Contact Killing of Bacterial Pathogens on Metallic Copper

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Abstract

Hospital-acquired infections (HAIs) are a serious health concern worldwide. Currently in New Zealand, about one in ten patients admitted to hospitals will acquire an infection while receiving treatments for other medical or surgical conditions. An emerging strategy for HAIs prevention is to use self-sanitising copper surfaces on items commonly touched in hospitals, which can provide sustained protection against microbial contamination. This is due to the fact that a wide range of microorganisms can be rapidly killed on copper in a process termed "contact killing". However, the mechanisms of copper-mediated contact killing are not fully understood; and moreover, the potential of bacterial pathogens to develop resistance to metallic copper has so far not been examined.

Here we hypothesize that bacteria are predominantly killed by a burst release of toxic copper ions resulted from chemical reactions between surface components of bacterial cell and metallic copper. To test this copper ion burst release hypothesis, we isolated and phenotypically characterized small colony variants (SCVs) derived from the two most common nosocomial pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Consistent to our expectation, SCV mutants overproducing exopolysaccharides (EPS) are more rapidly killed than wild type on the surfaces of pure copper (99.9% Cu) and brass (63.5% Cu). Similar results were obtained with a panel of mutants with altered production of cell surface components (EPS, lipopolysaccharides,

capsules, flagella and pili) in a non-pathogenic model organism of *Pseudomonas fluorescens* SBW25.

Next, a unique approach of experimental evolution was used to assess the potential emergence of bacterial resistance to metallic copper. Specifically, *P. fluorescens* SBW25 was subjected to daily passage of sub-lethal conditions on the surfaces of brass. After 100 daily transfers, the evolved strains had a slight increase of survival rate on brass; but importantly, ~97% of cells can still be killed on brass within one hour.

Taken together, our results clearly indicate that the rate of bacterial killing on copper is largely determined by surface components of a bacterial cell, providing support for the copper ion burst release hypothesis. Our primary data of experimental evolution showed that bacteria have limited ability to evolve resistance to metallic copper.

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"Let me tell you the secret that has led me to my goal.

My strength lies solely in my tenacity."

- Louis Pasteur-

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ATP	adenosine triphosphate
A_{550}	absorbance measured at 550 nm
bp	base pairs
CA	colanic acid
CF	cystic fibrosis
cfu	colony forming unit
CFW	calcofluor white
CFP	cyan fluorescent protein
cm	centimeter
CV	crystal violet
DF	dilution factor
DNA	deoxyribonucleic acid
dNTP	deoxyribo nucleotide tri-phosphate
DR	death rate

EDAX	energy dispersive x-ray spectroscopy
EPA	environmental protection agency
EPS	exopolysaccharides or extracellular polysaccharides
FS	fuzzy spreader
FTIR	synchrotron fourier-transform infrared spectromicroscopy
g	gram
Gem	gentamicin
h	hour
HAIs	hospital-acquired infections
ICP	inductively coupled plasma mass spectrometry
ICU	intensive care units
Km	kanamycin
kV	kilovolt
LB	luria-bertani
LSWS	large spreading wrinkly spreader

LPS	lipopolysaccharide
MIC	minimal inhibitory concentration
min	minutes
ml	milliliter
mM	millimolar
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
MSSA	methicillin sensitive *Staphylococcus aureus*
nm	nanometer
OD	optical density
PCR	polymerase chain reaction
pmol	picomole
ROS	reactive oxygen species
rpm	revolutions per minute
s	seconds

SCVs	small colony variants
SD	standard deviation
SEM	scanning electronic microscope
SiC	silicon carbide
TAC	tricarboxylic-acid cycle
TBE	tris-borate-ethylenediamine tetraacetic acid
UV	ultraviolet
VRE	vancomycin resistant *Enterococcus*
WHO	world health organization
WT	wild type
ºC	degrees celsius
µg	microgramme
µl	microliter
µm	micrometre
µM	micromolar