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Efficacy of selenium in controlling *Acinetobacter baumannii* associated wound infections

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ABSTRACT

Acinetobacter baumannii is a multi-drug resistant, nosocomial pathogen causing a variety of disease conditions, especially wound infections in humans. *A. baumannii*'s ability to form biofilms and colonize epithelial cells potentially makes it difficult to treat skin and soft-tissue infections of this pathogen. Thus, in light of the multidrug resistance and biofilm producing capacity, new strategies for controlling *A. baumannii* wound infections are necessary. This study investigated the efficacy of the essential mineral, selenium (Se) in inhibiting skin-colonizing and biofilm forming abilities of *A. baumannii in vitro*.

The effect of Se on *A. baumannii* adhesion and invasion of human skin keratinocytes (HEK001) was studied. Additionally, the efficacy of Se in inhibiting *A. baumannii* biofilm formation was determined using an *in vitro* collagen matrix wound model, and scanning electron microscopy (SEM) was done to visualize its potential antibiofilm effect. The effect of Se on critical *A. baumannii genes* for biofilm synthesis was also determined using real-time qPCR (RT-qPCR).

Selenium inhibited *A. baumannii* biofilm formation in the collagen-based wound model and reduced bacterial adhesion and invasion of HEK001 ($P < 0.05$). Scanning electron microscopy revealed that Se disrupted *A. baumannii* biofilm architecture. RT-qPCR results indicated that Se significantly down-regulated the transcription of genes associated with *A. baumannii* biofilm production ($P < 0.05$). Results suggest that Se could potentially be used to control *A. baumannii* wound infections but follow up investigation in an appropriate mammalian model is warranted.

1. Introduction

Multidrug resistant (MDR) *Acinetobacter baumannii* is a major nosocomial pathogen causing a wide range of clinical conditions with significant mortality rates [1]. *A. baumannii* is ranked as one of the most common fatal pathogens linked to infections associated with intensive care units [2], and is very difficult to treat due to its resistance to most of the currently available antibiotics [3,4]. The World Health Organization reported *A. baumannii* as one among the fatal ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) resistant to many antibacterial drugs [5]. *A. baumannii* possesses almost all known antimicrobial resistance mechanisms reported in bacteria, including plasmids, transposons, integrons, efflux pumps and reduced the permeability to some antibiotics [6]. In addition, biofilm formation ability of *A. baumannii* not only increases its potential for nosocomial spread, but also contributes to its antibiotic resistance [7,8]. Thus, with limited therapeutic options against *A. baumannii*, there is a critical need to explore novel strategies for controlling infections caused by this bacterium.

MDR *A. baumannii* has been implicated in persistent skin and soft tissue infections (SSTIs), especially in burn victims and combat-related injuries [9–12]. The treatment of wound infections typically consists of using gauze and cotton wool to protect wound from outside contamination [13], along with antibiotic creams or powders [14,15]. However, *A. baumannii's* resistance to antibiotics and the inefficacy of systemic antibiotics in reducing pathogen loads in granulation wounds warrant alternate approaches for treating and controlling wound infections caused by the pathogen [16,17].

Metals have been used as disinfecting agents since ancient times. In

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the past centuries, metals such as tellurium and magnesium oxides were used to treat leprosy, tuberculosis, gonorrhea and syphilis [18,19]. Vessels made from copper and silver were used for food preservation and disinfecting water [20]. Copper, zinc, and silver are often incorporated into antiseptic creams and cleaning agents [21]. Many transition metals and metalloids, such as copper, silver, and gallium have been reported as effective antimicrobials and antibiofilm agents against *Escherichia coli, Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus* [19].

Selenium (Se) is a metalloid, well-recognized as a dietary antioxidant, and its compounds are commonly used in nutrition and chemoprevention therapy [22]. It is an essential component of several enzymes, including glutathione reductase, and is recommended for daily dietary intake in humans by the Food and Drug Administration [23]. Several selenium-based formulations such as selenomethionine and sodium selenite (Na_2SeO_3) are available commercially as food supplements, anticancer agents, and immune stimulators. In addition, the biological activity and role of different selenium compounds against antibiotic resistant pathogens such as *S. aureus* have been investigated [22].

The current study investigated the efficacy of Se to inhibit *A. baumannii* adhesion to and invasion of human keratinocyte cells, and its antibiofilm effect using an *in vitro* collagen model were determined.

