. 20 µl of each antibiotic dilution was pipetted in duplicates in the wells of 96-well microtitre plates. 180 µl of diluted bacterial cultures of all three species was added to all wells with different antibiotic concentrations. Negative and positive controls were included in each run. The microtitre plates were incubated for 24 h with shaking at 37°C. After incubation, bacterial growth was observed in the form of cloudiness. The MIC was the lowest antibiotic concentration that resulted in clear wells. The same procedure was followed for determining the MICs of metals for *S. aureus* and *P. aeruginosa* using the same concentrations for pure Se, Ge and Li. The MBCs for metals were determined by sub-culturing the broth dilutions to nutrient agar plates and counting the viable cells. 180 µl of each antibiotic dilution was pipetted in the Petri dishes followed by the addition of 18 ml of pre-cooled MHA. After the plates were cooled and dried, they were inoculated with 100 µl of diluted (1:1000) bacterial cultures. The presence of bacterial colonies was recorded after an incubation of 24 h at 37°C.

Minimum biofilm eradication concentration assay

The MBEC assay, previously used in many studies (Arias-Moliz et al., 2012; Sepandj et al., 2004), allows the simultaneous determination of MBEC values for many test solutions. The assay entails the formation of identical biofilms on plastic pegs on the lid of the MBEC device (Innovotech, USA). The biofilms are exposed to test metals for a certain time period and then transferred to a 96-well recovery plate. After an incubation of 72 h, the MBEC values are determined.

Overnight cultures of *S. aureus* SH1000 and *P. aeruginosa* PA01 were prepared in MHB at 37°C with shaking. This was followed by uniformly spreading the overnight grown culture on sterile MHA Petri plates. A sub-culture was prepared by streaking the cells on MHA plates. The individual colonies from the subculture were added to MHB and the suspension's turbidity was adjusted to match the turbidity of a 1.0 McFarland standard, approximately 3×10^8 CFU/ml of suspension. This solution was further diluted to 1:30 where the diluent was 1/10 strength MHB. 20 µl of the 1:30 dilution of each bacterial strain was used to inoculate the trough of the MBEC^{TM}-HTP device, (Innovotech, Ca). The peg lid was fitted inside the trough and the assembled device was then placed on a rocker (3-5 rocks/min) to promote biofilm growth on the pegs of the lid. After 48 h of incubation at 37°C, the pegs on the device showed attached or adherent biofilms. The lid was removed from the device and immersed for 2 min in sterile 96-well plate containing 200 µl of 0.9% saline. This step was performed twice to rinse off loose bacterial cells adhering to the biofilm.

To determine biofilm formation, the selected pegs were cut off from the lid of the MBEC^{TM}-HTP plate using sterile and flamed pliers, and were immediately transferred to the wells of another sterile microtitre plate containing 200 µl of 0.9% saline. Aliquots of planktonic cultures collected from the trough of the MBEC^{TM}-HTP device were also placed in the assigned wells of the same microtitre plate. The cultures were sonicated on high settings for approximately 30 min by placing the microtitre plate on a table sonicator to disrupt the biofilms. Ten-fold serial dilutions were made for plaktonic cultures and biofilms, and were then plated on nutrient agar medium to observe growth.

The susceptibility tests were performed in a microtitre plate called challenge plate. After the biofilm formation on the pegs, the lid was immersed in the challenge plate containing 200 µl of serial dilutions (100, 50, 25, 12.5, 6.25, 3.75 mg/ml) of metals, and was allowed to incubate for 24 h. After the exposure of biofilms to metal solutions, the peg lid was rinsed twice as previously described. The peg lid was then placed in another 96-well plate, called the recovery plate. Each well of the recovery plate was filled with 200 µl of recovery/neutralizing solution. The recovery solution was prepared by mixing 1 g L-histidine, 1 g L-cysteine and 2 g reduced glutathione (Sigma-Aldrich,UK) in a total volume of 20 ml with double-distilled water. The mixture was filter-sterilized through 0.20 µm filter and stored at -20°C until use. Before use, the solution volume was brought up to 1 L using MHB supplemented with 20 g/l saponin and 10 g/l Tween-80. The pH of the final solution was adjusted to 7.0. The recovery plate was placed on top of a table sonicator. The biofilms were disrupted and dislodged from the lid pegs and transferred to the recovery plate by sonicating on high speed for 30 min. The peg lid was removed and discarded, whereas the recovery plate with the detached biofilms were covered and incubated for 72 h at 37°C. The MBEC values were determined by visually exami-

