STAPHYLOCOCCUS AUREUS BACTERIAL RESISTANCE TO SILVER NANOPARTICLE: THE EMERGENCE AND THE MECHANISMS OF RESISTANCE

by

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A thesis submitted in fulfilment of the requirements for the award of

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Certificate of Original Authorship

I, Elizabeth Valentin, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the ithree Institute, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Elizabeth Valentin

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To Roy

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Abstract

The widespread use of silver nanoparticle (NAg) in consumer products has prompted major concerns for bacterial resistance phenomena. Scholarly works have reported the natural ability of bacteria to develop resistance to the antibacterial nanoparticle. While the adaptation mechanisms have been studied in Gram-negative bacteria, it remains largely unexplored in Gram-positive bacteria. This work seeks to investigate the phenomena of NAg resistance in *Staphylococcus aureus*, a Gram-positive bacterium, to unravel the development and mechanisms of resistance in one of the WHO-listed priority pathogens.

Herein, the work found that *S. aureus* has the ability to evolve stable resistance traits, reducing the potency of NAg, upon prolonged exposure. Higher NAg concentrations are required to inhibit the bacterium growth when compared to the wild-type strain. A whole genome analysis of the resistant strain revealed mutation in gene that encodes the purine operon repressor protein. The mutation was thought to be responsible, at least in part, for the physiological adaptation of *S. aureus*, that is, the defence responses that manifest even in the absence of the nanoparticle. The role of this mutation was supported by transcriptomic analysis, whereby, increased expression of the whole purine synthesis operon were detected in the resistant strain. This, along with the observed changes in the expression of other metabolic genes (the pyrimidine, nitrogen, and capsular polysaccharide), highlight a metabolic adaptation to the likely disruptions in bacterial fitness.

The transcriptomic analysis also gives insights into the roles of specific cellular pathways, most likely responsible for the resistance characteristics. Our study found increased expression of genes that encode transporter proteins, ROS (reactive oxygen species) scavenger enzymes in the resistant strain and genes that are associated with the assembly of the iron-sulphur (Fe-S) clusters, one of the main cellular targets of NAg. For the latter, the upregulation was detected in the clusters' main synthesis genes (*suf* system) as well as in the iron and cysteine acquisition genes. Collectively, the adaptation mechanisms involve attempt by the bacterium to neutralize the over-production of ROS, while simultaneously recovering the damaged Fe-S clusters and facilitating silver efflux.

In summary, the knowledge generated herein provides insights into the emergence and molecular basis of NAg resistance in a bacterium that has been perceived to have no, or very low resistance tendency to NAg. The fundamental knowledge will allow

development of technologies to overcome the resistance phenomena and to inform strategies for an effective long term use of the nanoparticle.

List of publications

Journal article

E. Valentin, A. L. Bottomley, G. S. Chilambi, E. Harry, R. Amal, G. A. Sotiriou, S. A. Rice and C. Gunawan. (2020) Heritable Nanosilver Resistance in Priority Pathogen: A Unique Genetic Adaptation and Comparison with Ionic Silver and Antibiotic. *Nanoscale*, **12**, 2384 – 2392. DOI: 10.1039/C9NR08424J.

Conference articles

C. Gunawan*, E. Valentin, A. L. Bottomley, G. A. Sotiriou, S. A. Rice, R. Amal, E. J. Harry. Nanosilver Resistance in Priority Pathogen: Molecular Basis of Adaptation. **International Conference of Energy and Healthcare Materials (ICEHM)**, Sydney, Australia, 30 September-2 October 2020. (invited oral presentation)

C. Gunawan*, E. Valentin, R. Mann, O. McNeilly, A. L. Bottomley, G. A. Sotiriou, S. A. Rice, R. Amal, E. J. Harry. Genetically-Encoded Nanosilver Resitance in Priority Pathogen. **International Conference on Nanoscience and Nanotech**, Brisbane, Australia, 9-13 February 2020. (oral presentation)