2. Materials and methods

2.1. A. baumannii cultures and growth conditions

Two clinical wound isolates of *A. baumannii* (Navel-17 and OIFC-109) (BEI resources, Manassas, VA, USA) were used in the study. All bacteriological media used in the study, except Leeds MDR *Acinetobacter* agar, were purchased from Difco (Becton Dickinson, Sparks, MD, USA). Leeds MDR agar was procured from Hardy Diagnostics (Santa Maria, CA, USA). The bacterial isolates were cultured separately in 10 ml tryptic soy broth (TSB) overnight, followed by streaking on Leeds MDR *Acinetobacter* agar plates and incubation at 37 °C for 24 h. An individual colony from Leeds MDR *Acinetobacter* agar was sub-cultured twice in 10 ml of TSB at 37 °C overnight to obtain ~8 $\log_{10}$ CFU/ml. The bacterial cultures were then sedimented by centrifugation (3700 x g, 15 min, 4 °C), the pellet was washed twice and resuspended in sterile phosphate buffered saline (PBS, pH 7.2), and diluted appropriately in PBS to obtain ~5 to 6 $\log_{10}$ CFU/ml to be used as the inoculum. Subsequently, the bacterial population in the inoculum was confirmed by plating on tryptic soy agar (TSA) after incubation at 37 °C for 24 h.

2.2. Determination of sub-inhibitory concentration (SIC) and minimum inhibitory concentration (MIC) of Se against A. baumannii

The SIC and MIC of Se against *A. baumannii* were determined as previously reported [24]. Tryptic soy broth (10 ml) tubes containing 10–500 µl of a 50% (w/v) stock solution of sodium selenite (99% purity, Sigma-Aldrich, St. Louis, MO, USA) in increments of 10 µl were inoculated separately with two *A. baumannii* isolates at ~5 $\log_{10}$ CFU/ml, and incubated at 37 °C for 24 h. Tubes without any added Se, but inoculated with *A. baumannii* served as positive controls. After incubation, the samples were serially diluted (1:10) in PBS, plated on TSA, and incubated at 37 °C for 24 h before counting the colonies. The highest concentration of Se that did not inhibit *A. baumannii* growth after 24 h of incubation was selected as the SIC, while the lowest concentration of Se that inhibited bacterial growth after 24 h incubation was taken as the MIC.

2.3. Keratinocyte cell culture

Human skin keratinocyte, HEK001 (ATCC CRL-2404) was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in a 25 cm^2 cell culture flask containing keratinocyte whole medium (K-SFM) supplemented with human recombinant epidermal growth factor (Invitrogen, Carlsbad, CA, USA) at 37 °C in the presence of 5% CO_2.

2.4. Adhesion and invasion assay

The effect of MIC of Se on *A. baumannii* adhesion to and invasion of HEK001 keratinocyte cells was determined, as previously described [25]. Twenty-four well tissue culture plates (BD, Franklin Lakes, NJ, USA) were seeded with ~10^5 cells/well, and incubated at 37 °C for 24 h in 5% CO_2 incubator to form a monolayer. *A. baumannii* was grown to mid-log phase after six hours of incubation at 37 °C, washed and resuspended in K-SFM with the MIC of Se. Bacteria suspended in K-SFM and the media alone were used as positive and negative controls, respectively. Aliquots of 100 µl of the bacterial suspension containing ~6 $\log_{10}$ CFU/well (MOI 1:10) was inoculated in duplicates into the HEK001 monolayer and incubated at 37 °C in 5% CO_2 incubator for two hours. For the adhesion assay, the infected monolayers after incubation were washed three times with PBS, and the cells were lysed using 0.1% Triton X-100 (Invitrogen). The number of viable adhered bacteria was enumerated by serial dilution and culturing on TSA plates. For the invasion assay, the HEK001 monolayer was washed three times with PBS, followed by an additional incubation for two hours in K-SFM containing gentamicin (100 µg/ml) (Invitrogen) to kill the extracellular bacteria. Subsequently, the wells were washed three times with PBS and the cells were lysed using 0.1% Triton X-100 to release the intracellular bacteria. The invaded bacteria were enumerated by serial dilution in PBS and culturing on TSA plates.

2.5. Quantification of biofilm in vitro

In order to replicate the conditions of a chronic wound, an *in vitro* system that accommodates bacterial aggregates in a simulated wound fluid and collagen matrix model, as described by Werthen et al [26] was used. Briefly 24-well cell culture plates were coated with collagen solution (rat tail collagen type 1, BD Biosciences, San Jose, CA). A 10 ml collagen solution (2 mg/ml) contained 1 ml of 0.1% acetic acid, 2 ml collagen stock solution (10 mg/ml) and 6 ml cold simulated wound fluid (SWF, 50% fetal calf serum and 50% physiological sodium chloride in 0.1% peptone) and 1 ml of 0.1 M sodium hydroxide. One ml of the above solution was added to each 24-well plate, which was incubated at 37 °C for one hour. After complete polymerization, each *A. baumannii* isolate (~5 $\log_{10}$ CFU/ml) was suspended separately in SWF with or without the MIC of Se and added to each well in duplicates. The plates were incubated for 48 h, and the polymerized collagen was subsequently dissolved using collagenase solution (500 µl/ml in PBS), followed by incubation for 60 min at 37 °C. *A. baumannii* counts were determined using serial dilution and plating on TSA.