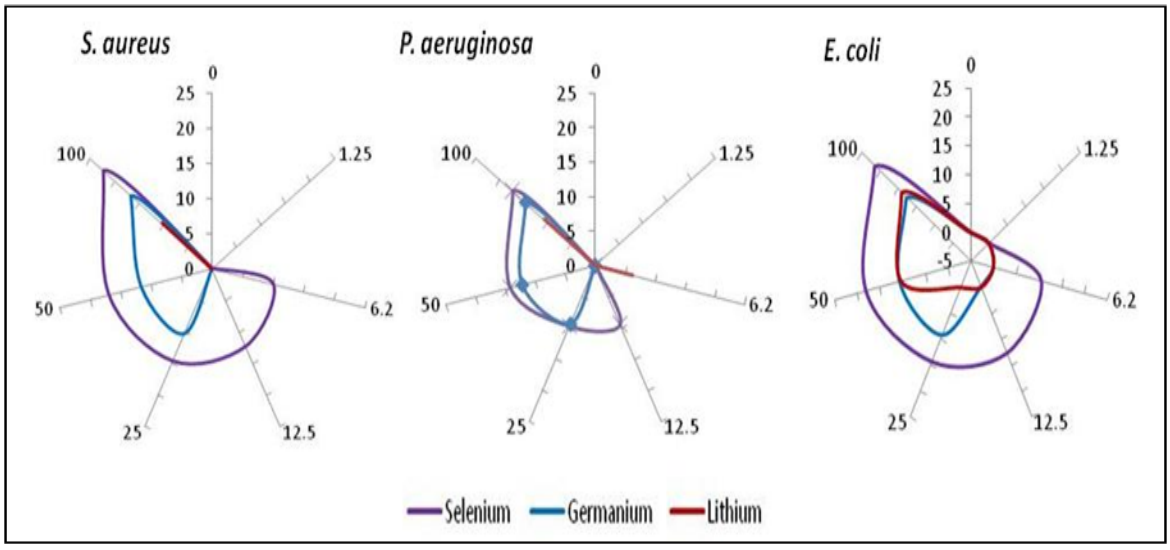

Figure 1. Zone of inhibition (mm) produced after growing bacteria with metals in agar medium. Values represent the mean of three replicates. Points were fitted using radar graph feature in Excel (Microsoft 2003).

ning the turbidity in the wells of the recovery plate. Clear wells indicated absence of biofilms and successful biofilm eradication.

RESULTS

Examining the primary antibacterial activity of pure metals using zone of inhibition assay

The antagonistic effects of selenium, germanium and lithium were tested against the three bacterial species using the well diffusion assay. The diameter of the measured zone of inhibition is directly proportional to the strength of inhibition. The primary antibacterial activity of the three metals varied among the three bacterial species tested and, as expected, showed a gradual increase with increasing metal concentrations (Figure 1). Amongst the three metals tested, selenium was found to be the most active against all three species, whereas lithium demonstrated the least inhibitory effects. Selenium inhibited the growth of *S. aureus* and *E. coli* at 6.2 mg, whereas the inhibitory amount of selenium for *P. aeruginosa* was 12.5 mg (Figure 1).

Germanium hindered the growth of all three bacteria at 25 mg. Lithium, being the least effective, showed inhibitory effects on *E. coli* at 50 mg, whereas *S. aureus* and *P. aeruginosa* were inhibited using 100 mg of lithium. The results show that the *E. coli* was the most sensitive bacteria to the action of selenium and lithium, whereas *P. aeruginosa* was the least susceptible since its growth was affected at double the concentration of selenium required to inhibit the growth of the other two species.