E. Valentin*, A. L. Bottomley, E. Harry, R. Amal, G. A. Sotiriou, S. A. Rice and C. Gunawan. The emergence of silver nanoparticle resistance to priority Gram-positive pathogenic bacteria. **1st International Young Scientist Forum (IYSF) of the 12th International Particle Toxicology Conference (IPTC),** Salzburg, Austria, 9-13 September 2019. (oral presentation)

C. Gunawan*, E. Valentin, R. Mann, O. McNeilly, A. L. Bottomley, G. A. Sotiriou, S. A. Rice, W. Y. Teoh, C. P. Marquis, R. Amal, E. J. Harry. Nanosilver and Priority Pathogen: Development of Resistance. **10th International Nanomedicine Conference**, Sydney, Australia, 24-26 June 2019. (oral presentation)

C. Gunawan*, E. Valentin, O. McNeilly, A. L. Bottomley, G. A. Sotiriou, S. A. Rice, W. Y. Teoh, C. P. Marquis, R. Amal, E. J. Harry. The Emergence if Bacterial Resistance to Nanoparticles. **6th International Conference on Antibiotics, Antimicrobials and Resistance**, Edinburg, UK, 11-12 October 2018. (invited oral presentation)

* presenting author

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Abbreviations

µ	Micro (10^{-6})
Ag^+	Ionic silver
Ag-r	Ag^+-resistant strain
ANSTO	Australia's Nuclear Science and Technology Organisation
ASV	Anodic stripping voltammetry
ATP	Adenosine monophosphate
ATR	Attenuated total reflection
B.	Bacillus
BET	Brunauer–Emmett–Teller
BOD	Biological oxygen demand
BPEI	Branched polyethyleneimine
Ca-MHB	Cation-adjusted Mueller Hinton broth
CLSI	The Clinical and Laboratory Standards Institute
CRISPRi	Clustered regularly interspaced short palindromic repeats interference
CSR	the Centre for Staphylococcal Research
d	Diameter
dCas9	dead Cas9
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
dTEM	Diameter based on transmission electron microscopy
E.	Escherichia
EDAX	Energy dispersive X-ray spectroscopy
EXAFS	Extended X-ray fine absorption structure
Fe-S cluster	Iron-sulphur cluster
FSP	Flame spray pyrolysis

FT-IR	Fouirer transform infrared
GSH	Glutathione
GSSG	Oxidized glutathione
h	Hour
HGT	Horizontal gene transfer
ICP-MS	Inductively coupled plasma spectrometry-mass spectrometry
IMP	Integral membrane protein
IMP	Inosine monophosphate
LB	Luria Bertani broth
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
MBC	Minimum bactericidal concentration
MHA	Cation-adjusted Mueller Hinton agar
MIC	Minimum inhibitory concentration
min	Minute
MRSA	Methicillin-resistant *S. aureus*
NAg	Silver nanoparticle
NAG	N-acetylglucosamine
NAg-r	NAg-resistant strain
NAM	N-acetylmuramic acid
NCBI	National Centre of Biotechnology Information
OD	Optical density
Omp	Outer membrane protein
ORF	Open reading frame
P.	Pseudomonas
PAM	Protospacer adjacent motive
PBP	Penicillin binding proteins

PCA	Principal component analysis
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PGRE	Pomegranate rind extract
PVP	Polyvinylpyrrolidone
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNA-seq	RNA-sequencing
ROS	Reactive oxygen species
RPM	Rotation per minute
S.	Staphylococcus
SCELSE	Singapore Centre for Environmental Life Science Engineering
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SERS	Surface–enhanced Raman spectroscopy
sgRNA	single guide RNA
SNV	Single nucleotide variants
TCA cycle	Citric acid cycle
TEM	Transmission electron microscopy
TRFLP	Terminal restriction fragment length polymorphism
UMP	Uridine monophosphate
UV	Ultra violet
WHO	World Health Organization
WT	Wild-type strain
wt%	Weight percentage
WTA	Wall teichoic acid
XRD	X-ray diffraction