2.6. Scanning electron microscopy

The three-dimensional structure of *A. baumannii* biofilm treated with or without Se was visualized using scanning electron microscopy [27,28]. *A. baumannii* exposed to the MIC of Se in TSB was allowed to form biofilm on collagen coated silicon wafer chips (Prod No. 16008, Ted Pella Inc.) by incubation at 37 °C for 48 h, followed by gently washing with PBS three times. Samples were then fixed in glutaraldehyde-paraformaldehyde-cacodylate buffer (pH 7) at 4 °C for 90 min. Following fixation, they were washed with 0.1 M sodiumcacodylate buffer (pH 7) and post-fixed in 1% osmium tetroxide at 4 °C overnight. The chips were then rinsed twice for 15 min in distilled water, dehydrated in serial concentrations of ethanol (30, 50, 70, 95, 100, and 100% EtOH, 15 min each), and dried using a critical point dryer (931 G L, Tousimis). The dried chips were then mounted on SEM

Table 1
List of primers used for RT-PCR analysis of *A. baumannii* biofilm genes.

Gene	Sequence (5'→3')
bfmR (F)	ATGTTGCCGGGTGCAGAT
(R)	CTGCACCCATTTCCAGACCA
csuA (F)	AGCTGTGGTAGCTTCACCAA
(R)	TGAATTATTGCCTTCTTGTTCTGT
abaI (F)	AGCAGTCAGGCTGTGTCATC
(R)	CCCGCAGCACGTAATAAACG
bap (F)	ACTGGACCGATGAGAGTGGA
(R)	TTGCCCACTTATCACGCCAT
rRNA-16S(F)	TGGCTCAGATTGAACGCTGGCGGC CGCTGGCGGC
(R)	TACCTTGTTACGACTTCACCCCA

stub using silver paint and sputter coated with gold/palladium (E5100, Polaron) and examined using a scanning electron microscope (FEI Nova Nano SEM 450).

2.7. Real-Time Quantitative PCR (RT-qPCR)

The effect of Se on the transcription of genes critical for biofilm production in *A. bauamannii* was investigated using RT-qPCR. The information on the primer sequences for biofilm-associated *A. baumannii* genes, including *bap*, *csuA*, *bfmR*, *and abaI* are provided in Table 1. The primers were designed as previously published [25,29,30], and custom synthesized primers were obtained from Integrated DNA Technologies (Foster City, CA, USA).

A. baumannii was grown to mid-log phase in TSB at 37 °C with or without the SIC of Se, and total RNA was extracted using RNeasy RNA isolation kit (Qiagen, CA, USA). The complementary DNA (cDNA) synthesized using the Iscript cDNA synthesis kit (Biorad, CA, USA) was used as the template for RT-qPCR. SYBR green reagents (Applied Biosystems, Inc., CA, USA) were used to detect the amplification products. RT-qPCR was performed with Step OnePlus™ Real Time PCR system (Applied Biosystems, Foster city, CA, USA) using the SYBR green assay (Applied Biosystems) under custom thermal cycling conditions with the normalized RNA as the template. Duplicate samples were analyzed and standardized against 16S rRNA gene expression. The relative fold change in gene expression was calculated using the $2-\Delta\Delta Ct$ method.

3. Statistical analysis

All experiments had duplicate samples for each treatment and control, and were replicated three times. The adhesion, invasion, and biofilm data were analyzed using a generalized linear model of proc genmod procedure of SAS 9.3 version (SAS institute, Cary, NC, USA). The differences between the means were compared using least significant difference (LSD) test and significance tested at $P < 0.05$.

4. Results

The SIC of Se against *A. baumannii* was 5.6 mM, while the MIC was 14 mM. The efficacy of Se in reducing colonization of keratinocytes by the two clinical strains of *A. baumannii* is presented in Fig. 1. The results revealed that Se was effective in reducing *A. baumannii* adhesion to and invasion of HEK001 cells compared to controls ($P < 0.05$). The MIC of Se reduced *A. baumannii* adhesion and invasion of keratinocytes by ˜1.8 to 2 log CFU/ml compared to controls ($P < 0.05$). Further, on the collagen matrix after 48 h, Se inhibited *A. baumannii* biofilm formation and reduced biofilm-associated bacterial counts by ˜2 to ˜3.5 $\log_{10}$ CFU/ml ($P < 0.05$) (Fig. 2).