Effects of pure metals on the cell viability of bacterial species

The cell viability of *S. aureus*, *P. aeruginosa* and *E. coli* was evaluated to test their ability to reproduce in a nutrient medium under favorable conditions after exposing them to metals. Each surviving cell should ideally develop into an individual colony after an overnight incubation in Mueller-Hinton Agar thus providing a precise measure of bacterial viability. Figure 2 summarizes the cell viability data for the three species. The cell viability of bacterial cultures decreased with increasing metal concentrations in the nutrient broth, with selenium showing the most drastic effects on all three species (Figure 2). The growth of *S. aureus* and *E. coli* declined by 95% at 6.25 mg/ml of selenium, whereas the *P. aeruginosa* cell viability reduced gradually from 34% at 6.25 mg/ml of selenium to 98% at 25 mg of selenium. All bacterial strains showed at least 89% growth reduction at 6.25 mg of germanium. Approximately, 2% of *S. aureus* and *P. aeruginosa* cells were still viable at 50 mg of germanium, but none survived the100 mg treatment. It is interesting to note that addition of up to 12.5 mg lithium in the broth medium showed an increase in the cell viability of *S. aureus* and *P. aeruginosa* (Table 2). However, the cells started to die at 25 mg of lithium, and no bacteria remained viable at 100 mg treatment. Interestingly, 30% of the *E. coli* cells survived at 100 mg lithium. Nevertheless, *E. coli*, once again, exhibited least resistance to test metals added in the growth medium, whereas *P. aeruginosa* was found to be the least sensitive to metal stress.

Determination of minimum inhibitory and bactericidal concentrations

Minimum inhibitory and bactericidal concentrations were determined for planktonic cultures of *S. aureus* and *P. aeruginosa* exposed to antibiotics and metals. The results showed that MICs of antibiotics ranged from 0.0078 to 32 µg (Table 1). *S. aureus* was found to be more sensitive to rifampicin than mupirocin, whereas *P. aeruginosa* was more sensitive to ciprofloxacin than rifampicin. The MICs and MBCs of metals also varied for the two species. All three metals were effective in inhibiting the bacterial growth of the two species at relatively low concentrations. However, both the species were observed to be most sensitive to selenium and least sensitive to lithium.

Determination of minimum biofilm eradication concentration

The minimum concentration of metals that results in eradication of bacterial biofilms was determined using MBEC assay. The formation of biofilms was confirmed by observing bacterial growth on plates inoculated with samples grown in metal-free culture. The minimum biofilm eradication concentrations (MBEC) of metals are

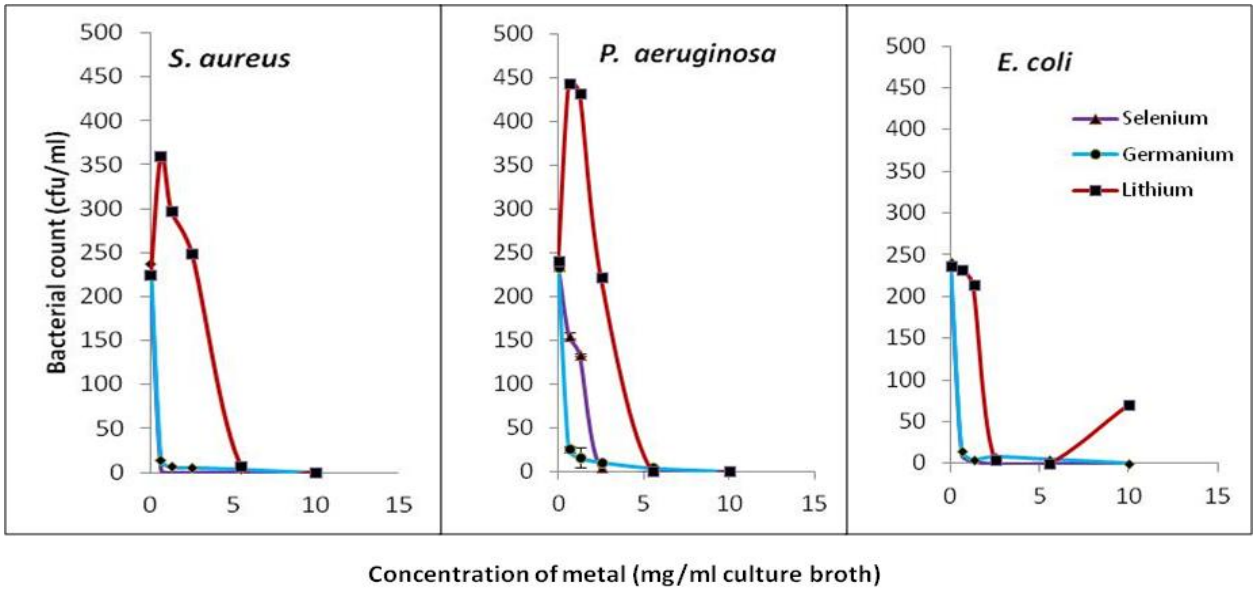

Figure 2. Growth of the different bacteria cultured in nutrient broth supplemented with varying concentrations ofselenium, germanium, and lithium. Points were fitted using scatter graph feature in Excel (Microsoft 2003).

Table 1. Minimum inhibitory concentrations and minimum bactericidal concentrations of antibiotics and metals for planktonic cultures of *S. aureus* and *P. aeruginosa*.

Antibiotic/ metal	*S. aureus* SH1000		*P. aeruginosa* PA01	
	MIC	MBC	MIC	MBC
Rifampicin	0.0078	-	32 µg/ml	-
Mupirocin	0.125 µg/ml	-	-	-
Ciprofloxacin	-	-	0.1250 µg/ml	-
Selenium	0.0156 mg/ml	3.125 mg	0.1953 mg/ml	6.25 mg/ml
Germanium	0.3125 mg/ml	6.250 mg	0.6250 mg/ml	6.25 mg/ml
Lithium	2 mg/ml	12.50 mg	3.1250 mg/ml	12.50 mg/ml

Table 2. Minimum biofilm eradication concentrations of metals for biofilms composed of *S. aureus* and *P. aeruginosa*.

Metal	Minimum biofilm eradication concentration (mg/ml)	
	S. aureus SH1000	*P. aeruginosa* PA01
Selenium	25	12.5
Germanium	50	25
Lithium	50	25

summarized in Table 2. Results of the assay show that 12.5 mg/ml of Se and 25 mg/ml each of Ge and Li was effective in eradicating all biofilm bacteria for *P. aeruginosa*. The MBEC of metals for *S. aureus* was almost double the MBEC for *P. aeruginosa*, implicating higher resistance of *S. aureus* to the metals evaluated. The MBC and MBEC data value comparison (Tables 1 and 2) for metals against the two species points out the higher susceptibility of planktonic bacteria to the effects of metals compared to biofilms.

Scanning electron microscope imaging of planktonic cells exposed to metals

Conventional SEM image analysis was performed to visualize the ultrastructural damage caused by metal treatment to bacterial cells. Planktonic cells of *S. aureus*, *P. aeruginosa* and *E. coli* were grown in media supplemented with 50 mg Se, Ge and Li for 16 h. The cultures were then washed, fixed and imaged using SEM. Figure 3