Concurring with biofilm assay findings, the SEM results revealed a disruption of the biofilm architecture in Se treated samples, where a significantly lesser biofilm-associated bacteria were observed (Fig. 3). It was also observed that Se significantly down-regulated the expression of all the tested biofilm associated genes in *A. baumannii* ($P < 0.05$), thereby supporting the findings from the *in vitro* wound biofilm assay as well as SEM (Fig. 4). The RT-PCR was done only with strain OIFC-109 as there was no significant variation between the two strains in any of the other experiments discussed.

5. Discussion

Approximately 60% of chronic wounds presented in hospitals have been reported to be associated with recalcitrant bacterial biofilms [31,32]. The biofilm formation of *A. baumannii* on human skin could potentially aid the bacterium to resist host immune defenses and antimicrobial interventions, thereby delaying the healing process [33–35]. Quaternary ammonium compounds are mostly employed as wound and skin antiseptics, as well as disinfectants in hospitals [36]. However, resistance related to antiseptics has also been widely reported recently in *A. baumannii* strains [36]. Thus, there is a need to explore novel strategies for reducing and controlling *A. baumannii* wound infections.

As *A. baumannii*'s ability to form biofilms poses a significant challenge to the treatment of wound infections, an *in vitro* wound model system in a simulated wound matrix was used to study the efficacy of Se in inhibiting *A. baumannii* colonization. The results from the adhesion and invasion studies revealed that both Se was effective in reducing *A. baumannii* adhesion to and invasion of HEK001 cells compared with the controls ($P < 0.05$). Furthermore, results from the *in vitro* SWF model assay indicated that Se significantly decreased *A. baumannii* biofilm as revealed by the lower biofilm-associated bacterial counts compared to control (Fig. 3). These results were supported by SEM, where Se-treated *A. baumannii* biofilm appeared disintegrated and fragmented.

The *A. baumanni* biofilm genes analyzed for the gene expression study included *csuA*, pilus usher-chaperone assembly system that mediates adherence [37]; *bfmR*, the two component regulatory system sensing external signals [38]; *bap*, biofilm associated protein necessary for mature biofilm formation [39] and *abaI*, the autoinducer synthase [40]. It was observed that Se significantly down-regulated the expression of all these biofilm associated genes ($P < 0.05$), thereby

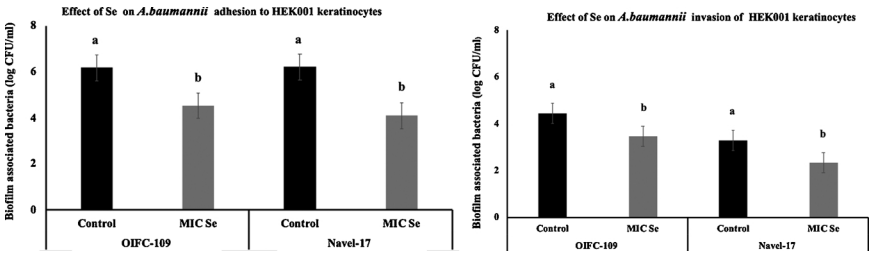

Fig. 1. Effect of Se on *A. baumannii* Naval-17 and OIFC-109 adhesion to and invasion of HEK001 keratinocytes. *A. baumannii* either alone or with MIC of Se was added to HEK001 monolayer. The adhered and invaded bacterial counts were determined by broth dilution. Bars with different superscripts differ from each other ($P < 0.05$).

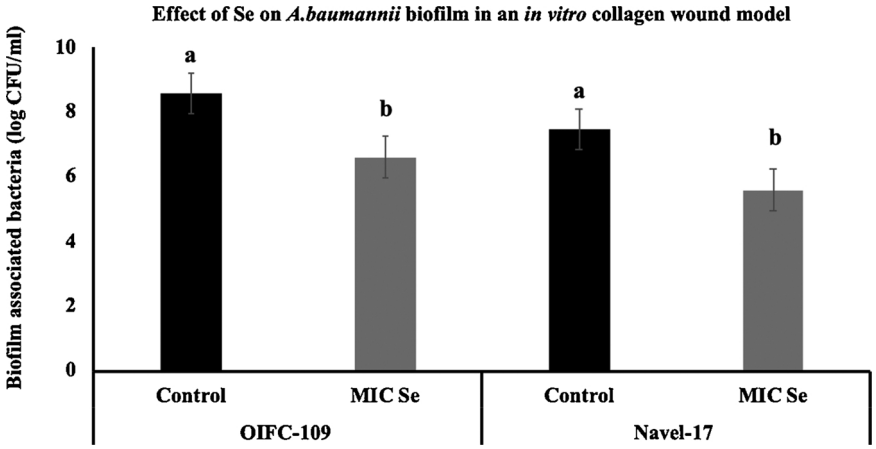

Fig. 2. Effect of Se on *A. baumannii* Naval -17 and OIFC 109 biofilm in an *in vitro* wound model. *A. baumannii* exposed to MIC of Se were allowed to form a biofilm on a collagen matrix at 37^{O}C. Bacterial counts were enumerated after 48 h of incubation. Bars with different superscripts differ from each other ($P < 0.05$).

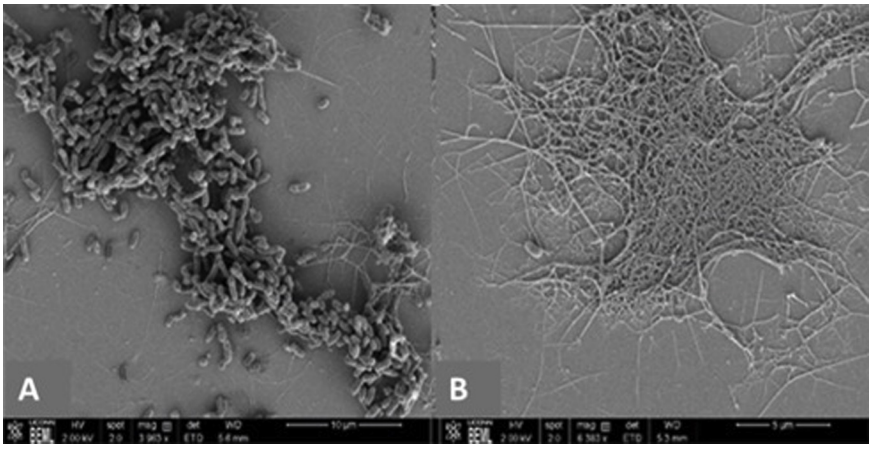

Fig. 3. Scanning electron micrographs of *A.baumannii* biofilm (A) without Se treatment, (B) after treatment with the MIC of Se.

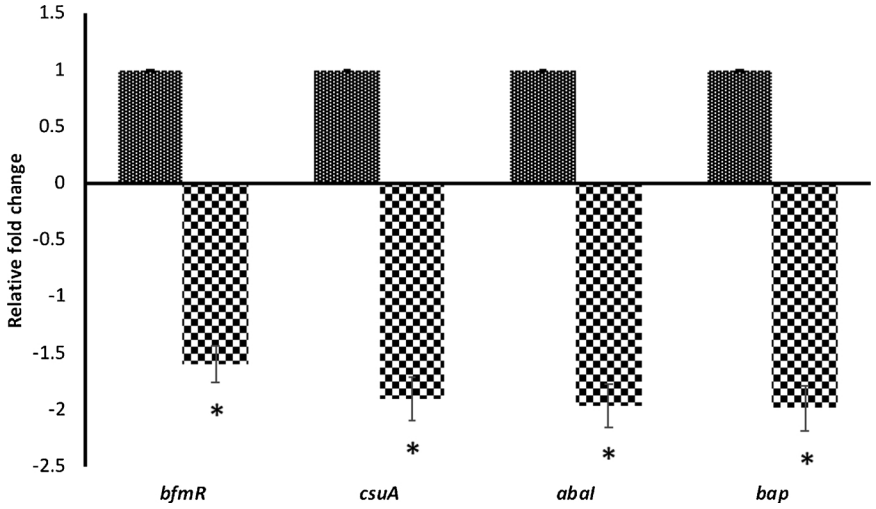

Fig. 4. Effect of Se on biofilm genes in *A. baumannii* OIFC 109. Bacteria not exposed to Se serve as control. *The treatments are significantly different from control at $P < 0.05$. [a] Fold change in gene expression relative to control.

supporting the findings from the *in vitro* wound biofilm assay as well as SEM.

Although, exact mechanisms are unknown, owing to its prooxidant property, Se generates superoxide radicals, which interact with many bacterial components, including membranes and nucleic acids, thereby exerting antimicrobial action [41]. Moreover, selenium compounds are reported to covalently attach to microbial surfaces and catalyze oxygen radical generation, thus reducing bacterial colonization and biofilm formation [42]. Previous study conducted in our laboratory showed that selenium exerted antibiofilm effect against enterohemorragic *Escherichia coli* O157:H7 by reducing bacterial attachment and formation of extracellular polysaccharide matrices, which provide the three-

dimensional structure to biofilms [43]. However, in-depth studies are required for a thorough understanding of the antibiofilm mechanisms of Se.