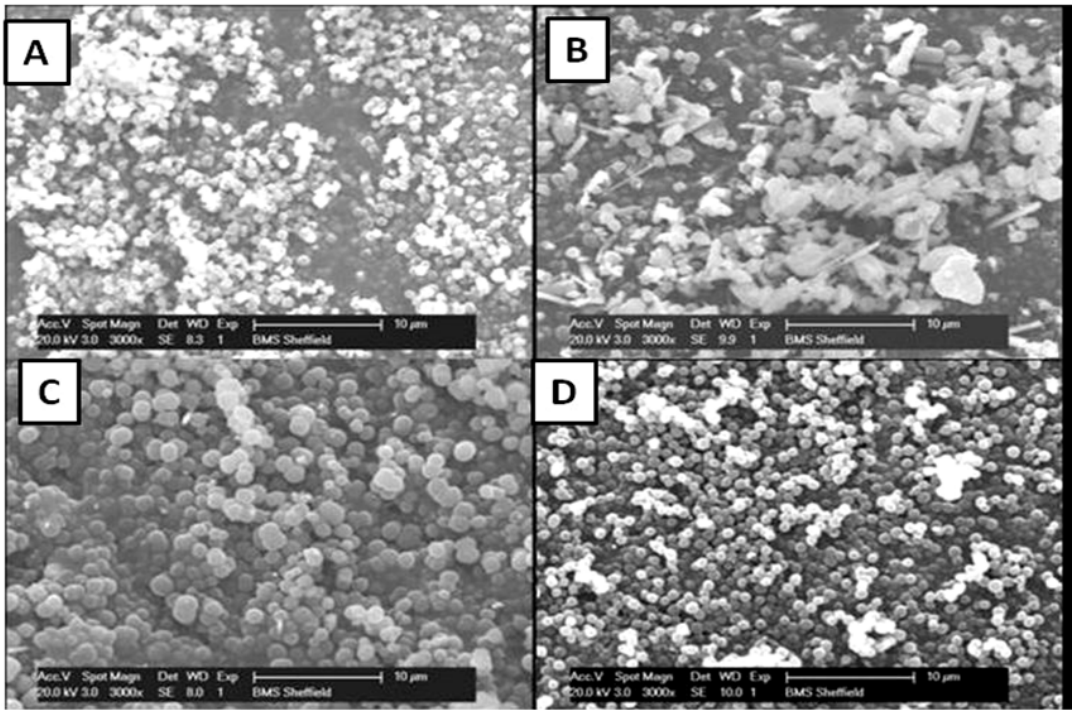

Figure 3. SEM image of planktonic *S. aureus* cells after 16 h of metal treatment in media supplemented with 50 mg of (A) selenium, (B) germanium, (C) lithium and (D) pure culture without metal.

shows the effects of these metals on *S. aureus* cultures. Several structural changes were observed in the treated cells (Figure 3A-C) compared to those grown in the metal-free culture (Figure 3D). *S. aureus* cells treated with Se appeared majorly damaged and dehydrated suggesting cell membrane disruption and leakage of cytoplasmic constituents (Figure 3A). *S. aureus* cells exposed to germanium-enriched media looked dehydrated and clustered (Figure 3B). Among the three metals, lithium seemed least effective in damaging the cell structure integrity of *S. aureus* (Figure 3C). The coccal cells were intact with thick cell layers which prevented the penetration of lithium into the cells.

Exposure of *P. aeruginosa* cells to metals also resulted in disintegration of cells with Se causing the most damage to the cell structure (Figure 4). Bacterial cells treated with Se lost their original rod shapes, most likely due to dehydration and shrinkage (Figure 4A). The metal could also be spotted in the image indicating its adsorption and integration within the bacterial cells. Germanium toxicity also led to dehydration of *P. aeruginosa* cells and disintegration of cellular layers (Figure 4B). However, some of the cells survived retaining their original rod shaped structure. This correlates well with a previous study showing *Pseudomonas* strain as one of the most tolerable bacterial strains against germanium(Van Dyke et al., 1989). As seen in Figure 4C, intact cells could be observed with some visible cell clumping indicating not much damage to *P. aeruginosa* cells by lithium exposure.

E. coli cells treated with Se were also damaged and dehydrated losing their original rod-shaped structure (Figure 5A). The presence of clumps indicated cellular debris from dead cells. Germanium treatment affected the cell number though the cell structures and sizes were retained (Figure 5B). Although, the *E.coli* cells exposed to lithium more or less resembled the control cells, there was some cell disintegration and clumping with reduced cell size (Figure 5C-D). Thick cell layers could be seen indicating lack of effective lithium penetration into the cells.

DISCUSSION

Increasing bacterial resistance to antibiotics has raised worldwide concern with an urgent need for antibiotic control programs and employment of novel and improved antimicrobials to limit pathogen resistance. Increased antibiotic resistance can be traced to many factors, including the overuse of antibiotics. Bacteria become resistance to antimicrobials as a result of increased bacterial adaptability by mutating their DNA sequence or exchanging their genetic material such that the antibiotic targets are altered. The formation of biofilms and the resultant multi-strain community of bacteria also make it difficult for the antibiotics to penetrate through individual cells. Large population of cells in a biofilm leads to depletion of nutrients resulting in oxidative stress. To cope up with that, the bacterial cells either enter a dormant stage or produce mutations, and eventually become resistant to antibiotics.

Such a situation where bacteria shows susceptibility at a certain concentration of antibacterial agent but then recovers at a higher concentration is referred to as “paradoxical effect” (Wainwright, 1994). The results for lithium presented here contradicted an earlier report on its antibacterial effects (Lieb, 2007), and thus need to be verified in future studies.

Scanning electron microscopy was employed for visualizing the effects of metals on bacterial cell structure. Comparing the MICs, bacterial species displayed higher

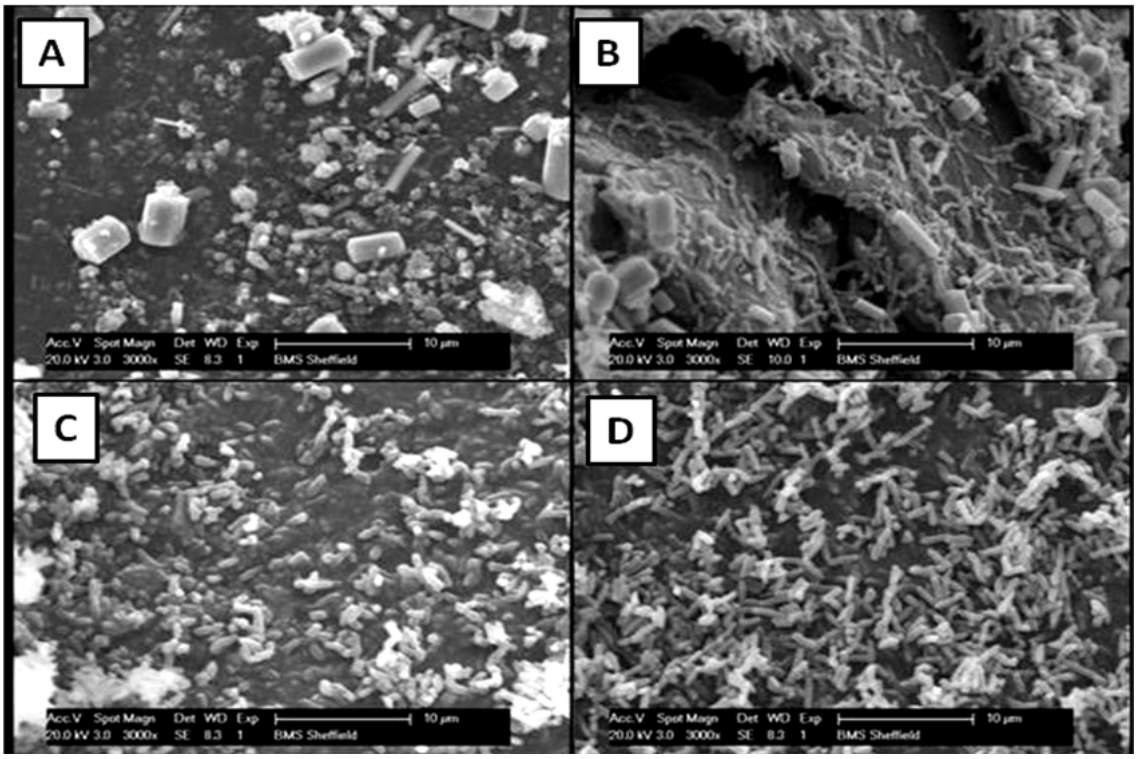

Figure 4. SEM image of planktonic *P. aeruginosa* cells after 16 h of metal treatment in media supplemented with 50 mg of (A) selenium, (B) germanium, (C) lithium and (D) pure culture without metal.