6. Conclusion

Collectively, results of this study indicated that Se was effective in reducing *A. baumannii* colonization of skin cells and inhibited bacterial biofilm formation on a collagen-based wound model. Selenium could potentially be used as an alternative to antibiotics for treating *A. baumannii* wound infections. However, additional studies on its safety and efficacy in an appropriate animal model are necessary before recommending its clinical application.

Ethical statement

The corresponding author confirms on behalf of all co-authors that this material has not been published in whole or in part elsewhere. The manuscript is being submitted with the final approve for publication from all co-authors.

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References

[1] J.S. Esterly, J. Wagner, M.M. McLaughlin, M.J. Postelnick, C. Qi, M.H. Scheetz, Evaluation of clinical outcomes in patients with bloodstream infections due to Gram-negative bacteria according to carbapenem MIC stratification, Antimicrob. Agents Chemother. 56 (2012) 4885–4890, https://doi.org/10.1128/AAC.06365-11.

[2] H. Giamarellou, Multidrug-resistant Gram-negative bacteria: how to treat and for how long, Int. J. Antimicrob. Agents 36 (2010) S50–S54, https://doi.org/10.1016/J.IJANTIMICAG.2010.11.014.

[3] L.L. Maragakis, T.M. Perl, T.M. Perl, Antimicrobial resistance: *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options, Clin. Infect. Dis. 46 (2008) 1254–1263, https://doi.org/10.1086/529198.

[4] I.K. Neonakis, D.A. Spandidos, E. Petinaki, Confronting multidrug-resistant *Acinetobacter baumannii*: a review, Int. J. Antimicrob. Agents 37 (2011) 102–109, https://doi.org/10.1016/J.IJANTIMICAG.2010.10.014.

[5] H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, M. Scheld, B. Spellberg, J. Bartlett, Bad bugs, No drugs: No ESKAPE! An update from The Infectious Diseases Society of America, Clin. Infect. Dis. 48 (2009) 1–12, https://doi.org/10.1086/595011.

[6] J. Vila, J. Pachón, Therapeutic options for *Acinetobacter baumannii* infections: an update, Expert Opin. Pharmacother. 13 (2012) 2319–2336, https://doi.org/10.1517/14656566.2012.729820.

[7] H.-W. Lee, Y.M. Koh, J. Kim, J.-C. Lee, Y.-C. Lee, S.-Y. Seol, D.-T. Cho, J. Kim, Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces, Clin. Microbiol. Infect. 14 (2008) 49–54, https://doi.org/10.1111/j.1469-0691.2007.01842.x.

[8] R.S. Rao, R.U. Karthika, S.P. Singh, P. Shashikala, R. Kanungo, S. Jayachandran, K. Prashanth, Correlation between biofilm production and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*, Indian J. Med. Microbiol. 26 (2008) 333–337, https://doi.org/10.4103/0255-0857.43566.

[9] D.M. Guerrero, F. Perez, N.G. Conger, J.S. Solomkin, M.D. Adams, P.N. Rather, R.A. Bonomo, *Acinetobacter baumannii* -associated skin and soft tissue infections: recognizing a broadening spectrum of disease, Surg. Infect. (Larchmt) 11 (2010) 49–57, https://doi.org/10.1089/sur.2009.022.

[10] A. Ali, J. Botha, R. Tiruvoipati, Fatal skin and soft tissue infection of multidrug resistant *Acinetobacter baumannii*: a case report, Int. J. Surg. Case Rep. 5 (2014) 532–536, https://doi.org/10.1016/j.ijscr.2014.04.019.

[11] S.G. Santucci, S. Gobara, C.R. Santos, C. Fontana, A.S. Levin, Infections in a burn intensive care unit: experience of seven years, J. Hosp. Infect. 53 (2003) 6–13 (Accessed November 3, 2018), http://www.ncbi.nlm.nih.gov/pubmed/12495679.

[12] B.R. Sharma, Infection in patients with severe burns: causes and prevention thereof, Infect. Dis. Clin. North Am. 21 (2007) 745–759, https://doi.org/10.1016/j.idc.2007.06.003 ix.

[13] V. Jones, J.E. Grey, K.G. Harding, Wound dressings, BMJ 332 (2006) 777–780, https://doi.org/10.1136/bmj.332.7544.777.

[14] D.S. Katti, K.W. Robinson, F.K. Ko, C.T. Laurencin, Bioresorbable nanofiber-based systems for wound healing and drug delivery: optimization of fabrication parameters, J. Biomed. Mater. Res. 70B (2004) 286–296, https://doi.org/10.1002/jbm.b.30041.

[15] T. Dai, Y.Y. Huang, S.K. Sharma, J.T. Hashmi, D.B. Kurup, M.R. Hamblin, Topical antimicrobials for burn wound infections, Recent Pat. Antiinfect. Drug Discov. 5 (2010) 124–151 (Accessed November 3, 2018), http://www.ncbi.nlm.nih.gov/pubmed/20429870.

[16] C.T. Selçuk, M. Durgun, B. Özalp, A. Tekin, R. Tekin, C. Akçay, U. Alabalık, Comparison of the antibacterial effect of silver sulfadiazine 1%, mupirocin 2%, acticoat and octenidine dihydrochloride in a full-thickness rat burn model contaminated with multi drug resistant *Acinetobacter baumannii*, Burns 38 (2012) 1204–1209, https://doi.org/10.1016/j.burns.2012.04.009.

[17] M.G. Thompson, C.C. Black, R.L. Pavlicek, C.L. Honnold, M.C. Wise, Y.A. Alamneh, J.K. Moon, J.L. Kessler, Y. Si, R. Williams, S. Yildirim, B.C. Kirkup, R.K. Green, E.R. Hall, T.J. Palys, D.V. Zurawski, D.V. Zurawski, Validation of a novel murine wound model of *Acinetobacter baumannii* infection, Antimicrob. Agents Chemother. 58 (2014) 1332–1342, https://doi.org/10.1128/AAC.01944-13.

[18] A.D. Frazer, Tellurium in the treatment of syphilis, Lancet 216 (1930) 133–134, https://doi.org/10.1016/S0140-6736(01)09037-7.

[19] J.A. Lemire, J.J. Harrison, R.J. Turner, Antimicrobial activity of metals: mechanisms, molecular targets and applications, Nat. Rev. Microbiol. 11 (2013) 371–384, https://doi.org/10.1038/nrmicro3028.

[20] J.W. Alexander, History of the medical use of silver, Surg. Infect. (Larchmt) 10 (2009) 289–292, https://doi.org/10.1089/sur.2008.9941.

[21] M.X. Chen, K.S. Alexander, G. Baki, Formulation and evaluation of antibacterial creams and gels containing metal ions for topical application, J. Pharm. (2016) 5754349, , https://doi.org/10.1155/2016/5754349 2016.

[22] E. Estevam, K. Witek, L. Faulstich, M. Nasim, G. Latacz, E. Domínguez-Álvarez, K. Kieć-Kononowicz, M. Demasi, J. Handzlik, C. Jacob, Aspects of a distinct cytotoxicity of selenium salts and organic selenides in living cells with possible implications for drug design, Molecules 20 (2015) 13894–13912, https://doi.org/10.3390/molecules200813894.

[23] R. Sunde, Z.T. Selenium, A.C. Ross, B. Caballero, R.J. Cousins, K.L. Tucker (Eds.), Mod. Nutr. Heal. Dis. 11th ed., Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2012, pp. 225–237.

[24] M. Surendran-Nair, A. Kollanoor-Johny, S. Ananda-Baskaran, C. Norris, J.-Y. Lee, K. Venkitanarayanan, Selenium reduces Enterohemorrhagic *Escherichia coli* O157:H7 verotoxin production and globotriaosylceramide receptor expression on host cells, Future Microbiol. 11 (2016), https://doi.org/10.2217/fmb.16.16.

[25] D.P. Karumathil, M. Surendran-Nair, K. Venkitanarayanan, Efficacy of Trans -cinnamaldehyde and eugenol in reducing *Acinetobacter baumannii* adhesion to and invasion of human keratinocytes and controlling wound infection *in vitro*, Phyther. Res. 30 (2016) 2053–2059, https://doi.org/10.1002/ptr.5713.

[26] M. Werthén, L. Henriksson, P.Ø. Jensen, C. Sternberg, M. Givskov, T. Bjarnsholt, An *in vitro* model of bacterial infections in wounds and other soft tissues, APMIS 118 (2010) 156–164, https://doi.org/10.1111/j.1600-0463.2009.02580.x.

[27] A. Narayanan, M.S. Nair, D.P. Karumathil, S.A. Baskaran, K. Venkitanarayanan, M.A.R. Amalaradjou, Inactivation of *Acinetobacter baumannii* biofilms on polystyrene, stainless steel, and urinary catheters by octenidine dihydrochloride, Front. Microbiol. 7 (2016), https://doi.org/10.3389/fmicb.2016.00847.