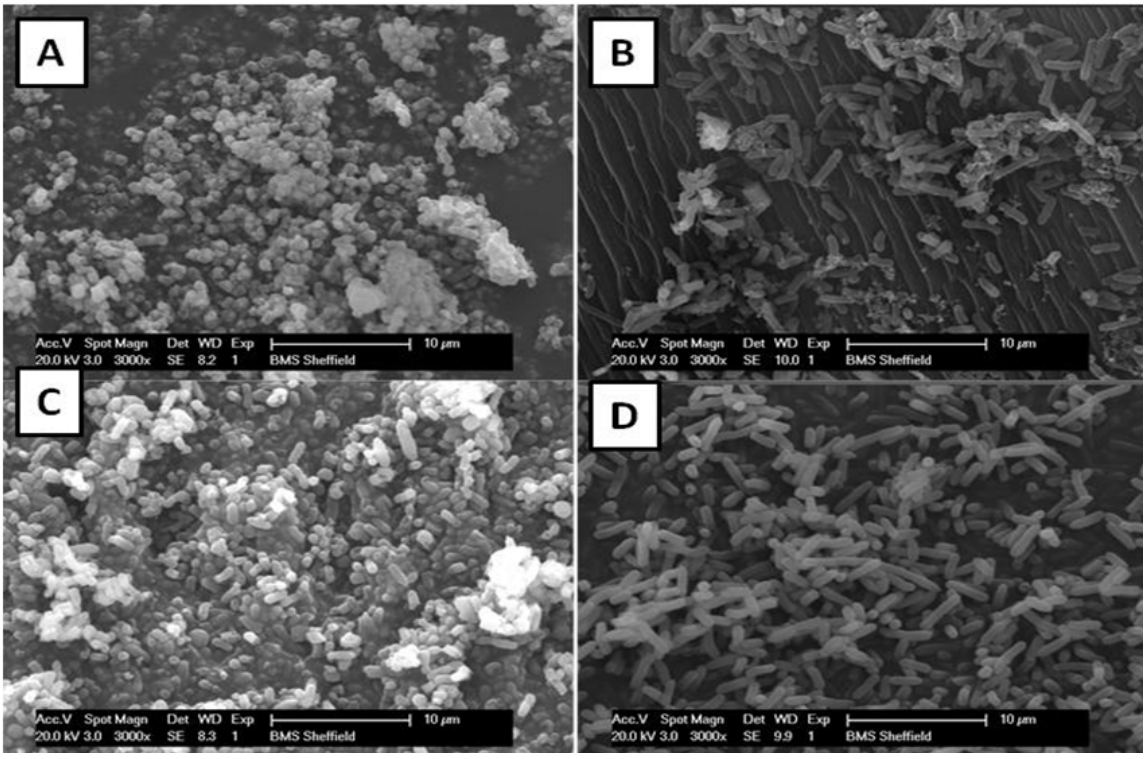

Figure 5. SEM image of planktonic *E. coli* cells after 16 h of metal treatment in media supplemented with 50 mg of (A) selenium, (B) germanium, (C) lithium and (D) pure culture without metal.

sensitivity towards antibiotics compared to metals, giving credence to previous reports suggesting metal toxicity as an outcome of prolonged exposure (Diaz-Ravina and Baath, 1996; Harrison et al., 2004). Metal accumulation is known for disrupting bacterial cell wall and other cell components (Yasuyuki et al., 2010b). Growing bacteria in different metal concentrations resulted in cell clumping, cell damage, formation of bacterial aggregates and dehydration, indicating damage in cell membrane and leakage of cytoplasmic constituents. The *P. aeruginosa* cells also lost their rod shaped structure especially when exposed to selenium (Figure 4A). Lithium appeared to have the least destructive effect on the cell structures, validating the earlier test results. The length of exposure to lithium could be a limiting factor for its weaker effects. In addition to the reports stating that the metal tolerance depends on the interaction of the metal and the bacterial strain(Van Dyke et al., 1989), it has also been shown that extending the exposure time to metals does not allow development of metal resistance, and eventually kills biofilms(Harrison et al., 2004). The differential response of bacterial strains to metals could also be attributed to the difference in their cell wall structure. Gram positive bacteria such as *S. aureus* have a thick peptioglycan layer that may reduce the entry of toxic metals. The lower susceptibility of *S. aureus* to metals compared to *P. aeruginosa*, as observed in Table 1, also correlates with this assumption. This is well in agreement with a previous study demonstrating higher vulnerability of *E. coli* to heavy metals compared to *S. aureus*(Yasuyuki et al., 2010b).

In conclusion, the report illustrates a significant step forward in the view of using pure metals as potent antibacterial agents, when administered in right amounts. Further studies should be conducted to reveal the cellular mechanisms involved in the antibacterial activities of metals and their possible applications in health and medicine. The data and information gained from such studies as reported here should pave the way for further investigations on the use of pure metals as effective antibacterial agents.

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