[28] M. Surendran Nair, P. Lau, K. Belskie, S. Fancher, C.-H. Chen, D.P. Karumathil, H.-B. Yin, Y. Liu, F. Ma, I. Upadhyaya, A. Upadhyay, R. Mancini, K. Venkitanarayanan, Potentiating the heat inactivation of *Escherichia coli* O157:H7 in ground beef patties by natural antimicrobials, Front. Microbiol. 7 (2016) 15, https://doi.org/10.3389/fmicb.2016.00015.

[29] C.W. Dorsey, M.S. Beglin, L.A. Actis, Detection and analysis of iron uptake components expressed by *Acinetobacter baumannii* clinical isolates, J. Clin. Microbiol. 41 (2003) 4188–4193 (accessed November 3, 2018), http://www.ncbi.nlm.nih.gov/pubmed/12958246.

[30] M.-L. Liou, P.-C. Soo, S.-R. Ling, H.-Y. Kuo, C.Y. Tang, K.-C. Chang, The sensor kinase BfmS mediates virulence in *Acinetobacter baumannii*, J. Microbiol. Immunol. Infect. 47 (2014) 275–281, https://doi.org/10.1016/j.jmii.2012.12.004.

[31] G.A. James, E. Swogger, R. Wolcott, E. deLancey Pulcini, P. Secor, J. Sestrich, J.W. Costerton, P.S. Stewart, Biofilms in chronic wounds, Wound Repair Regen. 16 (2008) 37–44, https://doi.org/10.1111/j.1524-475X.2007.00321.x.

[32] J.M. Martin, J.M. Zenilman, G.S. Lazarus, Molecular Microbiology: new dimensions for cutaneous biology and wound healing, J. Invest. Dermatol. 130 (2010) 38–48, https://doi.org/10.1038/jid.2009.221.

[33] S.F. Dallo, T. Weitao, Insights into *Acinetobacter* war-wound infections, biofilms, and control, Adv. Skin Wound Care 23 (2010) 169–174, https://doi.org/10.1097/01.ASW.0000363527.08501.a3.

[34] D.M. Guerrero, F. Perez, N.G. Conger, J.S. Solomkin, M.D. Adams, P.N. Rather, R.A. Bonomo, *Acinetobacter baumannii* -associated skin and soft tissue infections: recognizing a broadening spectrum of disease, Surg. Infect. (Larchmt) 11 (2010) 49–57, https://doi.org/10.1089/sur.2009.022.

[35] A. de Breij, E.M. Haisma, M. Rietveld, A. El Ghalbzouri, P.J. van den Broek, L. Dijkshoorn, P.H. Nibbering, Three-dimensional human skin equivalent as a tool to study *Acinetobacter baumannii* colonization, Antimicrob. Agents Chemother. 56 (2012) 2459–2464, https://doi.org/10.1128/AAC.05975-11.

[36] M. Mahzounieh, S. Khoshnood, A. Ebrahimi, S. Habibian, M. Yaghoubian, Detection of antiseptic-resistance genes in *Pseudomonas* and *Acinetobacter* spp. Isolated from burn patients, Jundishapur J. Nat. Pharm. Prod. 9 (2014).

[37] F. Longo, C. Vuotto, G. Donelli, Biofilm formation in *Acinetobacter baumannii*, New Microbiol. 37 (2014) 119–127 (Accessed November 3, 2018), http://www.ncbi.nlm.nih.gov/pubmed/24858639.

[38] J.A. Gaddy, L.A. Actis, Regulation of *Acinetobacter baumannii* biofilm formation, Future Microbiol. 4 (2009) 273–278, https://doi.org/10.2217/fmb.09.5.

[39] K.A. Brossard, A.A. Campagnari, The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells, Infect. Immun. 80 (2012) 228–233, https://doi.org/10.1128/IAI.05913-11.

[40] C. Niu, K.M. Clemmer, R.A. Bonomo, P.N. Rather, Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*, J. Bacteriol. 190 (2008) 3386–3392, https://doi.org/10.1128/JB.01929-07.
[41] Y. Seko, N. Imura, Active oxygen generation as a possible mechanism of selenium toxicity, Biomed. Environ. Sci. 10 (1997) 333–339.
[42] P. Tran, New Selenium Antimicrobials and Material Coating Against Bacteria and Bacterial Biofilms, Doctoral dissertation Texas Tech University, Lubbock, 2008.
[43] M. Surendran Nair, A. Upadhyay, S. Fancher, I. Upadhyaya, S. Dey, A. Kollanoor-Johny, et al., Inhibition and inactivation of *Escherichia coli* O157: H7 biofilms by Selenium, J. Food Prot. 81 (6) (2018) 926–933.